Title: Responses to Loud Acoustic Stimuli: Short and Long Timescale Effects on Cortical and Sub-Cortical Preparation Reflect Conditional Probability.

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

Running Head: Movement Preparation and Loud Acoustic Stimulation

[1] School of Psychology, Curtin University, Perth, Western Australia

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

[3] Fiona Stanley Hospital, Murdoch, Western Australia

Corresponding Authors: An T. Nguyen (an.nguyen1@curtin.edu.au) and Welber Marinovic (welber.marinovic@curtin.edu.au).

Abstract

Loud acoustic stimuli (LAS) can trigger prepared motor responses at very short latencies: the StartReact effect. In this study, we tested the proposal that responses to LAS in the StartReact effect could be explained by stimulus intensity effects combined with movement-related preparation changes on nervous system excitability. Using a simple auditory reaction time task, we induced different levels of preparation by systematically manipulating the conditional probability and temporal location of the auditory stimulus (LAS or non-intense tone). We then examined how preparation-levels influenced motor responses to LAS and non-intense tones, as well as cortical and sub-cortical excitability — reflected by electroencephalographic (EEG) activity before the onset of the auditory stimulus, and the eye-blink startle reflex.

On both tone and LAS trials, higher preparation conditions were accompanied with reductions in movement onset time, increased force, as well as enhanced cortical (in auditory and motor areas) and sub-cortical excitability. At the trial-level, we found that enhanced pre-stimulus EEG activity in sensory and motor areas was associated with earlier movement onset on tone trials, and shorter blink onset latencies were associated with earlier movement onset on LAS trials. The results show that movement preparation is associated with wide-spread changes in cortical and sub-cortical excitability, and that movement preparation (combined with stimulus-intensity effects) plays a critical role in shaping responses to both non-intense tones and LAS.

Keywords: Movement Preparation, StartReact Effect, Electroencephalography, Contingent Negative Variation, Eye-Blink Reflex, Startle

Introduction

Before the execution of a volitional movement, neural processes work to prepare the neuromotor system to perform that action (Requin, Brener, & Ring, 1991; Requin & Riehle, 1995). In reaction time (RT) tasks and anticipatory timing tasks, these processes establish a state of readiness to respond to a stimulus (R. Chen, Yaseen, Cohen, & Hallett, 1998a; Ibáñez, Hannah, Rocchi, & Rothwell, 2020; Schmidt, Lee, Winstein, Wulf, & Zelaznik, 2018). This readiness is marked by increased activity in response-related circuits both within the brain and the spinal cord (R. Chen, Yaseen, Cohen, & Hallett, 1998b; Eichenberger & Rüegg, 1984; Leocani, Cohen, Wassermann, Ikoma, & Hallett, 2000). This increased activity is associated with increased sub-cortical 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, Grefkes, Zilles, & Eickhoff, 2013; Toro et al., 1994). In RT and anticipatory timing tasks, prepared actions can be triggered at very low latencies by the presentation of an intense stimulus, such as a loud acoustic stimulus (LAS): the StartReact effect. It was initially theorised that these responses were mediated by a separate mechanism that by-passes the cortex, involving sub-cortical circuitry associated with the startle response (Carlsen, Chua, Inglis, Sanderson, & Franks, 2004; Valls-Solé, Rothwell, Goulart, Cossu, & Munoz, 1999). This proposal was based on the idea that mental representations which specify the parameters of the movement (motor programmes) are prepared in advance and transferred to sub-cortical structures for storage and execution (Carlsen et al., 2004; Valls-Solé, Rothwell, Goulart, Cossu, & Munoz, 1999). The presentation of the intense stimulus was thought to excite these structures as part of the startle response, involuntarily triggering the release of the motor programme while by-passing slower cortical triggering mechanisms involved in voluntary motor control.

However, recent studies suggest that the cortex may not be by-passed in the StartReact effect as initially suggested (Alibiglou & MacKinnon, 2012; Marinovic, Tresilian, de Rugy, Sidhu, & Riek, 2014; Stevenson et al., 2014). This idea aligns well with our proposal (see (Marinovic & Tresilian, 2016) that this phenomenon is mediated by voluntary control pathways and could be the result either of combined intersensory facilitation (Colonius & Diederich, 2012; Todd, 1912) and stimulus intensity effects (Cattell, 1886; Piéron, 1913), or just the latter. If this were the case, the dynamic changes that occur

cortically during movement preparation are likely to partially drive the manifestation of the StartReact effect. In activation models (See Figure 1), changes in excitability during movement preparation are visualised as the build-up of activation 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 modelled to increase activation in an additive manner as suggested by McInnes et al. (2020), proportional to the intensity of the stimulus. Responses are modelled 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.

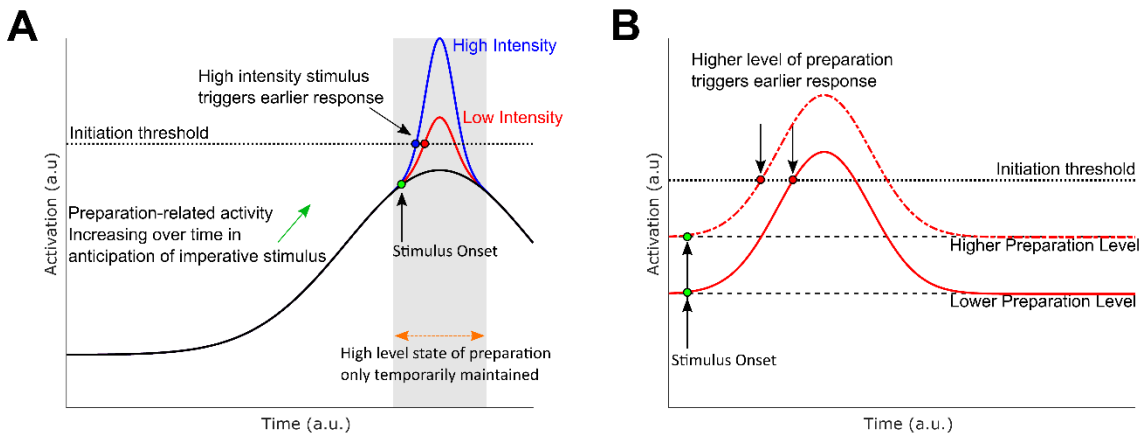

Figure 1. (**A**) *Conceptual visualisation of the Activation Model, depicting activation in the motor system from the start of the trial, leading up to a response. The black line represents preparation-related activity which gradually increases as expected time of the response approaches. The grey area 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**) Visualisation 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.*

In this model, variability in response times across trials is attributed to fluctuations in motor preparation, in part due to uncertainties about the precise time responses will be required. As high levels of preparation can only be maintained for a short duration (Alegria, 1974; Müller-Gethmann, Ulrich, & Rinkenauer, 2003), accurate responding relies on the appropriate timing of preparatory-related processes which are informed by acquired knowledge and experience. 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 reach a high level of preparation in tasks with temporal uncertainty (e.g. RT tasks with a randomly varying foreperiod), leading to longer and more varied RTs. Although motor preparation is less effective in tasks with temporal uncertainty, the level of preparation still plays a crucial role in shaping motor responses (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 sub-cortical excitability, studies have used LAS to probe the time-course of motor preparation in both systems. For example, cortical preparation-related activity reflected by contingent negative variation (CNV) – slow and centrally distributed negativity in the scalp electroencephalogram (EEG) implicated in the anticipation and preparation for an up-coming stimulus (Kononowicz & Penney, 2016) – was associated with RT in a predictable RT task (MacKinnon, Allen, Shiratori, & Rogers, 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, Blumenthal, & Adam, 2001; Marinovic, de Rugy, Lipp, & Tresilian, 2013; Valls-Solé, Rothwell, Goulart, Cossu, & Muñoz, 1999; Valls-Solé et al., 1995). However, research has yet to systematically examine whether both cortical and sub-cortical excitability are associated with the level of motor preparation, and whether they, in turn, influence movements in response to non-intense tones and LAS.

In this study, we sought to test the hypothesis that changes in cortical and sub-cortical excitability during motor preparation are involved in shaping motor responses to LAS. To do this, we used a modified auditory reaction time task where we altered the conditional probability and temporal position of the acoustic stimulus to induce different levels of motor preparation. To capture changes in cortical excitability during preparation, we used scalp

EEG to record brain activity reflected by the CNV. Sub-cortical excitability was assessed by recording of eye-blink startle reflexes elicited by the LAS, and indexed by EMG activity of the Orbicularis Oculi (OOc) muscle (Yeomans & Frankland, 1995). Motor responses produced by the flexion of the index finger were recorded by force sensors.

The study sought to investigate the following three questions. Firstly, we asked whether motor responses to LAS and tones are shaped by the same underlying processes. The motor preparation account predicts that RT should decrease as conditional probability increases, reflecting an increased readiness to respond. If responses to LAS and tones are indeed shaped by the same underlying processes, they both should show a similar decrease in RT. Secondly, we asked whether the state of cortical and sub-cortical excitability increases with the conditional probability of the acoustic stimulus (LAS and tones) — reflecting the level of motor preparation. The model assumes that changes in neural excitability reflect movement preparation. If this is true, we expected an increase in CNV amplitude (more negative) and facilitation of the eye-blink startle reflex as the level of preparation increases. Lastly, we asked whether variability in the motor response can be explained by fluctuations in cortical excitability. The model posits that excitability reflects temporal preparation, this in turn would influence the state of readiness to respond. If this is correct, then higher levels of excitability should be associated with faster response times for both LAS and tones on a trial-by-trial basis, both within and across preparation levels. For tone trials, CNV amplitude should be positively associated with response time (more negative CNV, shorter RT). For LAS trials, a similar CNV-RT association is expected as well as a positive relationship between the eye-blink reflex and RT (faster and/or larger eye-blinks, shorter RT).

Method

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 (< 75%), and four due to missing behavioural data. The final sample consisted of 23 participants ($M(SD)_{age}$ = 20.57(2.52) years, age range = 18 – 27 years, 19 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).

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 (Figure 1). This sequencing method allowed us to manipulate the conditional probability of the auditory stimulus, thereby modifying the level of motor preparation. As shown numerically in Table 1, stimuli presented early in the sequence have a lower conditional probability because most of the possible events are still available. Comparatively, stimuli presented later in the sequence are associated with a higher conditional probability because past events are no longer possible.

The tone was a 1700 Hz pure tone presented for 50 ms at 60 dBa, and the LAS was broadband white-noise presented for 50 ms at 104 dBa. Participants responded by pressing on a force sensor (SingleTact, Model: CS8-10N) 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, Brainard, & Pelli, 2007; Pelli, 1997) on an ASUS 24-inch LCD monitor (Model: VG248QE, running at 1920 x 1080 resolution and 120 Hz). The auditory stimuli were imported '.wav' files 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.

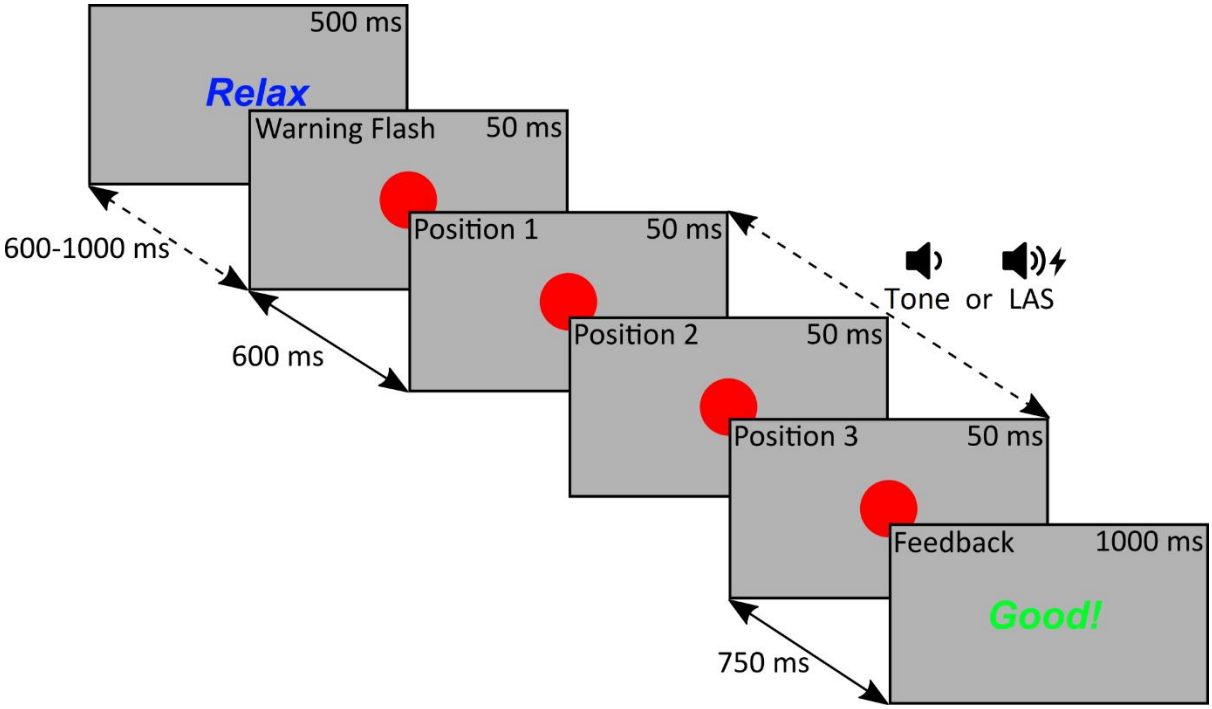

Figure 1. *A diagram showing the sequence of events on each trial. A red circle is briefly flashed on-screen four times and an acoustic stimulus, either the tone or loud acoustic stimulus (LAS) is randomly presented with the flash at Positions 1, 2 or 3. Participants respond to the stimulus by pressing on a force sensor and feedback is presented at the end of the trial. On a small portion of trials (not depicted on the diagram), the LAS is presented with the Warning Flash (Baseline LAS trial), or no sound is presented at all (Catch trials).*

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 the evolving chance of hearing an auditory stimulus at each position of the task presented on the bottom row.*

	Temporal Position of the Acoustic Stimulus			
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 **(5.55%)**	100/340 **(29.41%)**	100/240 **(41.67%)**	100/140 **(71.42%)**

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 centre of the display four times (50 ms duration with a stimulus onset asynchrony of 600 ms). The first flash served as the warning cue while 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 for 1000 ms, 750 ms after the final flash.

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-randomised 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, on tone trials, 'Good Timing' was presented if participants responded between 50 - 250 ms after stimulus-onset. 'Too early' or 'Too late' was presented if participants responded before 50 or after 250 ms. 'No response detected' was presented if no response was made within 600 ms of the tone. 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 to 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 analysed.

Force and EMG Data Reduction and Measurement:

Force data were continuously recorded for the duration of the trial, digitised 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 and colleagues (1993). Trials with response times outside 50 - 600 ms were excluded from further analysis, $M(SD)_{excluded}$ = 10.95(12.31) trials. We also measured the peak force of each response.

We recorded EMG activity from the right Orbicularis Oculi muscle using Ag/AgCl sintered electrodes in a pre-amplified bi-polar set-up. One electrode was placed under the pupil, the second was placed laterally and slightly higher than the first electrode, approximately 1 cm edge-to-edge and 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 digitised using the National Instruments DAQ.

The EMG data were processed offline using a semi-automated procedure in R. Firstly, the data were down-sampled 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) then decreased (down to 0.5 times signa n), until an onset was detected within 20 – 150 ms.

We also 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, Grothendieck, Ryan, Ulrich, & Andrews, 2020). 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 (*SD*) per

participant, 3.35 (4.49) trials were identified as non-response, 3.35 (4.91) trials were identified missing, 5.96 (6.06) trials were manually adjusted.

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 DC–100 Hz online filter. Data were recorded from 64 scalp electrodes arranged according to the 10-20 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 (CMS) and driven right leg (DRL) 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, Kreutz-Delgado, & Makeig, 2012), SASICA (Chaumon, Bishop, & Busch, 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 using separate low- and high-pass filters (using the 'pop_eegfiltnew' function in EEGLAB) with filter orders of 8448 and 86, respectively. The filtered data were then down sampled to 256 Hz.

Epochs were extracted on tone and LAS trials, time-locked to the presentation 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). This baseline was chosen to minimise the influence of different foreperiod lengths across Position on amplitude measures, emphasising conditional probability-related effects. To test the impact of baselining, we contrasted results between pre-trial and previous-flash baselines. The results of the pre-trial baseline lead to exaggerated differences across Position conditions on amplitude but did not have a meaningful impact on trial-by-trial relationships across variables. 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, $M(SD)_{removed}$ = 12.3(5.6) ICs. Trials containing voltages on analysed channels exceeding ± 75 µV were excluded, $M(SD)_{excluded}$ = 8.45(7.40) trials. 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. 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. 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.

A Surface Laplacian filter was applied using algorithms described in Perrin and colleagues (1989) (smoothing factor = $1e^{-5}$, order of Legendre polynomial = 10) to reduce volume conduction effects in EEG sensor space, resulting in a $\mu V/mm^2$ voltage scale. 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 central (Cz), auditory (T7,T8) and visual (Oz) areas.

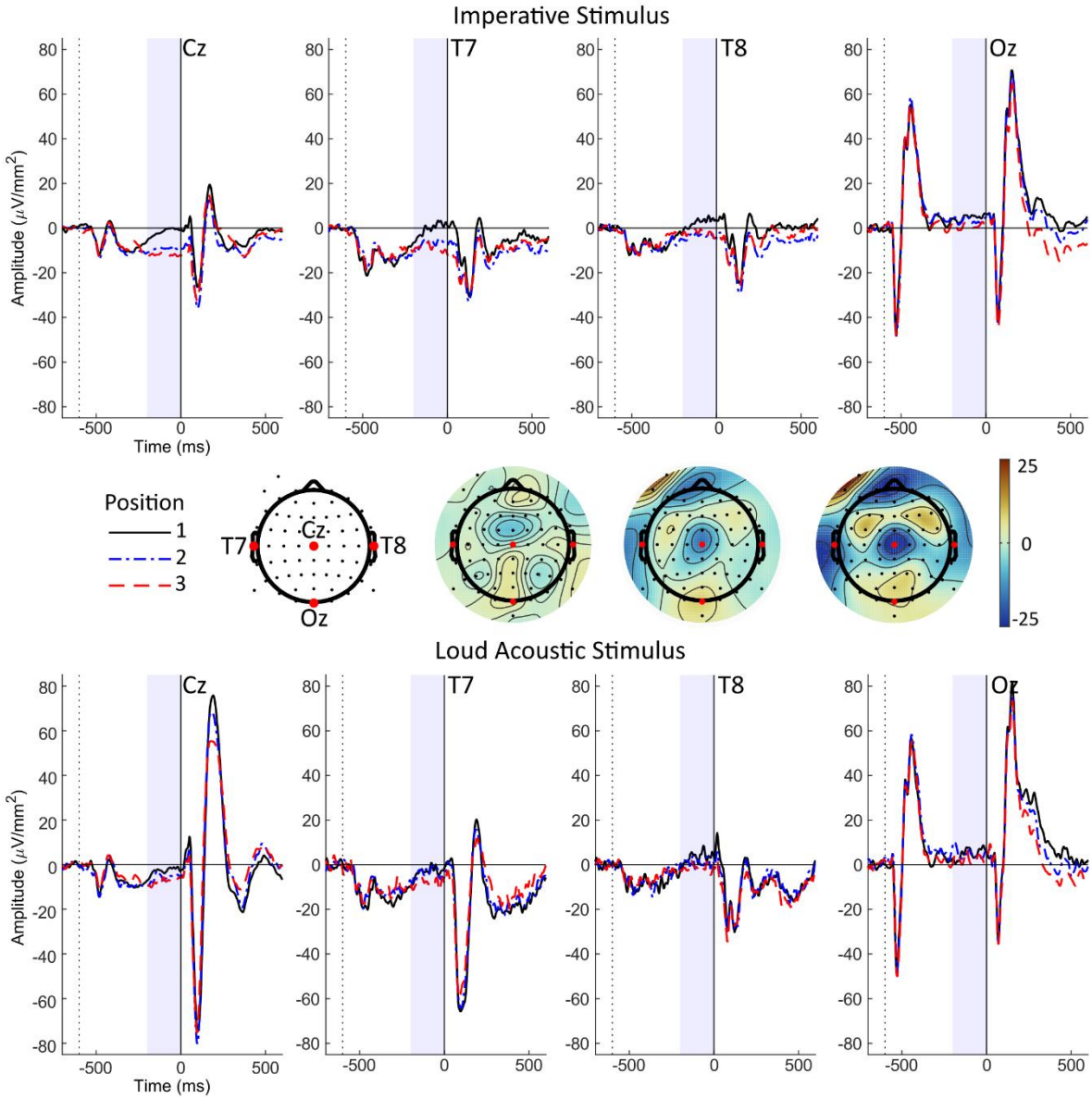

Figure 2. *Grand-averaged ERP waveforms for each Position (1 – solid black, 2 – dot-dashed blue, 3 – dashed red) from central (Cz), auditory (T7, T8) and visual areas (Oz) for tone (Top) and Loud Acoustic Stimulus trials (Bottom). Waveforms were time-locked to the onset of the auditory stimulus and were baseline-corrected from -100 to 0 ms before to the previous flash. The blue shaded area shows the interval where pre-stimulus mean amplitudes were measured. Topographical maps show the distribution of activity during the measured interval on standard tone trials.*

Statistical Analysis:

Statistical analyses were conducted in R statistics and R Studio using linear mixed models with the 'LmerTest' package (Kuznetsova, Brockhoff, Christensen, & Jensen, 2019). For all models, participant intercepts modelled as a random effect. We presented the results as *F*-values using the Satterthwaite's approximation method (Satterthwaite, 1941), using the 'anova' function. We used the 'summ' function in the 'jtools' package (Long, 2020) to calculate the estimated regression-weights (β) and used the 'r2beta' function from the 'r2glmm' package to calculate effect size as Partial R^2 (R_p^2) (Jaeger, 2017). For follow-up pairwise contrasts, we computed estimated marginal means using the 'emmeans' function from the 'emmeans' package (Lenth, Singmann, Love, Buerknerm, & Herve, 2020). We presented the results of these pairwise categorical comparisons as t-ratios (mean difference estimate divided by standard error) with degrees-of-freedom estimated using the Kenward-Roger method.

First, we characterised the finger response, eye-blink startle reflex and pre-stimulus electrophysiological activation effects. For the finger response, we analysed movement onset RT and peak force, modelling the dependent variable with Position (1,2,3), Stimulus-Type (tone, LAS) and their interaction as fixed-effects. For the eye-blink startle reflex, we analysed blink onset latency and amplitude on LAS trials, modelling the dependent variable with Position (Warning flash, Position 1, 2, 3) as the only fixed-effect. For pre-stimulus electrophysiology, we modelled pre-stimulus activity on central, auditory and visual areas on tone and LAS trials. For central and visual areas, we included Position (1, 2, 3), Stimulus-Type and their interaction as fixed effects. For the auditory regions, we also included Hemisphere (left, right) and its interactions as fixed effects.

We used linear mixed models to examine (1) whether trial-by-trial variability in the motor response to tone and LAS trials were associated with pre-stimulus cortical activity, (2) whether movement onset on LAS trials was associated with sub-cortical excitability reflected by blink onset latency, and (3) whether fluctuations in cortical and sub-cortical excitability were associated. To limit the number of reported analyses, trial-level analysis results which were not statistically significant ($p > .05$), such as those involving peak force, blink amplitude and pre-stimulus cortical activity at Oz, are not reported.

Results

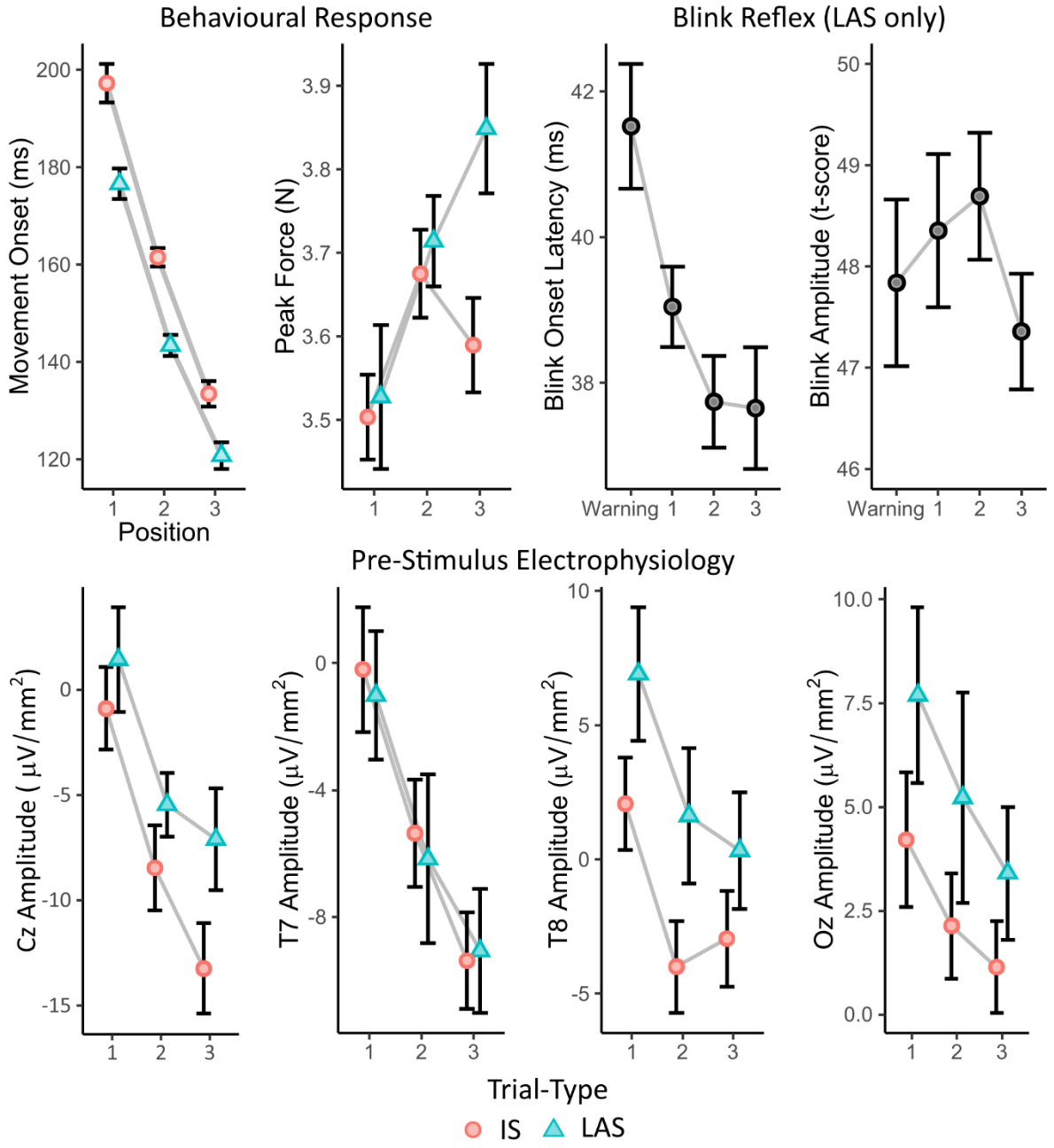

Figure 3. (**TOP LEFT**) *Plots depicting means and within-subject standard error bars for RTs and force across each Stimulus-Type (Tone, LAS) and Position (1, 2, 3).* (**TOP RIGHT**) *Blink onset latency and Blink amplitude on LAS trials (including the Warning flash).* (**BOTTOM**) *Pre-stimulus ERP amplitude at central (Cz), auditory (T7, T8) and visual areas (Oz).*

Finger Response:

Movement onset times decreased as the auditory stimulus was presented in later positions (main effect of position, $F(2, 6679.1) = 649.08$, $p < .001$, $R^2_p = .022$). This effect was present for both tone and LAS trials, when the two Stimulus-Types were separated (tone, $F(2,5361) = 986.97$, $p < .001$, $R^2_p = .27$; LAS, $F(2,1296.7) = 175.03$, $p < .001$, $R^2_p = 0.21$). Movement onset was overall earlier for LAS trials (main effect of Stimulus-Type, $F(1,6679.2) = 151.03$, $p < .001$, $R^2_p = .163$). We also observed a Stimulus-Type and Position Interaction (two-way interaction: $F(2,6679) = 6.33$, $p < .001$, $R^2_p = .002$). Follow-up pairwise contrasts between tone and LAS trials, at each position, showed that the effect of Stimulus-Type decreased as Position increased, possibly reflecting a floor effect as reactions approach their lower-limit (Position 1, estimated difference = 22.8 ms, *t-ratio*(6679) = 9.48, $p < .001$; Position 2, estimated difference = 17.0 ms, *t-ratio*(6679) = 7.21, $p < .001$; Position 3, estimated difference = 10.8 ms, *t-ratio*(6679) = 4.56, $p < .001$).

Peak force increased with Position (main effect of Position, $F(2,6679) = 17.56$, $p < .001$, $R^2_p = .005$). Interestingly, a significant Stimulus-Type x Position interaction was detected and follow-up contrasts showed that responses on LAS trials were only more forceful than on Tone trials at Position 3 (main effect of Stimulus-Type, $F(1,6679) = 11.76$, $p < .001$, $R^2_p = .002$; Trial-type x Position, $F(2,6679) = 5.70$, $p < .001$, $R^2_p = .002$; Tone vs. LAS at Position 1, estimated difference = 0.04 N, *t-ratio*(6679) = 0.56, $p = .601$; estimated difference = 0.05 N, Position 2, *t-ratio*(6679) = 0.65, $p = .519$; Position 3, estimated difference = 0.34 N, *t-ratio*(6679) = 4.75, $p < .001$). Further inspection of the data showed that this pattern was consistent across blocks.

Electrophysiological data:

Pre-stimulus activity reflected by the CNV at Cz became more negative as the position increased ($F(2,6506.4) = 23.36$, $p < .001$, $R^2_p = .007$). The effect of Position on CNV was present on both tone and LAS trials, when Stimulus-Types were separated (tone, $F(2,5222.8) = 50.04$, $p < .001$, $R^2_p = 0.019$; LAS, $F(2,1263.4) = 4.10$, $p = .017$, $R^2_p = .006$). Unexpectedly, a main effect of Stimulus-Type was observed, showing more negative amplitudes on tones compared to LAS trials (Stimulus-Type, $F(1,6509.1) = 6.32$, $p = .012$, $R^2_p = .001$). The Position by Stimulus-Type interaction was non-significant (Position x Stimulus-Type, $F(2,6505.6) = 2.28$, $p = .102$, $R^2_p = .001$).

To investigate the unexpected effect of Stimulus-Type, we tested whether the effect could be explained by differences in trial numbers between tone and LAS trials by re-analysing the data on a matched subset of trial numbers. The results indicate that the effect of Position was persistent, but the effect of Stimulus-Type was not (Position, $F(2,2548.4) = 13.08$, $p < .001$, $R^2_p = .010$; Stimulus-Type, $F(1,2543.7) = 0.89$, $p = .346$, $R^2_p = 001$; Position x Stimulus-Type, $F(2,2543.7) = 0.67$, $p = .512$, $R^2_p < 001$). Additional re-runs of the subset analysis showed that the presence of a Stimulus-Type effect was dependent on the random sampling of trials, but the effect of Position did not change. Although the effect of Stimulus-Type varied, the results did not have a meaningful impact on the overall interpretation. In addition, we examined whether sequence length was a predictor of CNV, and found that longer sequence lengths were indeed associated with smaller (more positive) CNV amplitude ($F(1,6524.5) = 5.56$, $p = .018$, $R^2_p = .001$), explaining the effect of Stimulus-Type was dependent on trial sampling. We then re-analysed the data, accounting for the tone sequence length between LAS as a random effect. The results showed that the effects of Stimulus-Type could be explained by sequence length (Preparation, $F(2,553.55) = 10.83$, $p < .001$, $R^2_p = .016$; Stimulus-Type, $F(1,98.17) = 0.11$, $p = .745$, $R^2_p = .001$; Position x Stimulus-Type, $F(2,550.83) = 0.232$, $p = .793$, $R^2_p < .001$).

Pre-stimulus cortical activity in temporal electrodes also decreased as position increased ($F(2,13035) = 21.06$, $p < .001$, $R^2_p = .003$). A main effect of hemisphere showed that amplitudes were more negative over the left hemisphere ($F(1,13030) = 30.60$, $p < .001$, $R^2_p = .002$). Similar to the CNV, an unexpected effect of Stimulus-Type was observed, showing more negative amplitudes on tone trials ($F(1,13041) = 4.45$, $p = .034$, $R^2_p < .001$). Inspection of the plots (Fig. 3) indicated that this was driven by differences in the right hemisphere. Although there were overall shifts in amplitude by hemisphere and Stimulus-Type, they did not interact with position effects (all two-way or three-way interactions, $p > .05$). Similar to the CNV, we examined whether Stimulus-Type effects could be explained by differences in trial numbers and tone sequence length. We re-analysed the data on a matched subset of trial numbers. The results showed that position and hemisphere effects persisted, but the effect of Stimulus-Type did not (Position, $F(2,5121.3) = 11.67$, $p < .001$, $R^2_p = .005$; Hemisphere, $F(1,5109.5) = 22.58$, $p < .001$, $R^2_p = .004$; Stimulus-Type, $F(1,5109.5) = 2.62$, $p = .106$, $R^2_p = .001$, all two-way and three-way interactions, $p > .05$). When considering tone sequence length as a random-effect, Stimulus-Type effects were also no-longer significant (Preparation, $F(2,745.4) = 14.89$, $p < .001$, $R^2_p = .036$; Hemisphere, $F(1,13022.9) = 30.6269$,

$p < .001$, $R^2_p = .002$; Stimulus-Type, $F(1,129.8) = 1.40$, $p = .239$, $R^2_p = .012$; all two-way and three-way interactions, $p > .05$)

For pre-stimulus cortical activity in the occipital mid-line electrode, no statistically significant effects were observed for Position and Stimulus-Type (Position, $F(2,6505.9) = 1.20$, $p = .301$, $R^2_p < .001$; Stimulus-Type = $F(1,6508.7) = 3.00$, $p = .083$, $R^2_p < .001$; Position x Stimulus-Type, $F(2,6505.1) = 0.10$, $p = .904$, $R^2_p < .001$).

Eye-Blink Startle Reflex (LAS trials only):

Blink onset latencies decreased as Position increased ($F(3,1356.6) = 19.37$, $p < .001$, $R^2_p = .041$). Follow-up pairwise comparisons showed a large initial effect from Warning to Position 1 which decreased in effect size across Position, reaching a plateau by Positions 2 and 3 (Warning vs. Position 1, estimated difference = 2.72 ms, *t-ratio*(1357) = 4.72, $p < .001$; Warning vs. Position 2, estimated difference = 3.94 ms, *t-ratio*(1357) = 6.81, $p < .001$; Warning vs. Position 3, estimated difference = 3.86 ms, *t-ratio*(1357) = 6.57, $p < .001$; Position 1 vs. 2, estimated difference = 1.21 ms, *t-ratio*(1357) = 2.20, $p = .123$; Position 1 vs. 3, estimated difference = 1.13 ms *t-ratio*(1356) = 2.03, $p = .138$; Position 2 vs. 3, estimated difference = 0.08 ms, *t-ratio*(1356) = 0.14, $p = .999$).

No statistically significant changes in blink amplitude across Positions were observed (main effect of Position, $F(3,1391) = 0.82$, $p = .481$, $R^2_p = .002$). To investigate whether habituation may have contributed to the absence of amplitude effects, we compared blink amplitude and latency across blocks. We found that blink amplitude decreased over the course of the experiment ($F(1,1505) = 89.36$, $p < .001$, $R^2_p = .056$, $\beta = -2.09$), but blink latency did not ($F(1,1485) = 0.07$, $p = .792$, $R^2_p < .001$, $\beta = .05$). We further examined whether blink amplitude effects could be seen in the first block but this did not yield a different result ($F(3,418) = 0.76$, $p = .514$, $R^2_p < .001$).

Relationship between cortical and sub-cortical excitability, and finger responses.

Cortical activation reflected by CNV amplitude was associated with movement onset times (Amplitude, $F(1,6509.1) = 22.97$, $p < .001$, $R^2_p = .004$, $\beta = 0.17$, Stimulus-Type, $F(1,6506.4) = 137.94$, $p < .001$, $R^2_p = .021$; Amplitude x Stimulus-Type, $F(1,6508.1) = 6.90$, $p = .009$, $R^2_p = .001$). Follow-up analyses showed that more negative CNV amplitudes were associated with earlier onset times for tone trials ($F(1,5224.4) = 74.99$, $p < .001$, $R^2_p = .014$, β

= .17). However, this effect did not reach statistical significance for LAS trials ($F(1,1278.5) = 3.28$, $p = .070$, $R^2_p = .003$, $\beta = .07$). In temporal areas, the same pattern was observed (Amplitude, $F(1,13039) = 22.41$, $p < .001$, $R^2_p = .002$, $\beta = .10$; Stimulus-Type, $F(1,13038) = 258.79$, $p < .001$, $R^2_p = .019$; Amplitude x Stimulus-Type, $F(1,13039) = 6.25$, $p = .012$, $R^2_p < .001$). Follow-up analyses revealed that more negative amplitude were associated with earlier onset times for tone but not LAS trials (tone, $F(1,10469) = 65.50$, $p < .001$, $R^2_p = .006$, $\beta = .10$; LAS, $F(1,2554.1) = 2.59$, $p = .108$, $R^2_p = .001$, $\beta = .04$). Regarding sub-cortical excitability, we found that earlier blink onset latency was associated with earlier movement onset time on LAS trials ($F(1,606.13) = 8.64$, $p = .003$, $R^2_p = .014$, $\beta = .053$). However, blink latency was not associated with the cortical response.

Discussion

In this study, we sought to investigate whether responses to LAS are mediated by the same voluntary control pathways as responses to non-intense tones and whether cortically mediated processes are involved in shaping responses to LAS. To do this, we manipulated the level of movement preparation throughout a trial and examined how it affected finger responses, cortical activity, and sub-cortical excitability.

Voluntarily prepared responses

Marinovic and Tresilian (2016) proposed that the StartReact effect on upper-limb movements can be mediated by the same voluntary control pathways as normal voluntary actions, with variations in response times and vigour attributed to fluctuations in readiness (see also (Alibiglou & MacKinnon, 2012; Stevenson et al., 2014). Such an effect would involve the engagement of a wide-spread cortical-sub-cortical network during movement preparation — a reasonable assumption for well-learned movement patterns (see (Berlot, Popp, & Diedrichsen, 2018).

We observed earlier onset times on LAS trials, with decreasing onset times to tones and LAS as they were presented in later positions. This decrease shows how uncertainty and foreperiod length influence the internal state of readiness, and its presence for both Stimulus-Types suggest that these responses are shaped by similar processes. One question that arises is whether these decreases across positions are driven by an increase in expectancy/conditional probability, or by an increase in foreperiod length. As both predict reductions in onset time, it is difficult to tease apart their contributions, but previous findings

by Leow et al. (2018) suggest that these reductions are attributable to both factors. Using the same experimental design, Leow et al. (2018) examined how the position of the auditory stimulus affected responses during predictable and unpredictable RT tasks. In the predictable task, stimuli were presented at different but known positions. Movement onset times were overall shorter in the predictable task, reflecting an expectancy effect, but most notably, onset times decreased with increasing foreperiod lengths in both tasks, on both Stimulus-Types. We also observed increased peak force in response to tones and LAS when these were presented in later positions. This finding is in contrast to those reported by Mattes and Ulrich (1997) who found that response force decreased as the temporal predictability of an imperative stimulus increased over time. Interestingly, our analysis also detected an interaction between position and Stimulus-Types across all blocks of trials. Notably, this effect appeared to be driven by a reduction in force on tone trials presented at the last position. We speculate this finding may reflect a reduction of preparatory activation in case no auditory stimulus is presented (i.e. a Catch trial), avoiding false-starts. However, the reduction in preparatory activity seemed to be reversed by the LAS presentation at the last position where we found the greatest increase in peak force in LAS trials.

Contingent Negative Variation, conditional probability and foreperiod length

Concerning cortical activation during preparation, we measured CNV amplitude which has been implicated in the anticipation and preparation of future events (Kononowicz & Penney, 2016). CNV amplitudes became more negative as stimuli were presented in later positions, for both Stimulus-Types. At the trial-level, more negative amplitudes were associated with earlier movement onsets on tone trials, demonstrating that fluctuations in movement onset are, in part, explained by fluctuations in cortical activity. However, this association only approached statistical significance on LAS trials (p = .070), reflecting either a lack of statistical power (due to fewer trials) or that the effect relies less on cortical structures. Although there may have been insufficient power to detect a trial-level effect, condition-level decreases in CNV for both Stimulus-Types is consistent with the interpretation that similar processes are responsible for shaping responses.

Like the effect of preparation-level on response time, the question of whether changes in CNV activity can be attributed to differences in expectancy or to foreperiod length also arises. To account for the effect of foreperiod length, amplitudes were baselined to the preceding visual flash. This baseline correction procedure minimises but does not eliminate

effects of foreperiod, because changes in CNV amplitude over time are known to be non-linear, plateauing over time (Hillyard, 1969). Hypothetically, if there was no effect of expectancy, the natural plateau in CNV on the post-baselined data would lead to decreasing (more positive) CNV amplitudes across positions. However, our results showed the opposite — more negative CNVs as position increased, demonstrating that there is an effect of expectancy, even while accounting for the effect of foreperiod length.

EEG signals in sensory areas during movement preparation

Recent research has shown interactions between auditory and motor regions of the brain during speech and musical rhythm perception in humans (J. L. Chen, Penhune, & Zatorre, 2008; J. L. Chen, Zatorre, & Penhune, 2006; Cheung, Hamiton, Johnson, & Chang, 2016). Work by Li and colleagues (2017) has further suggested that the auditory cortex is required not only to anticipate a sensory event but also produce appropriate motor responses in mice. To examine whether such association would be observed in our study, we analysed the effect of preparation-level on pre-stimulus activity in auditory and visual sensory areas of the cortex. Our expectations were guided by our predictions regarding CNV patterns, that is, pre-stimulus activity would show evidence of increased excitability across position and be associated with movement onset at the trial-level. In line with this prediction, activity at temporal electrodes above both hemispheres became more negative as stimuli were presented in later positions, for both Stimulus-Types. At the trial-level, more negative amplitudes were associated with earlier movement onset to tones. This result demonstrates that the state of readiness established by neural processes relating to movement preparation is not limited to motor-related circuits, including also sensory areas.

Although previous work by Bueti and Macaluso (2010) found that auditory expectations modulated occipital activity and were related to the probability of the auditory stimulus to occur, in our study, pre-stimulus activity in the occipital mid-line electrode did not change significantly across preparation-levels. This absence of an effect in visual areas likely reflects differences in the nature of the expected auditory stimuli. Bueti and Macaluso (2010) used sounds that had been previously associated with images such as the sound of hand-clapping or of a hammer-hammering, whereas we used simple tones or broad-band white noise which were not associated to any specific visual event (but see Swallow, Makovski & Jiang (2012). Overall, our findings indicate that the neural processes involved in preparation include the auditory cortex. Whether the results reflect a process involved in

facilitating the detection of an imperative stimulus, a required contribution to the level of preparation for action (Li et al., 2017), or both remains to be tested.

Eye-Blink Startle Reflex

We examined whether the cortically mediated processes involved in movement preparation also influence sub-cortical excitability reflected by the eye-blink startle reflex. In the same way that sensory and motor cortices are thought to be influenced by preparation-related processes, we hypothesised that sub-cortical structures downstream from motor cortex may also be prepared to facilitate the execution of motor responses. This led to predictions that the latency and amplitude of the eye-blink reflex would decrease and increase, respectively, as the LAS was presented in later positions. We also expected that eye-blink facilitation would be associated with increased cortical excitability and earlier movement onset times.

In line with our predictions, blink latency decreased as the LAS was presented in later positions. At the trial-level, we found a reliable association between blink latency and movement onset, suggesting that voluntarily mediated processes have a generalised effect extending to the circuits responsible for the eye-blink reflex. These results are in agreement with those obtained by (2001), who observed eye-blink reflex facilitation during a discrimination task (see also (Lipp, Alhadad, & Purkis, 2007). However, trial-level associations between blink latency and cortical activity were non-significant. The absence of this association could reflect a lack of statistical power, echoing the absence of associations between movement onset and cortical activity on LAS trials. Although it is inconclusive whether changes in sub-cortical excitability are driven by higher-order cortically mediated processes at the trial level, systematically modifying the level of motor preparation led to a similar pattern of change in the eye-blink reflex, EEG signals and the finger response. There was also evidence that neural excitability in sensory, motor, and sub-cortical circuits was linked to response time. We believe this is not a coincidence and the simplest explanation seems to be that these wide-spread, synced effects on voluntary and involuntary motor responses are caused by the same cascade of processes linked to movement preparation.

LAS expectation effects

An unexpected finding in our study was an effect of Stimulus-Type on CNV. As CNV was measured before the presentation of any auditory stimulus, one would not predict to

observe such an effect. To investigate further, we tested whether the sequence of tones between LAS trials may have influenced LAS expectancy. The nature of this expectancy effect could be either facilitatory or suppressive, depending on the context. It has been shown that anticipation of an uncertain and intense stimulus modulates sub-cortical excitability, reflected by blink amplitude (Davies & Craske, 2015; Grillon, Baas, Lissek, Smith, & Milstein, 2004). On one end, the more predictable the LAS becomes after several non-LAS trials in our study, the more suppressed sub-cortical pathways should be — an inhibitory effect. In contrast, we and others have previously reported that unpleasant visual stimuli can increase corticospinal excitability (Coelho, Lipp, Marinovic, Wallis, & Riek, 2010; Giovannelli et al., 2013), suggesting that the expectancy of aversive acoustic stimuli (e.g., LAS) could enhance the preparedness of the motor system for action — an excitatory effect. If participants kept track of the presentation of LAS trials over the experiment, longer sequences of tone trials would be associated with a greater conditional probability of the LAS, and vice-versa. Indeed, our analysis showed that sequence effects systematically affected the CNV signals, indicating the expectancy of an aversive auditory stimulus induced a lower preparatory state. This effect was observed on both central and temporal sites, indicating that participants considered LAS presentation history to suppress startle-related reactions which, in turn, also affected cortical preparation. Overall, this finding highlights the importance of considering trial-sequence effects (see also (Marinovic, Poh, de Rugy, & Carroll, 2017; Verstynen & Sabes, 2011), especially in tasks with a rare stimulus component, where the trial-sequence can have a significant impact on its expectancy.

Conclusion

In this study, we demonstrated that the level of readiness for action had similar effects on movement onset time to tones and LAS and discovered that it had a wide-spread effect on the excitability of the nervous system. At the condition-level, movement preparation influenced sensory and motor cortices for both Stimulus-Types, as well as sub-cortical circuits associated with the eye-blink startle reflex. At the trial-level, there was evidence that increased cortical and sub-cortical excitability was associated with shorter movement onset times. We also discovered a previously unknown inhibitory effect of trial history — driven by the probability of presentation of an intense auditory stimulus— on cortical activity. Collectively, the results are consistent with the interpretation that the StartReact effect reflects a well-orchestrated, dynamic relationship between cortical and sub-cortical areas in

the central nervous system that takes into account the differential probabilities of discrete events on both short (within-trial) and long (across-trials) timescales.

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