ORIGINAL ARTICLE

Evolving changes in cortical and subcortical excitability during movement preparation: A study of brain potentials and eye-blink reflexes during loud acoustic stimulation

An T. Nguyen[1] | James R. Tresilian[2] | Ottmar V. Lipp[3] | Dayse Tavora-Vieira[4] | Welber Marinovic[1]

[1]School of Population Health, Curtin University, Perth, Western Australia, Australia

[2]Department of Psychology, University of Warwick, Coventry, UK

[3]School of Psychology and Counselling, Queensland University of Technology, Brisbane, Queensland, Australia

[4]Fiona Stanley Hospital, Murdoch, Western Australia, Australia

Correspondence
Welber Marinovic, School of Population Health, Curtin University, Perth, WA, Australia.
Email: welber.marinovic@curtin.edu.au

Abstract

During preparation for action, the presentation of loud acoustic stimuli (LAS) can trigger movements at very short latencies in a phenomenon called the StartReact effect. It was initially proposed that a special, separate subcortical mechanism that bypasses slower cortical areas could be involved. We sought to examine the evidence for a separate mechanism against the alternative that responses to LAS can be explained by a combination of stimulus intensity effects and preparatory states. To investigate whether cortically mediated preparatory processes are involved in mediating reactions to LAS, we used an auditory reaction task where we manipulated the preparation level within each trial by altering the conditional probability of the imperative stimulus. We contrasted responses to non-intense tones and LAS and examined whether cortical activation and subcortical excitability and motor responses were influenced by preparation levels. Increases in preparation levels were marked by gradual reductions in reaction time (RT) coupled with increases in cortical activation and subcortical excitability – at both condition and trial levels. Interestingly, changes in cortical activation influenced motor and auditory but not visual areas – highlighting the widespread yet selective nature of preparation. RTs were shorter to LAS than tones, but the overall pattern of preparation level effects was the same for both stimuli. Collectively, the results demonstrate that LAS responses are indeed shaped by cortically mediated preparatory processes. The concurrent changes observed in brain and behavior with increasing preparation reinforce the notion that preparation is marked by evolving brain states which shape the motor system for action.

KEYWORDS
electroencephalography, event-related potentials, eye-blink reflex, motor control, movement preparation, startle, StartReact effect

1 | INTRODUCTION

Before the execution of a volitional movement, neural processes prepare the neuromotor system to perform that action (Requin & Riehle, 1995; Requin et al., 1991). In reaction time (RT) tasks and anticipatory timing tasks, these processes establish a state of readiness to respond to a stimulus (Chen et al., 1998; Ibanez et al., 2020; Schmidt & Lee, 2011). This readiness is marked by increased activity in response-related circuits both within the brain and the spinal cord (Chen et al., 1998; Eichenberger & Rüegg, 1984; Leocani et al., 2000). This increased activity is associated with increased subcortical excitability, such as stretch reflex excitability in response-related musculature (Sullivan & Hayes, 1987), as well as increased cortical excitability in the circuitry responsible for generating the volitional response (Hoffstaedter et al., 2013; Toro et al., 1994).

Prepared actions can be triggered at very low latencies by the presentation of an intense stimulus, such as a loud acoustic stimulus (LAS), in a phenomenon called the StartReact effect. It was initially theorized that these responses were mediated by a separate mechanism that bypasses the cortex, involving subcortical circuitry associated with the startle response (Carlsen et al., 2004; Valls-Solé et al., 1999). This proposal was based on the idea that mental representations (or motor programs) which specify the parameters of the movement are prepared in advance and transferred to subcortical structures for storage and execution (Carlsen et al., 2004; Valls-Solé et al., 1999). The presentation of an intense stimulus was thought to excite these structures as part of the startle response, involuntarily triggering the release of the motor program and bypassing slower cortical triggering mechanisms involved in voluntary motor control. Early behavioral evidence seemed to suggest that bypassing cortical triggering was a plausible mechanism by which responses could be triggered by LAS, particularly when sternocleidomastoid activity was present as these trials were very fast and produced with similar latencies even when the intensity of the acoustic stimuli varied from 93 to 123 dBa (Carlsen et al., 2007b; Maslovat et al., 2015). In addition, it was reported that LAS above 80 dBA could quickly inhibit the primary motor cortex (M1) (Furubayashi et al., 2000), which was inconsistent with the idea that the LAS could trigger voluntary actions via cortical mechanisms. More recently, however, it has been shown that the effects of acoustic stimuli on M1 excitability depend on the level of preparation for action: LAS during low levels of preparation inhibits M1, whereas it has the opposite effect during high levels of preparation (Marinovic et al., 2014). Although these results do not establish that cortical areas are involved in the triggering of quickly initiated actions

in response to intense stimuli, they indicate that some level of cortical involvement in the StartReact phenomenon is plausible (Marinovic & Tresilian, 2016).

Several other studies have suggested that the cortex may not be bypassed in the StartReact effect as initially suggested (Alibiglou & MacKinnon, 2012; Marinovic et al., 2014; Stevenson et al., 2014). This idea aligns with our proposal (see Marinovic & Tresilian, 2016) that this phenomenon is mediated by voluntary control pathways and could be the result of stimulus intensity effects (Cattell, 1886; Piéron, 1913), particularly for upper limb actions. If this were the case, the dynamic changes that occur cortically during movement preparation would be expected to drive the manifestation of the StartReact effect. In activation models (See Figure 1a), changes in excitability during movement preparation are visualized as the build-up of activation unfolds over time as the expected moment of response initiation approaches, and responses are initiated when activation reaches a certain threshold (Tresilian & Plooy, 2006). Stimulus-evoked activity is also modeled to increase activation in an additive manner as suggested by McInnes et al. (2020), proportional to the intensity of the stimulus. Responses are modeled to be shaped by a combination of these factors, where higher levels of stimulus intensity (Figure 1a) and preparation (Figure 1b) are associated with shorter RTs.

In this model, variability in RTs across trials is attributed to fluctuations in motor preparation, in part due to uncertainties about precisely when responses will be required. As high levels of preparation can only be maintained for a short duration (Alegria, 1974; Muller-Gethmann et al., 2003), accurate responding relies on the appropriate timing of preparatory-related processes which are informed by previous knowledge and experience, and current information. Preparation is relatively straightforward in predictable tasks (e.g., anticipatory timing tasks, and RT tasks with a fixed foreperiod) leading to overall shorter RTs. However, it is more difficult to maintain a high level of preparation in tasks with temporal uncertainty (e.g., tasks with a random foreperiod), leading to relatively longer and more varied RTs (Leow et al., 2018).

Consistent with this account, it has recently been shown that higher levels of temporal preparation were associated with graded decreases in RT to both non-intense tones and LAS in an unpredictable reaction task (Leow et al., 2018). Concerning cortical and subcortical excitability, studies have used LAS to probe the time course effects of movement preparation separately for each system. For example, cortical preparation-related activity reflected by the contingent negative variation (CNV) – a slow and centrally distributed negativity in the scalp electroencephalogram (EEG) implicated in the anticipation for an upcoming stimulus and movement preparation
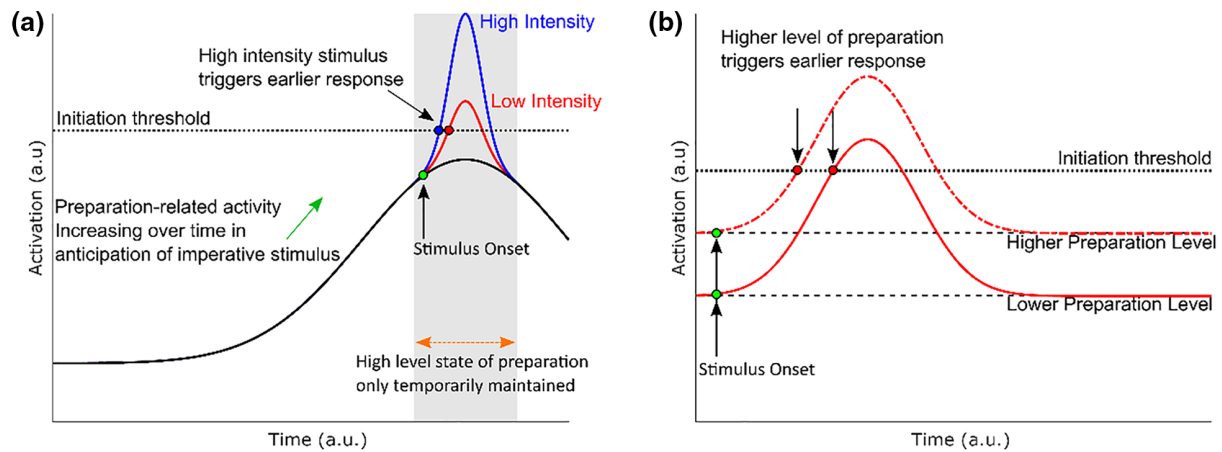
FIGURE 1 (a) Conceptual visualization of the activation model, depicting activation in the motor system from the start of the trial, leading up to a response. The black line represents preparation-related activity which gradually increases as expected time of the response approaches. The gray area shows that a high level of preparation can only be maintained for a short period of time. The red and blue lines represent the activity evoked by low- and high-intensity acoustic stimuli, respectively. This induced activity causes the net activity in the system to cross the initiation threshold (dotted black line), triggering the response, but activity in the high-intensity stimulus reaches the threshold earlier, producing an earlier response. (b) Visualization showing the influence of preparation level on response time, given the same stimulus (static levels of preparation used for simplicity). When the system is at a higher state of preparation, voluntary responses to stimuli can occur earlier because less additional activation is required to reach initiation threshold.

(Kononowicz & Penney, 2016) – was associated with RT in a predictable RT task (MacKinnon et al., 2013), and some have reported that reflex excitability is enhanced around the expected time of the response compared to baseline (Carlsen et al., 2004; Lipp et al., 2001; Marinovic et al., 2013; Valls-Solé et al., 1995, 1999). However, research has yet to systematically examine how both cortical and subcortical excitability are influenced by the level of movement preparation and whether they, in turn, are associated with the execution of the motor action.

1.1 | Current study

In this study, we sought to capture the evolution of cortical and subcortical excitability that occurs over time with motor preparation and examine its relationship with voluntary responses to LAS and non-intense stimuli. To study the evolving effects of motor preparation, we modified an auditory RT task to induce increasing levels of motor preparation within each trial. We expected that RTs to LAS would be significantly shorter than non-intense tones, reflecting an effect of stimulus intensity. As force production is associated with primary motor cortex (M1) activity (Ashe, 1997), additional M1 activity induced by the LAS was predicted to result in more forceful responses than those to non-intense tones (see also McInnes et al. (2020)). According to the activation model, responses to LAS and non-intense tones are expected to be similarly influenced by the level of preparation – such that RTs should decrease, and forces should increase as

the sounds are presented in later positions, demonstrating that increased readiness to respond leads to faster responses. Alternatively, it could be that RTs to LAS would be unaffected by cortical levels of preparation.

Regarding cortical excitability, we expected to observe an increasing negativity in the motor region as sounds are presented in later positions, reflecting increasing levels of preparation. This pattern should be observed on both tone and LAS trials, demonstrating cortical involvement in both contexts. Extending beyond the motor system, research has shown interactions between auditory and motor regions of the brain during speech and musical rhythm perception in humans (Chen et al., 2006, 2008; Cheung et al., 2016). Recent neuroimaging work by Gale et al. (2021) in humans and Li et al. (2017) in mice has further suggested that the auditory cortex is involved not only to anticipate a sensory event but also to produce appropriate motor responses. As such, we also sought to examine whether activity in sensory cortical areas (auditory and visual) would also evolve with preparation. Lastly, because the dorsal premotor cortex can provide both an excitatory input to M1 and inhibitory input to the spinal cord during early preparation (Duque & Ivry, 2009; Duque et al., 2012), we were also interested in determining the time course of subcortical excitability as the time to movement onset approached. We have previously observed an association between the time course of movement preparation and eye-blink responses – latency and amplitude – (Nguyen et al., 2021; see also Valls-Solé, 2012), and, therefore, we expected that subcortical excitability would increase as the level of motor preparation evolves over

time, reflected by decreases in blink latency and increases in blink amplitude.

2 | METHOD

2.1 | Participants

Thirty-one healthy adult participants consisting of university student and staff volunteers were recruited. Eight participants were excluded in total: two due to excessive EEG noise and artifacts, two due to low performance on 'Catch' trials (<70%), and four due to missing behavioral data. The final sample consisted of 23 participants (age $M(SD) = 20.43(2.57)$ years, age range = 18–27 years, 18 females). All participants reported being right-hand dominant, having normal or corrected to normal vision, no history of significant head trauma, and no diagnosed neurological conditions. All participants provided written informed consent before starting the experiment, and the protocol was approved by the human research ethics committee of Curtin University (approval code: HRE2018-0257).

2.2 | Modified auditory reaction task

Participants were instructed to quickly respond to an auditory stimulus (tone or LAS) that was randomly embedded in a sequence of four visual flashes (See Figure 2). This design allowed us to manipulate the level of motor preparation over the course of each trial, which can be represented by the increasing conditional probability (See Table 1). The tone was a 1700 Hz pure tone presented for 50 ms at 60 dBa, and the LAS was a broadband white noise stimulus presented for 50 ms at 104 dBa. Broadband white noise was chosen for the LAS as it is more likely to elicit a startle reflex than a pure tone (Carlsen, 2015). Participants responded to all sounds (except for LAS presented at the Warning Flash) by pressing on a force sensor (SingleTact, Model: CS8-10 N) with their right index finger. The force sensor was embedded in a custom-built device resembling a computer mouse. The task was presented using MATLAB 2015b and Psychtoolbox version 3.0.11 (Brainard, 1997; Kleiner et al., 2007; Pelli, 1997) on an ASUS 24-inch LCD monitor (Model: VG248QE, running at 1920 × 1080 resolution and 120 Hz). The auditory stimuli were presented through Sennheiser headphones (Model: HD25-1 II). The recorded rise time of both stimuli from the output of a soundwave was <1.25 ms. Sound intensity was measured with a Brüel & Kjaer sound-level meter (type 2205, A weighted) placed 2 cm from the headphone speaker.

On each trial, 'Relax' was presented for 500 ms followed by a blank screen ranging randomly from 600–1000 ms. A red circle (42 mm in diameter) was briefly flashed on the center of the display four times (50 ms duration with
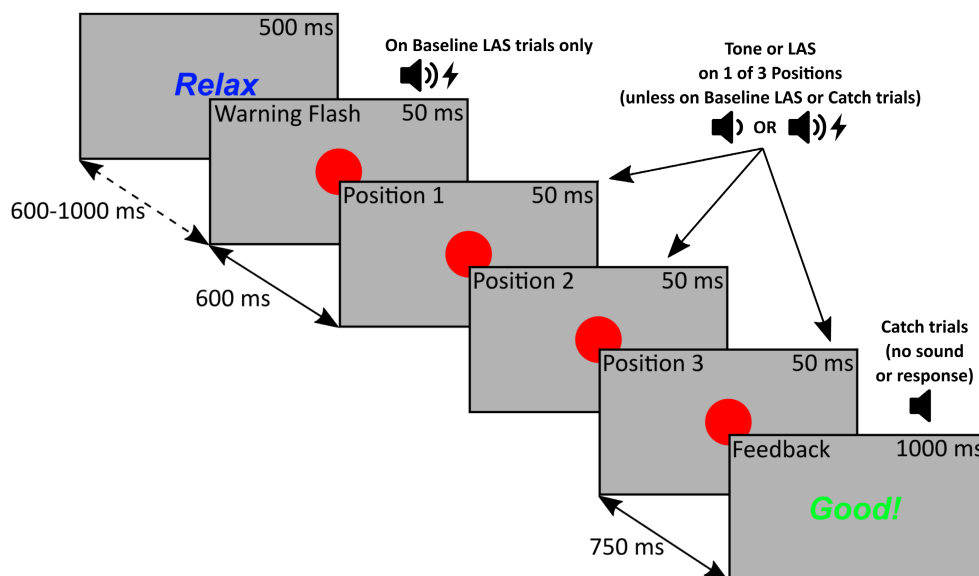

FIGURE 2 A diagram showing the sequence of events on each trial. A red circle was briefly flashed on-screen four times and an acoustic stimulus, either the tone or loud acoustic stimulus (LAS) was randomly presented synchronously with the flash at Positions 1, 2, or 3. Participants reacted to the sound by pressing on a force sensor, and feedback about timing and force was presented at the end of the trial. On a small portion of trials, the LAS was presented with the Warning Flash (Baseline LAS trial), or no sound is presented at all (Catch trials) No responses were required on these trials.

Stimulus type	Warning flash	Position 1	Position 2	Position 3
Tone	0/360	80/340	80/240	80/140
LAS	20/360	20/340	20/240	20/140
Total	20/360	100/340	100/240	100/140
Conditional probability of response required	0%	29.41%	41.67%	71.42%

TABLE 1 Summary showing the number of tone and loud acoustic stimulus (LAS) trials for each Position against the number of possible remaining events.

Note: Note the evolving chance of responding at each position of the task presented on the bottom row. No response was required for LAS at Warning Flash.

a stimulus onset asynchrony of 600 ms). The first flash served as the warning cue as the following three flashes served as potential positions where the tone could be presented (referred to as Positions 1, 2, and 3). Occasionally, a LAS was presented instead of the tone. Visual feedback was presented 750 ms after the final flash for 1000 ms. On some trials, the LAS was paired with the warning flash to elicit a baseline measure of the eye-blink startle reflex. To discourage anticipatory responding to the final flash, we also included Catch trials where no auditory stimulus was presented, and no response was required. The order of trials was pseudo-randomized such that the LAS was never presented on two consecutive trials.

The experimental portion of the task consisted of 360 trials, split into 4 blocks of 90 trials with self-paced breaks between blocks. In total, there were 240 tone trials (66.67% of total trials, 80 trials for each Position), 80 LAS trials (22.22% of total trials, 20 for each Position, including the warning flash), and 40 Catch trials (11.11% of total trials). Before the experimental task, participants were provided with verbal and on-screen instructions, example demonstrations of the trial sequence, the tone and LAS, followed by a practice block consisting of 18 trials in a fixed sequence with the same trial proportions as the experimental blocks.

With respect to feedback, 'Good Timing' was presented on tone trials if participants responded between 50–250 ms after stimulus onset. Otherwise, 'Too early' or 'Too late' was presented. 'No response detected' was presented if no response was made within 600 ms. On Catch trials, 'Good' was presented if no response was detected; otherwise, 'Oops!' was presented. On LAS trials, no feedback on performance was presented, and 'Probe trial' was presented in place of feedback. A point system was also implemented to encourage task engagement. Three points were awarded for timely responses on tone trials, but points were not deducted for inaccurate or absent responses. No points were awarded or deducted for Catch or LAS trials, and points were reset after each block. Points were not recorded or analyzed.

2.3 | Force and EMG data reduction and measurement

Force data were continuously recorded for the duration of the trial, digitized at 2000 Hz using a National Instruments data acquisition device (Model: USB-6229). The data were filtered using a low-pass second-order Butterworth filter with a cut-off frequency of 20 Hz. We measured movement onset, relative to the onset of the auditory stimulus, calculated from the tangential speed time series derived from the force data using the algorithm recommended by Teasdale et al. (1993). Trials with response times outside 50–600 ms were excluded from further analysis, $M(SD) = 10.95(12.31)$ trials (~2%). We also measured the peak force of each response. As participants were free to exert a comfortable amount of force over the course of the study (max of 10 N), we rescaled peak force within individuals using the 'scale' function in R (base package). Raw peak force values were standardized using a t-score transformation (mean 50 and SD 10).

We recorded EMG activity from the right Orbicularis Oculi muscle using Ag/AgCl sintered electrodes in a pre-amplified bipolar setup. One electrode was placed below the right eye, and the second was placed laterally and slightly higher than the first electrode, ~1 cm edge-to-edge. A ground electrode was placed on the right mastoid region. We used a Neurolog Systems Digitimer Pre-Amplifier (Model: NL820) and Amplifier (Model: NL905), with a 50–1000 Hz pass-band filter and Gain set to 1000. The data were also digitized using the National Instruments DAQ.

The EMG data were processed offline using a semi-automated procedure in R. Firstly, the data were downsampled to 1000 Hz, rectified using the 'rectification' function in 'biosignalEMG' package (Guerrero & Macias-Diaz, 2018). The Bonato et al. (1998) method was used to automatically detect blink onset latency on the rectified data, using the 'onoff_bonato' function in the 'biosignalEMG package' (sigma n = standard deviation of activity 0–200 ms prior to the LAS). If no onset was detected,

the threshold was gradually increased (up to 3 times sigma n) and then decreased (down to 0.5 times signal n), until an onset was detected within 20–150 ms.

We measured baseline-to-peak EMG amplitude occurring after blink onset on the smoothed data using a 5-point moving average with the 'rollapply' function in the 'zoo' package (Zeileis et al., 2020). Similar to peak force, EMG amplitudes were normalized within individuals by calculating t-scores ($M = 50$, $SD = 10$) to account for individual differences in blink amplitude (Blumenthal et al., 2005). Each trial was manually screened, and corrections were made to onset and peak latencies where necessary. Acceptable onset latencies were within 20 to 80 ms from LAS onset, trials outside this window were excluded from further analyses of blink data (Blumenthal et al., 2005). Trials with a flat EMG response were classified as 'non-response trials', trials containing excessive noise, artifacts, or voluntary activation before 20 ms were classified as 'missing' trials. Non-response and missing trials were not included in further analyses of blink data. On average, 3.35(4.49) trials (~4.2%) per participant were identified as non-response, 3.35(4.91) trials (~4.2%) were identified missing, and 5.96(6.06) trials (~7.5%) were manually adjusted.

2.4 | EEG data acquisition, pre-processing, and ERP measurement

EEG data were recorded continuously throughout the experimental blocks. Data were acquired using a Biosemi ActiveTwo EEG system and ActiView (ver. 7.07) at a sampling rate of 2048 Hz with a 100 Hz low-pass online filter. Data were recorded from 64 scalp electrodes arranged according to the 10–5 system with additional electrodes placed adjacent to the outer canthi of the left eye and on the left infraorbital region. For online referencing, the Biosemi EEG system uses active electrodes with Common Mode Sense and Driven Right Leg electrodes providing a reference relative to the amplifier reference voltage.

EEG data were processed offline in MATLAB 2018a using EEGLAB (Delorme & Makeig, 2004), AMICA (Palmer et al., 2012), SASICA (Chaumon et al., 2015), and ERPLAB (Lopez-Calderon & Luck, 2014) plugins. The data were re-referenced to the average of the 64 scalp electrodes, filtered from 0.1–40 Hz with separate low- and high-pass filters, using the 'pop_eegfiltnew' function in EEGLAB. The filtered data were then down-sampled to 256 Hz.

Epochs were extracted on tone and LAS trials, time-locked to the onset of the sound. Epochs spanned for the entire trial (−5000 to 3000 ms) and baseline amplitudes were corrected to the 100 ms interval before the previous flash (i.e., −700 to −600 ms relative to the sound). A close baseline was chosen to minimize the influence of different foreperiod length on amplitude measures, allowing us to focus on the updating expectations from one Position to the next. To correct for blinks, horizontal saccades, and other artifacts, independent component analysis was conducted and independent components (ICs) containing artifacts were manually identified with the guidance of SASICA and removed, $M(SD) = 12.3(5.6)$ ICs.

A Surface Laplacian filter was applied using algorithms described in Perrin et al. (1989) (smoothing factor = $1\,\mathrm{e}^{-5}$, order of Legendre polynomial = 10) to reduce volume conduction effects in EEG sensor space, resulting in a $\mu V/mm^2$ voltage scale (see Kayser and Tenke (2015) for a recent review on this technique). Trials containing voltages on analyzed channels exceeding $\pm 100 \mu V/mm^2$ were excluded, $M(SD) = 8.45(7.40)$ trials (~2.3%). After trial rejection, the average (SD) number of trials retained on Tone trials were 76.57(3.34), 76.43(3.30), and 75.09(4.48) for Position 1, 2, and 3, respectively (~95%). For LAS trials, an average of 17.91(3.16), 19.00(1.57) and 19.00(1.28) trials were retained for each respective Position (~89–95%). For EEG and blink latency analyses, non-response and missing blinks were also excluded resulting in a retained average of 15.48(4.28), 15.7(4.38), and 14.74(4.95) trials for respective position (~75%).

To examine preparation-related changes in the brain, we measured ERP mean amplitude over a 200 ms interval preceding the Tone at the trial level. Voltages were measured at sites corresponding to motor (Cz), auditory (T7 and T8 average), and visual (Oz) areas. Electrodes Cz and T7/T8 were selected where pre-stimulus activity in the corresponding regions was maximal (See Figure 3c, noting how Cz and T7/T8 reflect distinct negative distributions). As the visual area did not show much pre-stimulus activity, Oz was selected based on the maximal response to the visual flashes.

2.5 | Statistical analysis

Statistical analyses were conducted in R statistics and R Studio using generalized and linear mixed models with the 'GAMlj' package (Gallucci, 2019). To avoid transforming the reaction times (Lo & Andrews, 2015), we used the '*gamljGlmMixed*' function with a Gamma regression and an identity link function of the reaction time as the dependent variable. All other variables were analyzed using linear mixed models ('*gamljMixed*' function). For follow-up pairwise contrasts, *p*-values for multiple comparisons were corrected using the Holm method. When we thought it was relevant to establish the lack of statistically reliable effects, we performed a
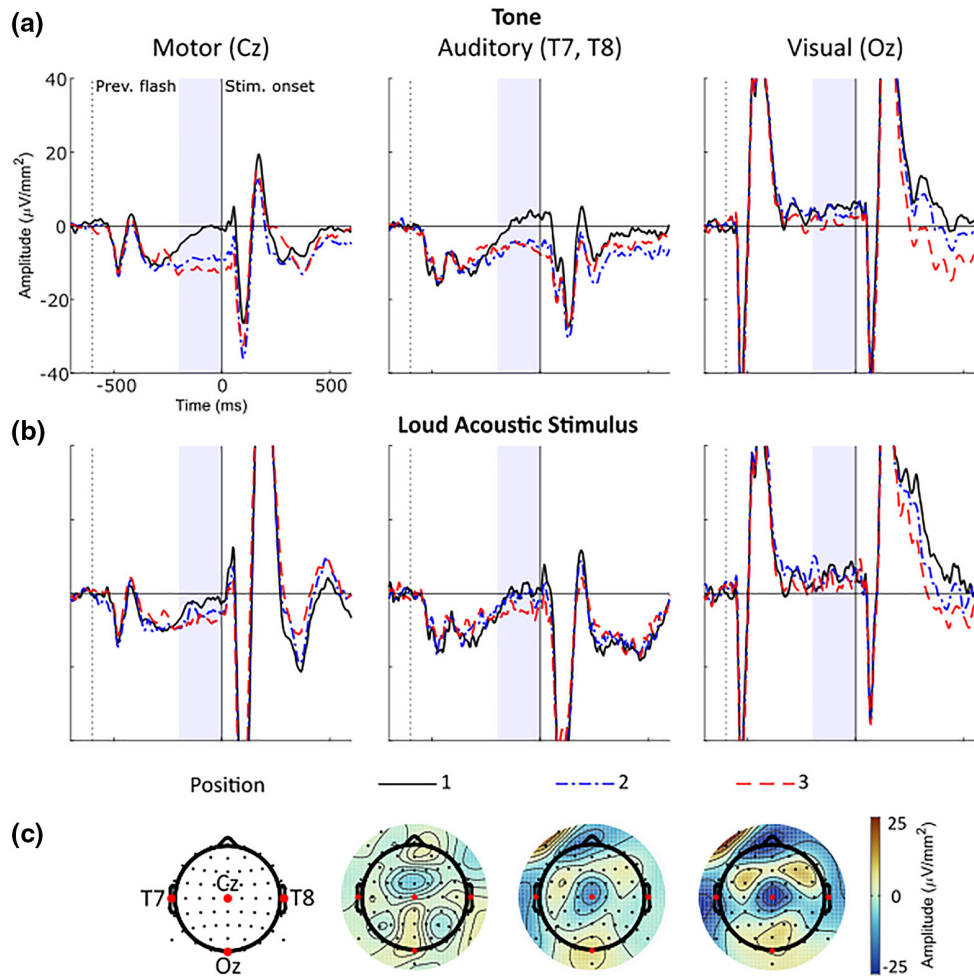
FIGURE 3 Grand-averaged waveforms at each Position for tone (a) and loud acoustic stimulus trials (b), at scalp sites corresponding to motor (Cz), auditory (T7, T8), and visual areas (Oz). Waveforms are aligned to the onset of the auditory stimulus and baseline-corrected to −100 to 0 ms relative to the previous flash. The blue-shaded area shows the interval where pre-stimulus mean amplitudes were measured. (c) Topographical maps show the distribution of activity during the measured interval on tone trials.

Bayesian alternative to linear mixed models using the '*generalTestBF*' function (BayesFactor package), which can provide evidence both for our hypotheses or the null (H0).

For the finger response and ERP data, we modeled the dependent variable (movement onset RT, peak force, pre-stimulus amplitude) with Position (1,2,3), Stimulus Type (tone, LAS), and their interaction as fixed effects. For the eye-blink startle reflex, we analyzed the dependent variable (blink onset latency, blink amplitude) on LAS trials in two ways. First, we provide a more descriptive analysis that includes all conditions where the LAS was presented (Warning flash, Position 1, 2, and 3). This preliminary analysis allows us to compare eye-blink metrics when participants were not prepared (Warning flash) and prepared for action (Positions 1, 2, and 3). More relevant to our hypothesis concerning the time course of preparation, we also analyzed eye-blink variables excluding the Warning flash condition and focused our analysis on the polynomial

trends. For these analyses, LAS Position (Warning flash, Position 1, 2, 3) was the only fixed effect. For all models, participants were modeled as random intercepts.

3 | RESULTS

3.1 | Finger response

3.1.1 | Response time

Generalized linear mixed modeling revealed that RTs were shorter on LAS trials ($X^2(1, N = 23) = 207.01$, $p < .0001$) and decreased as sounds were presented later ($X^2(2, N = 23) = 1958.42$, $p < .0001$), reflecting effects of stimulus-intensity and preparation level. A two-way interaction showed that RT differences decreased as position increased, possibly reflecting a floor effect as reactions approach their lower limit ($X^2(1, N = 23) = 21.41$, $p < .0001$;

RT differences across positions systematically decreased as responses became more likely: 21.9 ms $_{p<.001}$ → 15.5 ms $_{p<.001}$ → 10.2 ms $_{p<.001}$; see Figure 4b).

According to the cortical bypassing hypothesis, not all responses to a LAS presented at 104 dB are expected to activate the subcortical mechanisms responsible for the StartReact effect (Carlsen et al., 2007a; but also see Marinovic & Tresilian, 2016; McInnes et al., 2020). It is possible that this mechanism was only engaged on a subset of express (e.g., very fast) LAS responses. As such, effects of motor preparation would be absent on express responses, as this mechanism bypasses voluntary control

processes. To investigate this, (1) we compared cumulative distribution functions (CDFs) of RTs between tone and LAS trials and (2) we separately analyzed a subset of fast responses (5th percentile). CDFs have been demonstrated to be a more sensitive and reliable way of quantifying RT effects in the context of StartReact compared to traditional methods which only analyze a small subset of trials in which a startle reflex in the sternocleidomastoid muscle is observed (McInnes, Castellote, et al., 2021).

The results of the CDF analysis were consistent with our main analysis (see Figure 5), we observed main effects of Stimulus Type ($F_{(1,1298)} = 243.70$) and Position
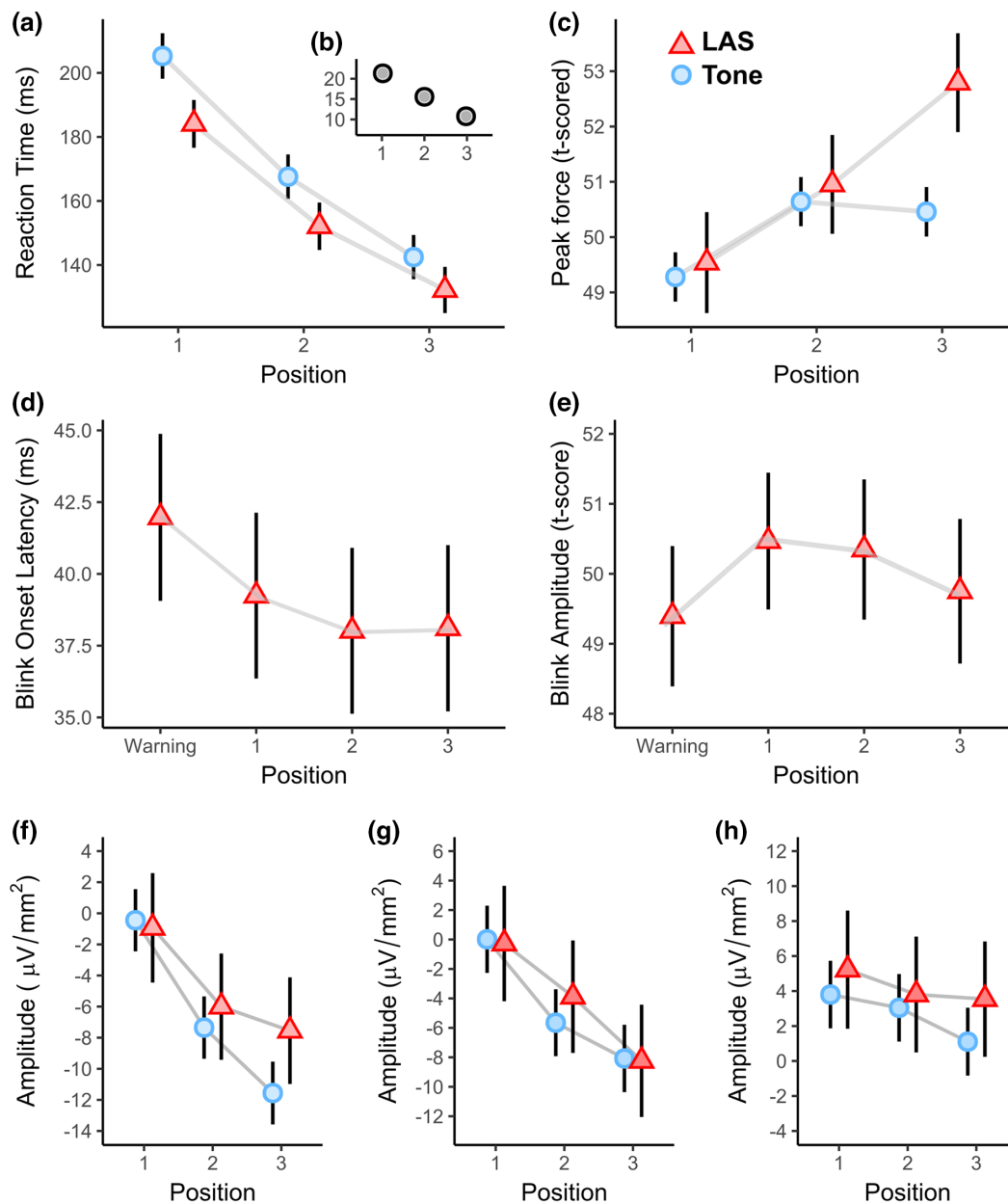

FIGURE 4 Models' estimated means for (a) RT, (b) RT differences between tone and LAS trials across the three positions, (c) peak force (t-scores), (d) blink onset latency, (e) blink amplitude (t-scores), and pre-stimulus cortical activity at (f) motor, (g) auditory, and (h) visual areas. Error bars represent the 95% confidence interval of the models' estimated means.
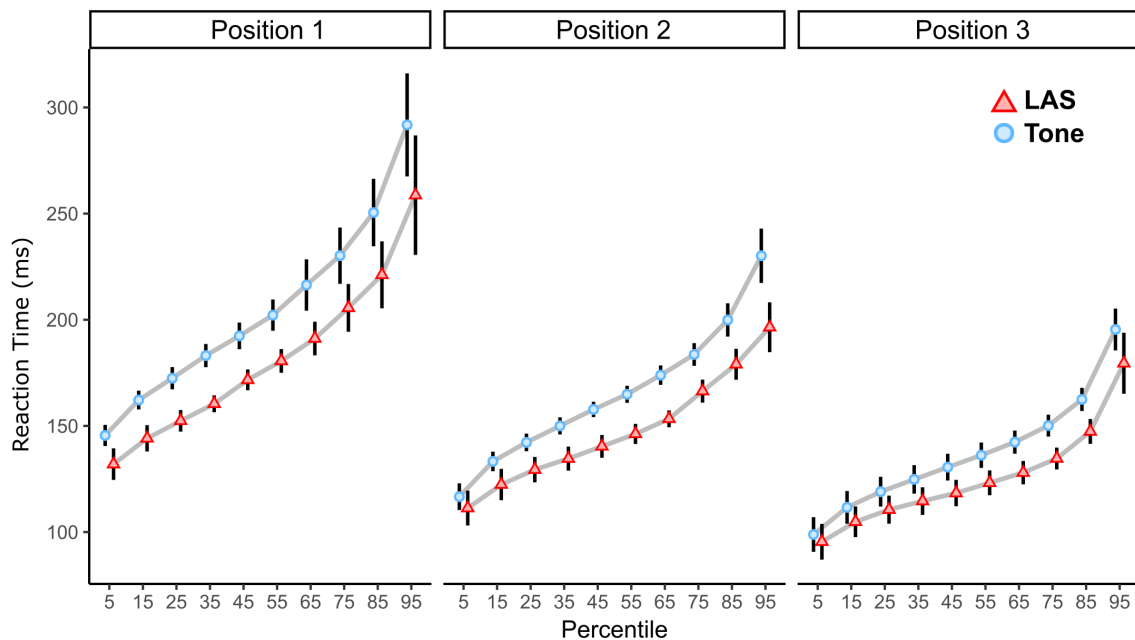
FIGURE 5 Cumulative distribution functions for RT, showing grand means with 95% confidence intervals each percentile (5–95%) and stimulus type (tone, LAS) at each Position (1–3).

($F_{(2,1298)}$ = 1058.62, $p < .0001$) as well as an interaction between them ($F_{(2,1298)}$ = 8.89, $p < .0001$). Notably, percentiles did not influence the two-way interaction (stimulus type × position × percentile, $F_{(18,1298)}$ = 0.16, $p = .999$), suggesting that the effects of stimulus type and position are evident throughout the entire RT distribution. In our analysis of express responses (5th percentile), we observed the same main effects as in our main analysis (stimulus type, $F_{(1,110)}$ = 15.24, $p < .0001$; Position, $F_{(2,110)}$ = 161.16, $p < .0001$), but the interaction only approached significance (stimulus type × position, $F_{(2,110)}$ = 2.64, $p = .07$). As the latency of express responses to LAS were expected to be unaffected by slow cortical triggering mechanisms, we also analyzed the fastest percentile of LAS trials only. Here, we would expect to observe no changes in movement onset time as a function of LAS position if the fastest responses bypass cortical triggering. We found that even at the fastest percentile (5th percentile), movement onset was systematically affected by LAS position ($F_{(2,44)}$ = 57.17, $p < .0001$; all pairwise comparisons were statistically significant: All $p < .001$).

3.1.2 | Peak force

Overall, the force of responses increased as Position increases ($F_{(2,6701)}$ = 18.9, $p < .0001$). The main effect of stimulus type was also statistically reliable ($F_{(2,6701)}$ = 10.77, $p < .01$), but the follow-up for the interaction (stimulus type × Position, $F_{(2,6701)}$ = 5.40, $p = .004$) showed that peak

force was only enhanced on LAS trials (relative to tone trials) at Position 3 (see Figure 4c). Further inspection of the data showed that this pattern was consistent across all blocks.

3.2 | Eye-Blink startle reflex (LAS trials only)

3.2.1 | Blink onset latency

As shown in Figure 4d, we observed a reduction in blink latency from Warning to later Positions ($F_{(3,1357)}$ = 19.44, $p < .0001$). This decrease diminished across position, with the largest difference between the Warning and 1st position, with smaller reductions there-after (Est. mean differences = 2.72 ms $_{p < .001}$ → 1.22 ms $_{p = .078}$ → −0.08 ms $_{p = .87}$). As the LAS at the warning time occurred much earlier than the LAS at other positions, we conducted the same statistical analysis including only LAS blinks at positions 1, 2, and 3. In particular, we were interested in determining whether there was a linear or quadratic trend as the time of movement onset approached. This analysis showed that the linear trend was close to significance, ($F_{(2,1052)}$ = 1.91, $p = .056$), but the quadratic trend was not, ($F_{(2,1051)}$ = 1.34, $p = .18$). The omnibus ANOVA only approached statistical significance ($F_{(2,1051)}$ = 2.76, $p = .06$). Following a suggestion of one of our reviewers, we also analyzed blink latency differences between the Warning position, when no response was required, and Position 1, when a response was required but the level of preparation was relatively lower.

This additional analysis found a statistically reliable difference between means (Est. means: Warning = 42.1 ms vs. Position 1 = 39.4 ms; Difference = 2.69; $p < .001$). Follow-up analyses showed a positive association between RT and blink latency, where shorter RTs were associated with earlier blink onset latencies ($X^2(1, N = 23) = 6.10$, $p = .013$). This effect remained when also accounting for LAS Position. However, there was no statistically significant association with peak force when accounting for Position ($X^2(1, N = 23) = .38, p = .57$).

3.2.2 | Blink amplitude

Although blink amplitude seemed to increase from Baseline (warning, see Figure 4e), we did not observe an effect of Position for blink amplitude ($F_{(3,1503)} = 0.99$, $p = .396$). A follow-up Bayesian analysis showed decisive evidence for the null effect (BF01 = 145). The analysis excluding the warning position yielded similar results, with no clear trends (linear trend: $F_{(2,1116)} = 0.93$, $p = .35$; quadratic trend: $F_{(2,1108)} = 0.51$, $p = .61$). The omnibus ANOVA also failed to reach statistical significance ($F_{(2,1127)} = 0.54$, $p = .58$). As for blink latency, we analyzed blink amplitude differences between the Warning position and Position 1. This additional analysis failed to show a reliable difference between means (Est. means: Warning = 49.39 vs. Position 1 = 50.46; Difference = 1.07; $p = .12$).

3.3 | Electrophysiological data

3.3.1 | Motor area

Overall, pre-stimulus amplitude at Cz became more negative as Position increased ($F_{(2,6420)} = 23.38, p < .0001$) (see Figure 4f). However, no significant effect of stimulus type ($F_{(2,6422)} = 2.29, p = .13$) or two-way interaction was observed ($F_{(2,6419)} = 1.47, p = .22$). Follow-up analyses revealed a positive association between Cz activity and RT ($X^2(1, N = 23) = 26.64, p < .0001$).

3.3.2 | Auditory area

Similarly, pre-stimulus amplitude at T7 and T8 also decreased as Position increased ($F_{(2,6215)} = 15.34, p < .0001$) (see Figure 4g). However, no significant effect of stimulus type ($F_{(1,6215)} = 0.14, p = .701$) or two-way interaction was observed ($F_{(2,6213)} = .31, p = .72$). Follow-up analyses revealed a positive association between activity in T7/T8 and RT ($X^2(1, N = 23) = 29.93, p < .0001$).

3.3.3 | Visual area

Unlike effects observed in motor and auditory areas, no statistically significant effects or interactions were observed at Oz (Position, $F_{(2,6418)} = 1.50, p = .22$; stimulus type, $F_{(1,6422)} = 2.23, p = .13$; Interaction, $F_{(2,6417)} = 0.22$, $p = .79$) (see Figure 4h). A follow-up Bayesian analysis found that all models (main effects and interactions) provided more evidence for the null hypothesis (BF01 ranging from 9.2 [substantial evidence] to 31,224 [decisive evidence]).

4 | DISCUSSION

In this study, we examined whether changes in cortical preparation could modulate the responses to intense and non-intense acoustic stimuli in the same manner, via the normal cortical execution pathways (Valls-Solé et al., 1999). We also probed how cortical preparation could affect the manifestation of reflexive responses, controlled by subcortical circuits (Yeomans et al., 2002). Our hypothesis, derived from the activation model described in the introduction, was that the StartReact effect is not completely mediated by startle-reflex pathways and that cortical changes – assessed via EEG – would systematically affect the onset of prepared actions, irrespective of the intensity of the imperative stimulus (tone or LAS). To test the hypothesis that LAS responses can be explained by a combination of preparation state and stimulus intensity, we induced different levels of preparation by altering the conditional probability of the imperative stimulus over the course of each trial. According to the activation model, we predicted that higher preparation levels would be associated with increased neural activation and reduced response times – demonstrating that motor actions are set up by preparatory processes which alter the overall state of the nervous system for action. We also predicted RTs to LAS would be reduced compared to tones – but would show the same effects of preparation level on RT and neural activation.

As expected, as preparation levels increased over the course of a trial, voluntary (reduced RT, increased force) and to a lesser extent reflexive responses (linear trend for eye-blink latency across Positions 1 to 3 only approached statistical significance, $p = .056$) were facilitated. These effects were preceded by increased neural activation over motor and auditory areas (increased negativity in motor and auditory scalp areas), but not visual areas. With respect to the StartReact effect, shorter RTs were observed on LAS trials, and the same pattern of preparation-level effects on RT and neural activation was present on both tone and LAS trials. Collectively, these results comment on the

nature of movement preparation as (1) a quickly evolving process shaped by expectations about when the action is likely to be required (see also Churchland et al., 2006; Crammond & Kalaska, 2000; Elsayed et al., 2016; Ibanez et al., 2020; McInnes, Lipp, et al., 2021), and (2) a distributed process that also engages circuits beyond those involved in the execution of the motor action like the auditory cortex (Chen et al., 2006; Gale et al., 2021). The results also suggest that even the fastest reactions to LAS can be explained by a combination of established phenomena and that the existence of a separate subcortical pathway that bypasses the motor cortex is not necessary to explain the StartReact effect – at least for cortically mediated actions such as individual finger movements (Marinovic & Tresilian, 2016). Although our results are consistent with express responses being triggered via the normal cortical pathways, we stress that these results do not rule out that the facilitation of voluntary action in the presence of LAS can be partially achieved by recruiting subcortical pathways, in particular the reticulospinal system (Baker, 2011; Baker & Perez, 2017; Honeycutt et al., 2013; Riddle et al., 2009; Rothwell, 2006). This acknowledgment is particularly relevant for lower limb movements (Nonnekes et al., 2014) and upper-limb actions performed by stroke survivors (Honeycutt & Perreault, 2012; Honeycutt et al., 2015; Marinovic et al., 2016).

4.1 | Motor actions are prepared strategically according to the stimulus probability

The effect of the preparation-level manipulation on RT and neural activation demonstrates how motor actions and the underlying processes that set up the system are shaped by expectations about when a response is likely required. Although there were an equal number of trials for each temporal position (i.e., the global probabilities were the same), RTs decreased with each position. This suggests that participants were updating their expectations based on the absence of tones, gaining more confidence that the tone will occur together with the next flash – following the evolving conditional probability of the imperative stimulus (see Table 1). This RT reduction likely reflects a strategic trade-off between speed and accuracy, as opposed to physiological limits on how quickly actions can be prepared (see Haith et al., 2016; Lara et al., 2018). Limiting the level of preparation at earlier positions may serve to prevent premature and incorrect responses.

This idea is consistent with recent findings by Leow et al. (2018) who isolated the effects of expectancy by using the same flash-based design to contrast predictable and unpredictable (same as ours) variants. In their

predictable task, imperative stimuli were also presented at different but known temporal positions. Across the board, RTs were significantly shorter in the predictable compared to the unpredictable task. Notably, mean tone RT at the first position of the predictable task was ~145 ms, which is comparable to our tone RTs at the final position (~140 ms) – demonstrating that the nervous system can reach a high level of preparedness by the first flash (within 600 ms). Classical experiments studying the lower limits of preparation have shown that optimal RTs can be reached around 200–300 ms after the warning cue (Alegria, 1974; Muller-Gethmann et al., 2003) – about half of the time of the first flash. The fact that the nervous system appears to be under-prepared in early positions when there is temporal uncertainty demonstrates that preparation is not simply about the programming and passive storage of motor commands to be later released, rather a state that must be actively controlled and maintained. The gradual reduction in RT suggests that high levels of preparation were difficult to be maintained for the duration of a trial in our study – otherwise we would observe consistent RTs across preparation levels. The slower RT in early positions likely reflects a balance between speed, accuracy, and effort given the low likelihood of the required response (~29%).

In activation models, preparation is conceptualized described as a continuous but single-stage process where an action is triggered when activation crosses the initiation threshold. However, some researchers have also proposed two-process models which separate preparation and initiation processes (Haith et al., 2016). Preparation and initiation processes are theorized as parallel-but-staggered processes that specify the 'what' and 'when' of movements, which have been used to explain why voluntary responses can take ~50–100 ms longer to initiate compared to reactions to perturbations (Marinovic et al., 2017; Oostwoud Wijdenes et al., 2011), how actions may be initiated before preparation is complete, and other phenomenon like self-paced movements. In the context of our study, this framework can offer two alternate interpretations: Firstly, given that our task uses a very simple action (simple finger flexion) which may not require preparation, differences in RT across preparation level may reflect a modulation of the initiation process as opposed to the preparation of the metrics of the movement. Secondly, reactions to LAS may be faster because the stimulus-intensity effect is specifically speeding up the initiation process. Although the distinction between preparation and initiation does not meaningfully change our interpretation that preparation is associated with evolving and widespread changes in brain dynamics, the distinction between processes may be important in future studies considering more complex movements and high-urgency situations – when

actions might be initiated before movement preparation is complete. In that regard, we have recently reported that actions initiated with very short preparation times do not undergo the typical suppression of the corticospinal system (McInnes, Lipp, et al., 2021), a phenomenon observed for reaction time, anticipatory and self-paced actions (Ibanez et al., 2020). We have proposed that preparatory suppression might be related to the protection of the motor action from interference (McInnes, Lipp, et al., 2021), so it would be interesting to investigate how the gradual increase in preparatory activity observed in our task relates to changes in corticospinal excitability.

Further commenting on the strategic nature of movement preparation, we observed a peculiar yet consistent reduction in peak force on the final position on tone trials. This reduction might reflect the engagement of inhibitory mechanisms to avoid false starts in the event of a Catch trial. Interestingly, this reduction was not observed for LAS trials, rather we observed the greatest increase in peak force on LAS trials at the final position. The pattern of force results is difficult to interpret as this enhancement was specific to the final position, but one possibility is that response force might only be facilitated by intense stimuli when preparation levels are relatively high. Although this effect remains to be further examined, it highlights the potential impact of Catch trials on task strategy and therefore movement preparation.

4.2 | Activity in motor and auditory areas reveals the distributed nature of movement preparation

Interestingly, the dynamic nature of preparation-level effects on cortical activation was evident in motor as well as auditory scalp regions, demonstrating that preparation is a distributed process. This suggests that the ability to react in our task was not only dependent on the state of the motor system but also the state of sensory areas which could allow for earlier detection of the imperative stimulus leading to earlier responses. This finding is in line with recent fMRI work by Gale et al. (2021) showing that activation of the auditory cortex is associated with the initiation of motor actions in humans even when actions are cued visually instead of acoustically (see also work on mice, Li et al., 2017).

Given that our task also had a visual component, we also hypothesized a preparation-related increase in activation at visual areas – in line with previous work by Bueti and Macaluso (2010) who found that auditory expectations also modulated activity in visual areas during movement preparation. However, this effect was not reliable, which may be due to the lack of task-relevant information provided by the visual flashes (i.e., flashes only provided generic but not specific information about the appearance of imperative stimuli). Alternatively, the absence may be related to the specific use of sounds which may elicit multi-sensory representations. For example, Bueti and Macaluso (2010) used sounds such as those of hand-clapping or of a hammer-hammering which can be visually imagined, but we used pure tones and broad-band white noise which are not naturally associated with such visual imagery (but see Swallow et al., 2012). Overall, the presence of preparation-level effects in motor and auditory but not the visual area demonstrates that as movement preparation is a distributed process, it appears to engage task-relevant more so than non-relevant areas of the brain. Note, however, that as the channel-level topographic distributions are consistent with our predictions, due to limitations of the EEG technique (e.g., low spatial resolution, volume conduction, and challenges associated with the inverse problem), we are unable to identify the specific brain structures involved. Future studies using fMRI or MEG could provide further insight about the specific contribution of different brain regions to movement preparation.

4.3 | Movement preparation also influences the excitability of sub-cortical circuits

Lastly, our task provides new insights regarding the effects of movement preparation on startle-related circuits. To date, numerous studies have used LAS as a probe to study the time course of changes in subcortical excitability during movement preparation by delivering the LAS at different times: before, with, or after the imperative stimulus. Collectively, there is evidence for significant modulation of the eye-blink reflex shortly before and after the presentation of the imperative stimulus in reaction-based tasks (e.g., Lipp et al., 2001, 2007; Marinovic et al., 2013). In anticipatory timing tasks, modulation of the eye-blink reflex seemed to reflect the phenomenon known as pre-movement inhibition (McInnes, Lipp, et al., 2021; Nguyen et al., 2021). However, the specific time course and direction of these effects do vary, with some studies reporting null effects for the eye-blink reflex (Kumru & Valls-Solé, 2006; Kumru et al., 2006). A major difficulty with interpreting these discrepant findings is that response requirements and contextual parameters can vary significantly across studies which can have dramatic impacts on the time course of preparation (e.g., choice response vs. single response, jittered vs. fixed inter-stimulus intervals, equiprobable vs. skewed stimulus/response probabilities, and presence of catch trials).

Our current design offers a different approach to studying movement preparation by allowing us to systematically manipulate the amount and the time course of preparation, as opposed to standardizing the presentation of trials (in fixed-cue RT and anticipatory tasks). Using this design, we found some evidence that changes in subcortical excitability may occur alongside changes in the motor response and cortical activation during preparation. In classical models of the StartReact effect, cortical and subcortical circuits are given different roles – where subcortical circuits only become relevant after preparation is 'complete' and the resultant motor program is transferred subcortically for storage and triggering (Valls-Solé et al., 1999). However, our data demonstrate that changes in subcortical excitability might be part of the entire preparation process – possibly serving to facilitate the transmission of the motor action (see e.g., Cohen et al., 2010).

Although blink latency was associated with preparation-level, blink amplitude was not. This discrepancy may be attributed to onset latency and peak amplitude measures capturing different times in the EMG signal. It is known that intense stimuli can elicit two distinct eye-blink components: the auditory eye-blink reflex and the auditory startle reflex. The auditory eye-blink reflex occurs at short latencies and is thought to be mediated by mesencephalic circuits, and the auditory startle reflex triggers a later response along with a generalized skeletomuscular response – thought to originate from bulbopontine circuits, distinct from those associated with the auditory eye-blink (Brown et al., 1991). Given that blink latency captures the onset of EMG activity, it is likely to capture the auditory eye-blink reflex whereas the peak amplitude is more likely to capture the auditory startle reflex (if larger). Although these measures may reflect activity of separate circuits, there is some overlap. In Nguyen et al. (2021), we were able to show evidence of eye-blink suppression in both amplitude and latency. In addition to discrepancies caused by task differences, not all studies report both blink amplitude and latency which makes it difficult to evaluate the two metrics. Nevertheless, although the linear trend for blink latency fell short of statistical significance ($p = .056$), our data provide preliminary evidence that the excitability of subcortical startle circuits might be modulated by the level of preparation as predicted.

5 | CONCLUSION

The results of this study demonstrate that responses to LAS can be partially explained by a combination of multisite (e.g., motor, and auditory) preparation states and stimulus intensity. RT and neural activation evolved with the increasing conditional probabilities of the imperative stimulus, suggesting that preparation was based on the updating expectations occurring throughout the course of each trial – reflecting a strategic optimization between speed and accuracy. As predicted by the activation model, preparation effects were evident on both LAS and tones. Our task design provides a useful method for systematically manipulating movement preparation which allows us to show its evolving and widespread (but selective changes) effects on the nervous system.

AUTHOR CONTRIBUTIONS
An T. Nguyen: Conceptualization; data curation; formal analysis; investigation; methodology; software; visualization; writing – original draft; writing – review and editing. **James R. Tresilian:** Conceptualization; methodology; writing – original draft; writing – review and editing. **Ottmar V. Lipp:** Conceptualization; funding acquisition; writing – original draft; writing – review and editing. **Dayse Tavora-Vieira:** Conceptualization; writing – original draft; writing – review and editing. **Welber Marinovic:** Conceptualization; formal analysis; funding acquisition; methodology; project administration; supervision; writing – original draft; writing – review and editing.

DATA AVAILABILITY STATEMENT
The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

ORCID
An T. Nguyen https://orcid.org/0000-0001-5966-9903
Welber Marinovic https://orcid.org/0000-0002-2472-7955

REFERENCES
Alegria, J. (1974). The time course of preparation after a first peak: Some constraints of reacting mechanisms. *The Quarterly Journal of Experimental Psychology*, *26*(4), 622–632. https://doi.org/10.1080/14640747408400455

Alibiglou, L., & MacKinnon, C. D. (2012). The early release of planned movement by acoustic startle can be delayed by transcranial magnetic stimulation over the motor cortex. *Journal of Physiology*, *590*(4), 919–936. https://doi.org/10.1113/jphysiol.2011.219592

Ashe, J. (1997). Erratum to "Force and the motor cortex": [Behavioural Brain Research 86 (1997) 1–15] 1PII of original

article: S0166-4328(96)00145-31. *Behavioural Brain Research*, *87*(2), 255–269. https://doi.org/10.1016/S0166-4328(97)00752-3

Baker, S. N. (2011). The primate reticulospinal tract, hand function and functional recovery. *Journal of Physiology*, *589*(Pt 23), 5603–5612. https://doi.org/10.1113/jphysiol.2011.215160

Baker, S. N., & Perez, M. A. (2017). Reticulospinal contributions to gross hand function after human spinal cord injury. *Journal of Neuroscience*, *37*(40), 9778–9784. https://doi.org/10.1523/JNEUROSCI.3368-16.2017

Blumenthal, T. D., Cuthbert, B. N., Filion, D. L., Hackley, S., Lipp, O. V., & Van Boxtel, A. (2005). Committee report: Guidelines for human startle eyeblink electromyographic studies. *Psychophysiology*, *42*(1), 1–15. https://doi.org/10.1111/j.1469-8986.2005.00271.x

Bonato, P., D'Alessio, T., & Knaflitz, M. (1998). A statistical method for the measurement of muscle activation intervals from surface myoelectric signal during gait. *IEEE Transactions on Biomedical Engineering*, *45*(3), 287–299. https://doi.org/10.1109/10.661154

Brainard, D. H. (1997). The psychophysics toolbox. *Spatial Vision*, *10*(4), 433–436. https://doi.org/10.1163/156856897X00357

Brown, P., Rothwell, J. C., Thompson, P. D., Britton, T. C., Day, B. L., & Marsden, C. D. (1991). New observations on the normal auditory startle reflex in man. *Brain*, *114*(4), 1891–1902. https://doi.org/10.1093/brain/114.4.1891

Bueti, D., & Macaluso, E. (2010). Auditory temporal expectations modulate activity in visual cortex. *NeuroImage*, *51*(3), 1168–1183. https://doi.org/10.1016/j.neuroimage.2010.03.023

Carlsen, A., Chua, R., Inglis, J. T., Sanderson, D. J., & Franks, I. M. (2004). Prepared movements are elicited early by startle. *Journal of Motor Behavior*, *36*(3), 253–264. https://doi.org/10.3200/JMBR.36.3.253-264

Carlsen, A., Dakin, C. J., Chua, R., & Franks, I. M. (2007a). Startle produces early response latencies that are distinct from stimulus intensity effects. *Experimental Brain Research*, *176*(2), 199–205. https://doi.org/10.1007/s00221-006-0610-8

Carlsen, A. N. (2015). A broadband acoustic stimulus is more likely than a pure tone to elicit a startle reflex and prepared movements. *Physiological Reports*, *3*(8), e12509. https://doi.org/10.14814/phy2.12509

Carlsen, A. N., Dakin, C. J., Chua, R., & Franks, I. M. (2007b). Startle produces early response latencies that are distinct from stimulus intensity effects [research support, non-U.S. Gov't]. *Experimental Brain Research*, *176*(2), 199–205. https://doi.org/10.1007/s00221-006-0610-8

Cattell, J. M. (1886). The influence of the intensity of the stimulus on the length of the reaction time. *Brain*, *8*(4), 512–515. https://doi.org/10.1093/brain/8.4.512

Chaumon, M., Bishop, D. V., & Busch, N. A. (2015). A practical guide to the selection of independent components of the electroencephalogram for artifact correction. *Journal of Neuroscience Methods*, *250*, 47–63. https://doi.org/10.1016/j.jneumeth.2015.02.025

Chen, J. L., Penhune, V. B., & Zatorre, R. J. (2008). Moving on time: Brain network for auditory-motor synchronization is modulated by rhythm complexity and musical training. *Journal of Cognitive Neuroscience*, *20*(2), 226–239. https://doi.org/10.1162/jocn.2008.20018

Chen, J. L., Zatorre, R. J., & Penhune, V. B. (2006). Interactions between auditory and dorsal premotor cortex during synchronization to musical rhythms [research support, non-U.S. Gov't].

NeuroImage, *32*(4), 1771–1781. https://doi.org/10.1016/j.neuroimage.2006.04.207

Chen, R., Yaseen, Z., Cohen, L. G., & Hallett, M. (1998). Time course of corticospinal excitability in reaction time and self-paced movements. *Annals of Neurology*, *44*(3), 317–325. https://doi.org/10.1002/ana.410440306

Cheung, C., Hamiton, L. S., Johnson, K., & Chang, E. F. (2016). The auditory representation of speech sounds in human motor cortex. *eLife*, *5*, e12577. https://doi.org/10.7554/eLife.12577

Churchland, M. M., Yu, B. M., Ryu, S. I., Santhanam, G., & Shenoy, K. V. (2006). Neural variability in premotor cortex provides a signature of motor preparation. *Journal of Neuroscience*, *26*(14), 3697–3712. https://doi.org/10.1523/JNEUROSCI.3762-05.2006

Cohen, O., Sherman, E., Zinger, N., Perlmutter, S., & Prut, Y. (2010). Getting ready to move: Transmitted information in the corticospinal pathway during preparation for movement [review]. *Current Opinion in Neurobiology*, *20*(6), 696–703. https://doi.org/10.1016/j.conb.2010.09.001

Crammond, D. J., & Kalaska, J. F. (2000). Prior information in motor and premotor cortex: Activity during the delay period and effect on pre-movement activity. *Journal of Neurophysiology*, *84*(2), 986–1005. https://doi.org/10.1152/jn.2000.84.2.986

Delorme, A., & Makeig, S. (2004). EEGLAB: An open source toolbox for analysis of single-trial EEG dynamics including independent component analysis. *Journal of Neuroscience Methods*, *134*(1), 9–21. https://doi.org/10.1016/j.jneumeth.2003.10.009

Duque, J., & Ivry, R. B. (2009). Role of corticospinal suppression during motor preparation. *Cerebral Cortex*, *19*(9), 2013–2024. https://doi.org/10.1093/cercor/bhn230

Duque, J., Labruna, L., Verset, S., Olivier, E., & Ivry, R. B. (2012). Dissociating the role of prefrontal and premotor cortices in controlling inhibitory mechanisms during motor preparation. *Journal of Neuroscience*, *32*(3), 806–816. https://doi.org/10.1523/JNEUROSCI.4299-12.2012

Eichenberger, A., & Rüegg, D. G. (1984). Relation between the specific H reflex facilitation preceding a voluntary movement and movement parameters in man. *Journal of Physiology*, *347*, 545–559. https://doi.org/10.1113/jphysiol.1984.sp015082

Elsayed, G. F., Lara, A. H., Kaufman, M. T., Churchland, M. M., & Cunningham, J. P. (2016). Reorganization between preparatory and movement population responses in motor cortex. *Nature Communications*, *7*(1), 13239. https://doi.org/10.1038/ncomms13239

Furubayashi, T., Ugawa, Y., Terao, Y., Hanajima, R., Sakai, K., Machii, K., Mochizuki, H., Shiio, Y., Uesugi, H., Enomoto, H., & Kanazawa, I. (2000). The human hand motor area is transiently suppressed by an unexpected auditory stimulus. *Clinical Neurophysiology*, *111*(1), 178–183. http://www.ncbi.nlm.nih.gov/pubmed/10656526

Gale, D. J., Areshenkoff, C. N., Honda, C., Johnsrude, I. S., Flanagan, J. R., & Gallivan, J. P. (2021). Motor planning modulates neural activity patterns in early human auditory cortex. *Cerebral Cortex*, *31*(6), 2952–2967. https://doi.org/10.1093/cercor/bhaa403

Gallucci, M. (2019). *GAMLj: General analyses for linear models.* https://gamlj.github.io/

Guerrero, J. A., & Macias-Diaz, J. E. (2018). *biosignalEMG: Tools for Electromyogram Signals (EMG) analysis.* https://cran.r-project.org/web/packages/biosignalEMG/

Haith, A. M., Pakpoor, J., & Krakauer, J. W. (2016). Independence of movement preparation and movement initiation. *Journal of Neuroscience*, *36*(10), 3007–3015. https://doi.org/10.1523/JNEUROSCI.3245-15.2016

Hoffstaedter, F., Grefkes, C., Zilles, K., & Eickhoff, S. B. (2013). The "what" and "when" of self-initiated movements. *Cerebral Cortex*, *23*(3), 520–530. https://doi.org/10.1093/cercor/bhr391

Honeycutt, C. F., Kharouta, M., & Perreault, E. J. (2013). Evidence for reticulospinal contributions to coordinated finger movements in humans. *Journal of Neurophysiology*, *110*(7), 1476–1483. https://doi.org/10.1152/jn.00866.2012

Honeycutt, C. F., & Perreault, E. J. (2012). Planning of ballistic movement following stroke: Insights from the startle reflex. *PLoS One*, *7*(8), e43097. https://doi.org/10.1371/journal.pone.0043097

Honeycutt, C. F., Tresch, U. A., & Perreault, E. J. (2015). Startling acoustic stimuli can evoke fast hand extension movements in stroke survivors. *Clinical Neurophysiology*, *126*(1), 160–164. https://doi.org/10.1016/j.clinph.2014.05.025

Ibanez, J., Hannah, R., Rocchi, L., & Rothwell, J. C. (2020). Premovement suppression of corticospinal excitability may be a necessary part of movement preparation. *Cerebral Cortex*, *30*(5), 2910–2923. https://doi.org/10.1093/cercor/bhz283

Kayser, J., & Tenke, C. E. (2015). Issues and considerations for using the scalp surface Laplacian in EEG/ERP research: A tutorial review. *International Journal of Psychophysiology*, *97*(3), 189–209. https://doi.org/10.1016/j.ijpsycho.2015.04.012

Kleiner, M., Brainard, D., & Pelli, D. (2007). *What's new in Psychtoolbox-3?* https://pure.mpg.de/rest/items/item_1790332/component/file_3136265/content

Kononowicz, T. W., & Penney, T. B. (2016). The contingent negative variation (CNV): Timing isn't everything. *Current Opinion in Behavioral Sciences*, *8*, 231–237. https://doi.org/10.1016/j.cobeha.2016.02.022

Kumru, H., Urra, X., Compta, Y., Castellote, J., Turbau, J., & Valls-Solé, J. (2006). Excitability of subcortical motor circuits in Go/noGo and forced choice reaction time tasks. *Neuroscience Letters*, *406*(1), 66–70. https://doi.org/10.1016/j.neulet.2006.07.012

Kumru, H., & Valls-Solé, J. (2006). Excitability of the pathways mediating the startle reaction before execution of a voluntary movement. *Experimental Brain Research*, *169*(3), 427–432. https://doi.org/10.1007/s00221-005-0156-1

Lara, A. H., Elsayed, G. F., Zimnik, A. J., Cunningham, J. P., & Churchland, M. M. (2018). Conservation of preparatory neural events in monkey motor cortex regardless of how movement is initiated. *eLife*, *7*, e31826. https://doi.org/10.7554/eLife.31826

Leocani, L., Cohen, L. G., Wassermann, E. M., Ikoma, K., & Hallett, M. (2000). Human corticospinal excitability evaluated with transcranial magnetic stimulation during different reaction time paradigms. *Brain*, *123*(Pt 6), 1161–1173. http://www.ncbi.nlm.nih.gov/pubmed/10825355

Leow, L. A., Uchida, A., Egberts, J. L., Riek, S., Lipp, O. V., Tresilian, J., & Marinovic, W. (2018). Triggering mechanisms for motor actions: The effects of expectation on reaction times to intense acoustic stimuli. *Neuroscience*, *393*, 226–235. https://doi.org/10.1016/j.neuroscience.2018.10.008

Li, J., Liao, X., Zhang, J., Wang, M., Yang, N., Lv, G., Li, H., Lu, J., Ding, R., Li, X., Guang, Y., Yang, Z., Qin, H., Jin, W., Zhang, K.,

He, C., Jia, H., Zeng, S., Hu, Z., ... Chen, X. (2017). Primary auditory cortex is required for anticipatory motor response. *Cerebral Cortex*, *27*(6), 3254–3271. https://doi.org/10.1093/cercor/bhx079

Lipp, O. V., Alhadad, S. S., & Purkis, H. M. (2007). Startle blink facilitation during the go signal of a reaction time task is not affected by movement preparation or attention to the go signal. *Neuroscience Letters*, *427*(2), 94–98. https://doi.org/10.1016/j.neulet.2007.09.018

Lipp, O. V., Blumenthal, T. D., & Adam, A. R. (2001). Attentional modulation of blink startle at long, short, and very short lead intervals. *Biological Psychology*, *58*(2), 89–103. https://doi.org/10.1016/s0301-0511(01)00109-0

Lo, S., & Andrews, S. (2015). To transform or not to transform: Using generalized linear mixed models to analyse reaction time data. *Frontiers in Psychology*, *6*. https://doi.org/10.3389/fpsyg.2015.01171

Lopez-Calderon, J., & Luck, S. J. (2014). ERPLAB: An open-source toolbox for the analysis of event-related potentials. *Frontiers in Human Neuroscience*, *8*, 213. https://doi.org/10.3389/fnhum.2014.00213

MacKinnon, C. D., Allen, D. P., Shiratori, T., & Rogers, M. W. (2013). Early and unintentional release of planned motor actions during motor cortical preparation. *PLoS One*, *8*(5), e63417. https://doi.org/10.1371/journal.pone.0063417

Marinovic, W., Brauer, S. G., Hayward, K. S., Carroll, T. J., & Riek, S. (2016). Electric and acoustic stimulation during movement preparation can facilitate movement execution in healthy participants and stroke survivors. *Neuroscience Letters*, *618*, 134–138. https://doi.org/10.1016/j.neulet.2016.03.009

Marinovic, W., de Rugy, A., Lipp, O. V., & Tresilian, J. R. (2013). Responses to loud auditory stimuli indicate that movement-related activation builds up in anticipation of action. *Journal of Neurophysiology*, *109*(4), 996–1008. https://doi.org/10.1152/jn.01119.2011

Marinovic, W., Tresilian, J., Chapple, J. L., Riek, S., & Carroll, T. J. (2017). Unexpected acoustic stimulation during action preparation reveals gradual re-specification of movement direction. *Neuroscience*, *348*, 23–32. https://doi.org/10.1016/j.neuroscience.2017.02.016

Marinovic, W., & Tresilian, J. R. (2016). Triggering prepared actions by sudden sounds: Reassessing the evidence for a single mechanism. *Acta Physiologica*, *217*(1), 13–32. https://doi.org/10.1111/apha.12627

Marinovic, W., Tresilian, J. R., de Rugy, A., Sidhu, S., & Riek, S. (2014). Corticospinal modulation induced by sounds depends on action preparedness. *Journal of Physiology*, *592*(Pt 1), 153–169. https://doi.org/10.1113/jphysiol.2013.254581

Maslovat, D., Franks, I. M., Leguerrier, A., & Carlsen, A. N. (2015). Responses to startling acoustic stimuli indicate that movement-related activation is constant prior to action: A replication with an alternate interpretation. *Physiological Reports*, *3*(2), e12300. https://doi.org/10.14814/phy2.12300

McInnes, A. N., Castellote, J. M., Kofler, M., Honeycutt, C. F., Lipp, O. V., Riek, S., Tresilian, J. R., & Marinovic, W. (2021). Cumulative distribution functions: An alternative approach to examine the triggering of prepared motor actions in the StartReact effect. *European Journal of Neuroscience*, *53*(5), 1545–1568. https://doi.org/10.1111/ejn.14973

McInnes, A. N., Corti, E. J., Tresilian, J. R., Lipp, O. V., & Marinovic, W. (2020). Neural gain induced by startling acoustic stimuli

is additive to preparatory activation. *Psychophysiology*, *57*(3), e13493. https://doi.org/10.1111/psyp.13493

McInnes, A. N., Lipp, O. V., Tresilian, J. R., Vallence, A. M., & Marinovic, W. (2021). Premovement inhibition can protect motor actions from interference by response-irrelevant sensory stimulation. *Journal of Physiology*, *599*(18), 4389–4406. https://doi.org/10.1113/JP281849

Muller-Gethmann, H., Ulrich, R., & Rinkenauer, G. (2003). Locus of the effect of temporal preparation: Evidence from the lateralized readiness potential. *Psychophysiology*, *40*(4), 597–611. http://www.ncbi.nlm.nih.gov/pubmed/14570167

Nguyen, A. T., Jacobs, L. A., Tresilian, J. R., Lipp, O. V., & Marinovic, W. (2021). Preparatory suppression and facilitation of voluntary and involuntary responses to loud acoustic stimuli in an anticipatory timing task. *Psychophysiology*, *58*(2), e13730. https://doi.org/10.1111/psyp.13730

Nonnekes, J., Oude Nijhuis, L. B., de Niet, M., de Bot, S. T., Pasman, J. W., van de Warrenburg, B. P., Bloem, B. R., Weerdesteyn, V., & Geurts, A. C. (2014). StartReact restores reaction time in HSP: Evidence for subcortical release of a motor program. *Journal of Neuroscience*, *34*(1), 275–281. https://doi.org/10.1523/JNEUROSCI.2948-13.2014

Oostwoud Wijdenes, L., Brenner, E., & Smeets, J. B. J. (2011). Fast and fine-tuned corrections when the target of a hand movement is displaced. *Experimental Brain Research*, *214*(3), 453–462. https://doi.org/10.1007/s00221-011-2843-4

Palmer, J. A., Kreutz-Delgado, K., & Makeig, S. (2012). *AMICA: An adaptive mixture of independent component analyzers with shared components*. Swartz Center for Computational Neuroscience, University of California San Diego, Tech. Rep.

Pelli, D. G. (1997). The VideoToolbox software for visual psychophysics: Transforming numbers into movies. *Spatial Vision*, *10*(4), 437–442. https://doi.org/10.1163/156856897X00366

Perrin, F., Pernier, J., Bertrand, O., & Echallier, J. F. (1989). Spherical splines for scalp potential and current density mapping. *Electroencephalography and Clinical Neurophysiology*, *72*(2), 184–187. https://doi.org/10.1016/0013-4694(89)90180-6

Piéron, H. (1913). II. Recherches sur les lois de variation des temps de latence sensorielle en fonction des intensités excitatrices. *L'Année Psychologique*, *20*(1), 17–96. https://doi.org/10.3406/psy.1913.4294

Requin, J., Brener, J., & Ring, C. (1991). Preparation for action. In J. R. Jennings & M. G. H. Coles (Eds.), *Handbook of cognitive psychophysiology: Central and autonomic nervous system approaches* (pp. 357–448). John Wiley & Sons.

Requin, J., & Riehle, A. (1995, Nov). Neural correlates of partial transmission of sensorimotor information in the cerebral cortex. *Acta Psychologica*, *90*(1–3), 81–95. https://doi.org/10.1016/0001-6918(95)00039-w

Riddle, C. N., Edgley, S. A., & Baker, S. N. (2009). Direct and indirect connections with upper limb motoneurons from the primate reticulospinal tract. *Journal of Neuroscience*, *29*(15), 4993–4999. https://doi.org/10.1523/JNEUROSCI.3720-08.2009

Rothwell, J. C. (2006). The startle reflex, voluntary movement, and the reticulospinal tract. *Supplement in Clinical Neurophysiology*, *58*, 223–231. http://www.ncbi.nlm.nih.gov/pubmed/16623334

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

Stevenson, A. J., Chiu, C., Maslovat, D., Chua, R., Gick, B., Blouin, J. S., & Franks, I. M. (2014). Cortical involvement in the StartReact effect. *Neuroscience*, *269*, 21–34. https://doi.org/10.1016/j.neuroscience.2014.03.041

Sullivan, S. J., & Hayes, K. C. (1987). Changes in short and long latency stretch reflexes prior to movement initiation. *Brain Research*, *412*(1), 139–143. https://doi.org/10.1016/0006-8993(87)91448-x

Swallow, K. M., Makovski, T., & Jiang, Y. V. (2012). Selection of events in time enhances activity throughout early visual cortex. *Journal of Neurophysiology*, *108*(12), 3239–3252. https://doi.org/10.1152/jn.00472.2012

Teasdale, N., Bard, C., Fleury, M., Young, D. E., & Proteau, L. (1993). Determining movement onsets from temporal series. *Journal of Motor Behavior*, *25*(2), 97–106. https://doi.org/10.1080/00222895.1993.9941644

Toro, C., Deuschl, G., Thatcher, R., Sato, S., Kufta, C., & Hallett, M. (1994). Event-related desynchronization and movement-related cortical potentials on the ECoG and EEG. *Electroencephalography and Clinical Neurophysiology*, *93*(5), 380–389. https://doi.org/10.1016/0168-5597(94)90126-0

Tresilian, J. R., & Plooy, A. M. (2006). Effects of acoustic startle stimuli on interceptive action. *Neuroscience*, *142*(2), 579–594. https://doi.org/10.1016/j.neuroscience.2006.06.029

Valls-Solé, J. (2012). Assessment of excitability in brainstem circuits mediating the blink reflex and the startle reaction. *Clinical Neurophysiology*, *123*(1), 13–20. https://doi.org/10.1016/j.clinph.2011.04.029

Valls-Solé, J., Rothwell, J. C., Goulart, F., Cossu, G., & Munoz, E. (1999). Patterned ballistic movements triggered by a startle in healthy humans. *Journal of Physiology*, *516*(Pt 3), 931–938. https://doi.org/10.1111/j.1469-7793.1999.0931u.x

Valls-Solé, J., Sole, A., Valldeoriola, F., Munoz, E., Gonzalez, L. E., & Tolosa, E. S. (1995). Reaction time and acoustic startle in normal human subjects. *Neuroscience Letters*, *195*(2), 97–100. http://www.ncbi.nlm.nih.gov/pubmed/7478277

Yeomans, J. S., Li, L., Scott, B. W., & Frankland, P. W. (2002). Tactile, acoustic and vestibular systems sum to elicit the startle reflex. *Neuroscience and Biobehavioral Reviews*, *26*(1), 1–11. http://www.ncbi.nlm.nih.gov/pubmed/11835980

Zeileis, A., Grothendieck, G., Ryan, J. A., Ulrich, J. M., & Andrews, F. (2020). *zoo: S3 infrastructure for regular and irregular time series* (*Z's ordered observations*). https://cran.r-project.org/web/packages/zoo/