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RESEARCH ARTICLE | *Control of Movement*

Dissociation of muscle and cortical response scaling to balance perturbation acceleration

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Payne AM, Hajcak G, Ting LH. Dissociation of muscle and cortical response scaling to balance perturbation acceleration. *J Neurophysiol* 121: 867–880, 2019. First published December 5, 2018; doi:10.1152/jn.00237.2018.—The role of cortical activity in standing balance is unclear. Here we tested whether perturbation-evoked cortical responses share sensory input with simultaneous balance-correcting muscle responses. We hypothesized that the acceleration-dependent somatosensory signals that drive the initial burst of the muscle automatic postural response also drive the simultaneous perturbation-evoked cortical N1 response. We measured in healthy young adults (n = 16) the initial burst of the muscle automatic postural response (100–200 ms), startle-related muscle responses (100–200 ms), and the perturbation-evoked cortical N1 potential, i.e., a negative peak in cortical EEG activity (100–200 ms) over the supplementary motor area. Forward and backward translational support-surface balance perturbations were applied at four levels of acceleration and were unpredictable in timing, direction, and acceleration. Our results from averaged and single-trial analyses suggest that although cortical and muscle responses are evoked by the same perturbation stimulus, their amplitudes are independently modulated. Although both muscle and cortical responses increase with acceleration, correlations between single-trial muscle and cortical responses were very weak. Furthermore, across subjects, the scaling of muscle responses to acceleration did not correspond to scaling of cortical responses to acceleration. Moreover, we observed a reduction in cortical response amplitude across trials that was related to a reduction in startle-related—but not balance-correcting—muscle activity. Therefore, cortical response attenuation may be related to a reduction in perceived threat rather than motor adaptation or changes in sensory inflow. We conclude that the cortical N1 reflects integrated sensory inputs simultaneously related to brain stem-mediated balance-correcting muscle responses and startle reflexes.

NEW & NOTEWORTHY Reactive balance recovery requires sensory inputs to be transformed into appropriate balance-correcting motor responses via brain stem circuits; these are accompanied by simultaneous and poorly understood cortical responses. We used single-trial analyses to dissociate muscle and cortical response modulation with perturbation acceleration. Although muscle and cortical responses share sensory inputs, they have independent scaling mechanisms. Attenuation of cortical responses with experience reflected attenuation of brain stem-mediated startle responses rather than the amplitude of balance-correcting motor responses.

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adaptation; balance N1; EEG; EMG; habituation; posture; startle

INTRODUCTION

It is unclear how cortical activity is related to balance-correcting behavior. The earliest muscle activation after a balance disturbance is a monosynaptic spinal stretch reflex, followed by a larger burst of balance-correcting muscle activity, called the automatic postural response (APR), mediated by brain stem sensorimotor circuits (Carpenter et al. 1999; Jacobs and Horak 2007). Although the initial stretch reflex response is quite small, the much larger balance-correcting APR muscle activity is initiated at ~100-ms latency (Carpenter et al. 1999), with its initial burst of activity scaling with perturbation acceleration (Welch and Ting 2009, 2008) due to proprioceptive sensory inputs (Lockhart and Ting 2007). Unpredictable balance disturbances can also serve as a startling stimulus (Campbell et al. 2013; Oude Nijhuis et al. 2010), evoking brain stem-mediated startle reflex muscle activity (Brown et al. 1991), simultaneous with the balance-correcting APR, particularly during the first few trials (Nonnekes et al. 2015; Siegmund et al. 2008). Similarly, the earliest cortical event-related potentials (ERPs) after a balance disturbance include a small and variable positive peak (P1) followed by a large and robust negative peak (N1). The cortical balance N1 peak occurs between 100 and 200 ms after perturbation onset, with the largest amplitude at central and fronto-central midline scalp electrodes, and has been localized to the supplementary motor area (Marlin et al. 2014; Mierau et al. 2015). Recent advances in technology have made it possible to directly measure cortical electrical activity during reactive balance recovery (Bolton 2015) and to perform trial-by-trial analyses (Mierau et al. 2015). Here we focus on the possible relationship between the cortical balance N1 and the initial burst of the muscle APR. Because of the similarity of their latencies, the cortical balance N1 cannot directly cause the initial burst of the muscle APR or startle-related muscle activity; rather, these phenomena are triggered by the same event and may be modulated common sensory inputs.

Prior studies examining averaged responses have suggested that the amplitudes of cortical and muscle responses to balance perturbations are modulated by shared somatosensory inputs.

The earliest studies of cortical responses to balance perturbations compared treadmill perturbations applied during walking and standing. Both the cortical balance N1 and the initial burst of the muscle APR were smaller and later when evoked during walking compared with standing (Dietz et al. 1984b). The inhibition of group I somatosensory afferents during walking was posed as a possible explanation for the observation of a shared delay and attenuation of cortical and muscle responses to perturbations during walking versus standing. Consistent with this hypothesis, ischemic block of group I somatosensory afferents was shown to attenuate both cortical and muscle responses to perturbations during standing as well as somatosensory evoked cortical potentials (SEPs) evoked by electrical stimulation of the tibial nerve (Dietz et al. 1985b). Moreover, these amplitude and latency differences in cortical and muscle responses during perturbations to walking are not apparent before the age of 6 yrs and develop with the suppression of stretch reflexes during walking (Berger et al. 1987). Further support of a common peripheral site of origin was the demonstration that both cortical and muscle responses to balance perturbation were delayed by ~30 ms in a patient with slow peripheral conduction velocities (Dietz et al. 1985a). More recently, in a lean-and-release balance perturbation paradigm, the cortical balance N1 and initial burst of the muscle APR scaled to perturbation amplitude (Mochizuki et al. 2010).

Trial-by-trial variations of the cortical balance N1 and the initial burst of the muscle APR could arise from dynamic processes over the course of an experiment such as habituation, adaptation, and learning. All of the above studies relied on averaging across multiple trials (~100 trials in Dietz et al., 30–60 trials in Mochizuki et al.), which would mask any time or history dependence of cortical and muscle responses. Recently, with single-trial analysis, a gradual reduction of cortical balance N1 amplitude was shown across a series of 10 identical perturbations that were unpredictable in timing (Mierau et al. 2015). Such a systematic reduction in the cortical balance N1 across identical, consecutive trials suggests that the processes underlying the cortical balance N1 are dynamic across trials and suggests a need to conduct single-trial analyses to examine its potential function and relationship to muscle responses. Our prior work demonstrated that nonrandom sources of trial-by-trial variation in the initial burst of the muscle APR include trial-by-trial variation in postural response strategy (Torres-Oviedo and Ting 2010), center of mass kinematics, i.e., the acceleration, velocity, and displacement of the body relative to the base of support (Safavynia and Ting 2012; Welch and Ting 2009), and adaptation of the underlying sensorimotor transformation (Horak and Nashner 1986; Welch and Ting 2014). If the cortical balance N1 shares the ascending sensory input with the brain stem sensorimotor circuit underlying the initial burst of the muscle APR, then the cortical balance N1 could also share these sources of trial-by-trial variation, leading to the prediction that cortical and muscle response amplitudes would be correlated across single trials. Indeed, Mierau and colleagues found a weak correlation between single-trial cortical and muscle responses despite the fact that muscle responses did not also decrease systematically across trials (Mierau et al. 2015). However, the experimental paradigm used did not explicitly alter sensory inputs across trials, so the question of whether changes in sensory inflow cause correlated changes in the cortical balance N1 and the initial burst of the muscle APR remains unanswered.

We previously demonstrated that the initial burst of the muscle APR scales with perturbation acceleration and is encoded by somatosensory inputs, but it is not known whether the cortical balance N1 shares this acceleration dependence. Using a series of unpredictable translational support-surface perturbations with randomized peak acceleration amplitude, a simple delayed-feedback model of kinematic errors explained the balance-correcting muscle response in humans (Welch and Ting 2009, 2008) and cats (He et al. 1991; Lockhart and Ting 2007). The initial burst of the muscle APR was explained by center of mass acceleration error, which depends on the imposed level of perturbation acceleration. We further showed in cats that this acceleration-dependent initial burst of the muscle APR was absent or reduced after loss of group I somatosensory afferents from pyridoxine-induced peripheral neuropathy (Lockhart and Ting 2007; Stapley et al. 2002). Acceleration dependence of the cortical N1 has only been tested in three subjects perturbed while seated, two of whom showed acceleration-dependent scaling of the cortical N1 (Staines et al. 2001). We are not aware of any prior studies demonstrating dependence of the cortical N1 on perturbation acceleration during standing.

We hypothesized that if the cortical balance N1 shares group I somatosensory afferent inputs with the initial burst of the muscle APR, then its amplitude would scale with peak perturbation acceleration in standing balance on a trial-by-trial basis. To test whether cortical and muscle responses are similarly scaled to sensory information, we compared single-trial amplitudes of muscle and cortical responses to balance perturbations that varied in peak acceleration magnitude. Healthy young adults were tested in a series of randomized support-surface balance perturbations that varied in perturbation acceleration in forward and backward directions. We predicted that the cortical balance N1 would increase with perturbation acceleration and would be correlated with the initial burst of the muscle APR across trials because of shared sensory inputs. Because of the possibility for motor responses following the APR to involve a transcortical sensorimotor response (Jacobs and Horak 2007), we also performed exploratory analyses relating the cortical balance N1 to longer-latency muscle activity. Our results suggest that muscle and cortical responses to translational support-surface perturbations share sensory inputs but have independent scaling mechanisms.

METHODS

Participants

Seventeen healthy young adults were recruited from Emory University and the surrounding population to participate in an experiment that was approved by the Emory University Institutional Review Board. All subjects gave written informed consent before participating. One subject was excluded from analysis for deviation from the experimental protocol. The remaining 16 subjects (9 women, 7 men) used in our analyses were 26 yr (SD 5) old and 171 cm (SD 13) tall and weighed 72 kg (SD 11).

Experimental Protocol

To test the effect of varying perturbation acceleration on evoked cortical and muscle activity, we presented subjects with a series of ramp-and-hold perturbations in which the floor was displaced during

quiet standing across a range of perturbation accelerations. Perturbations were delivered with a custom-designed perturbation platform (Factory Automation Systems, Atlanta, GA). Sixty-four perturbations were delivered to each subject, divided evenly between four levels of peak acceleration (0.23–0.66 g) and between forward and backward directions. All perturbations reached a peak velocity of 40 cm/s and a total displacement of 10 cm (Fig. 1).

Perturbations were unpredictable in timing, direction, and acceleration magnitude. Perturbations were presented in eight blocks, with each block containing one replicate of each of the eight distinct perturbations (2 directions × 4 accelerations) in random order. The blocks were also randomized into three different block orders across subjects. The total duration of the 64-perturbation series was 16.8 min (SD 4.4), with a 5-min rest enforced for durations > 15 min to prevent fatigue. Intertrial intervals (as measured from perturbation onset to perturbation onset) were 16 s (SD 4, range 7 s to 1.7 min, not counting the 5-min rest period when present).

To minimize recording artifacts, perturbations were initiated only when electroencephalography (EEG) activity was relatively quiescent, based on visual inspection of a monitor displaying the online EEG recording. Subjects were instructed to cross their arms across their chest and to try to maintain balance without taking a step while staring at a central location in a 73 × 106-cm poster of a mountain landscape on a wall 4.5 m in front of them. Subjects were verbally reminded to relax and look forward when electromyography (EMG) activity or large eye movements were visually apparent in ongoing EEG recording. Subjects were asked if they would like to take a break if alpha oscillations became visually apparent in the ongoing EEG recording. Subjects were allowed to blink freely.

Reflective markers placed on the head, neck (C7), hips (left and right anterior and superior iliac crest), knees, ankles, and feet were sampled at 100 Hz by a 10-camera Vicon 3D motion analysis system to track body motion in three dimensions. Stepping responses were noted during collection and manually confirmed by checking motion data. To assess the potential for movement artifacts during the window in which cortical data were quantified, we assessed absolute motion of the head and heel markers in the anterior-posterior plane at 100 ms and 200 ms after perturbation onset relative to the moment of perturbation onset. One subject was excluded from kinematic analyses ($n = 15$) because of data miscalibration.

EEG Collection

Thirty-two active EEG electrodes (ActiCAP; Brain Products, Germany) were placed on the scalp according to the International 10-20 system of EEG electrode placement, with the exception of electrodes TP9 and TP10, which were placed directly on the mastoid bones beneath the EEG cap for off-line rereferencing. ActiCAP active electrodes improve signal quality by performing impedance conversion with powered circuits integrated into the electrodes that transform high input resistance due to the scalp into a lower output resistance to reduce the impact of external sources of interference as the signals travel from the electrodes to the amplifier. Active electrode sites were prepared by rubbing the scalp with a blunt-tipped needle, which was subsequently used to apply conductive electrode gel (SuperVisc 100-g High Viscosity Electrolyte Gel for active electrodes; Brain Products). Mastoid electrode sites were additionally prepared by scrubbing the skin with an alcohol pad. Impedances at primary electrode sites (i.e., Cz and mastoids) were <10 kΩ before the start of data collection. All other active electrode sites were similarly prepared, but impedance values were not generally <10 kΩ before the start of data collection because of time limitations and were in many cases out of range of the impedance measurement.

To enable subtraction of blink and eye movement artifacts, electrooculography (EOG) data were collected with bipolar pairs of passive electrodes (E220x; Brain Products); a ground electrode was placed in the center of the forehead. Electrodes were prepared with high-chloride abrasive gel (ABRALYT HiCl 250-g high-chloride-10% abrasive electrolyte gel; Brain Products), and electrode sites were prepared by scrubbing the skin with an alcohol pad. Vertical EOG was measured between a pair of electrodes that vertically bisected the pupil of the right eye.

EEG and EOG data were amplified on a BrainAmp DC amplifier (Brain Products) sampling at 1,000 Hz, with a 16-bit A/D converter. Online filtering consisted of a first-order low-pass filter with a cutoff (−12 dB point) of 0.016 Hz with a 6 dB/octave slope and a fifth-order Butterworth low-pass filter with a cutoff of 1,000 Hz with a 30 dB/octave slope.

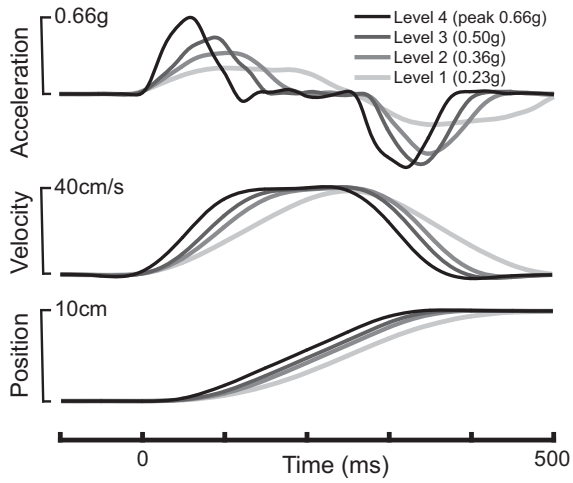

Fig. 1. Perturbation kinematics. Peak accelerations varied between 0.23, 0.36, 0.50, and 0.66 g, with a peak velocity of 40 cm/s, and a total displacement of 10 cm. Larger accelerations are indicated with darker lines.

EEG Data Preprocessing

The raw (nonsegmented) EEG data were high-pass filtered off-line with a third-order zero-lag Butterworth filter with a cutoff frequency (−3 dB point) of 0.05 Hz, with a slope of 18 dB/octave. EEG data were then centered on zero by subtracting the mean value from each channel for each subject before applying a similarly designed low-pass filter with a cutoff frequency of 25 Hz. EEG data from electrode Cz were then rereferenced to the average of the two mastoid electrodes and epoched into segments of 2.4 s, beginning 400 ms before perturbation onset. The 2.4-s duration of the epochs was selected as the longest time window for which both EEG and EMG data existed across all trials, ensuring that edge effects of filtering and artifact detection and correction (described below) would not overlap with the primary time windows of interest. Vertical EOG electrode voltages were filtered and segmented identically to EEG but were not rereferenced.

Blinks and vertical eye movement artifacts were subtracted with the Gratton and Coles algorithm (Gratton et al. 1983), which uses sequential linear regressions and subtractions to remove non-event-related correlations between vertical EOG activity and EEG activity at the Cz electrode due to blinks and eye movement. Segmented data were then baseline corrected by subtracting the mean value of a 100-ms time window ending 50 ms before the onset of platform acceleration.

EMG Collection

Surface EMGs (Konigsberg Instruments, Pasadena, CA) were collected from tibialis anterior (TA), medial gastrocnemius (MG), and sternocleidomastoid (SC) muscles bilaterally. TA and MG were selected because of their roles as primary agonist muscles responding to forward and backward perturbations, and SC was selected as a

primary indicator of startle responses (Brown et al. 1991; Campbell et al. 2013; Nonnekes et al. 2015). EMG signals were analog filtered online with a 500-Hz low-pass filter and sampled at 1,000 Hz. Skin was scrubbed with an alcohol pad and shaved if necessary before electrode placement. Silver-silver chloride disk electrode pairs were placed with 2-cm interelectrode distance.

EMG Data Preprocessing

Raw EMG signals were segmented into epochs of 2.4 s, beginning 400 ms before perturbation onset. Segmented EMG signals were high-pass filtered off-line with a third-order zero-lag Butterworth filter with a cutoff frequency (−3 dB point) of 35 Hz, with a slope of 18 dB/octave. EMG signals were then centered on zero by subtracting the mean from each epoch and were subsequently half-wave rectified. Rectified EMG signals were then low-pass filtered with a similarly designed Butterworth filter with a cutoff of 40 Hz. Bilateral EMG signals were then averaged across left and right sides.

Justification of Time Window of Analysis for EEG and EMG Data

The time window for primary analyses for both EEG and EMG data was defined a priori as 100–200 ms after the onset of perturbation acceleration. This time window begins at 100 ms because the onset of TA activity occurs ~100 ms after the onset of perturbation, with the initial burst of TA activity reflecting perturbation acceleration at a 100-ms delay (Welch and Ting 2009). Accordingly, perturbations were designed to reach peak perturbation acceleration within the first 100 ms after perturbation onset. This same time bin is also ideal for analysis of the cortical balance N1, which occurs ~150 ms after the onset of platform acceleration (Marlin et al. 2014; Mierau et al. 2015). A later 200–300 ms time bin used in secondary analyses was not defined a priori.

Quantification of EEG

Subject-averaged cortical ERPs were created by averaging cortical activity across all trials within subjects at electrode Cz time-locked to perturbation onset. Likewise, condition-averaged cortical ERPs were created for each subject by averaging cortical activity across the eight trial replicates within each of the four levels of perturbation acceleration in each direction at electrode Cz. The N1 peak was defined and quantified as the absolute peak amplitude in microvolts (of a negative peak) in a time window from 100 ms to 200 ms after the onset of platform motion for subject- and condition-averaged ERPs as well as single-trial data. N1 peak latency was also quantified as the time between perturbation onset and N1 peak in subject- and condition-averaged ERPs.

Quantification of EMG

Muscle responses to perturbation were likewise quantified as the peak amplitude of EMG activity in the same time bin from 100 ms to 200 ms after the onset of platform motion, on both single trials and condition averages. On single trials and condition averages, peak measures of the muscle responses were normalized to have a maximum value of 1 within each subject.

Quantification of Signal-to-Noise Ratios

On single trials and on condition averages, we quantified the peak value between 100 and 200 ms (as described above) and the standard deviation of the baseline period (−150 to −50 ms). These single-trial values were averaged within subjects. and then each subject's average peak measure was divided by the subject's average baseline standard deviation as a measure of single-trial signal-to-noise ratio for each subject. These measurements were also repeated on condition averages. We report these signal-to-noise ratio values as the mean and standard deviation across subjects for single trials and condition averages.

Statistical Analyses

Step frequency. To examine the effect of perturbation acceleration and direction on the frequency of stepping responses, we performed an ANOVA on the number of trials in which subjects took steps at each level of perturbation acceleration in each direction. Post hoc Tukey tests were used for all multiple comparisons. ANOVAs were performed in SAS statistical software.

Differences in cortical response amplitudes and latencies between subjects. We used a paired *t*-test to compare subject-averaged cortical response amplitudes and latencies between forward and backward perturbation directions to justify combining cortical responses across forward and backward perturbations in subject averages. We then used univariate linear regressions to assess correlations between the subject-averaged N1 amplitude or latency and between-subjects measurements, including height, weight, age, and number of trials in which steps were taken. Given four comparisons, we apply a Bonferroni correction to obtain a significance threshold of $\alpha = 0.05/4 = 0.0125$ for regressions on subject-averaged cortical responses. All linear regressions were performed in SAS, and all R^2 values are reported as adjusted R^2 values. Given apparent relationships between cortical response amplitude, subject height, and frequency of stepping, we also used a univariate linear regression to assess correlation between subject height and frequency of stepping responses.

Effect of acceleration on cortical response amplitudes in condition averages and single trials. To examine the effect of perturbation acceleration and direction on cortical response amplitudes, we performed a balanced mixed-model ANOVA (with acceleration and direction as within-subject factors, including possible acceleration × direction interaction, accounting for subject as a random effect) on cortical response amplitudes on condition averages. We further quantified the effect of acceleration by using a univariate linear regression to assess the correlation between *z*-transformed single-trial cortical response amplitudes and peak acceleration measured on single trials across subjects, both within and across perturbation directions. The *z*-transformation was performed before this regression to remove between-subjects variance, in order to quantify the within-subject effect of acceleration. Unlike the ANOVA, which used integer values to code for the four acceleration conditions, linear regressions used the maximum acceleration recorded in the first 100 ms of perturbations on single trials. To account for the combination of multiple subjects in the univariate linear regression, we report the *P* value from a corresponding generalized linear model including subject and subject × acceleration interaction terms (*P* values were identical within the precision reported). To further assess the distribution of the acceleration effect across subjects, we additionally performed linear regressions between single-trial cortical response amplitudes and perturbation acceleration within each subject individually. We report the number of subjects showing a significant correlation between cortical response amplitude and perturbation acceleration (at $\alpha = 0.05$) and the mean and standard deviation of the significant R^2 values. The regressions between cortical response amplitude and perturbation acceleration within individuals used data without prior *z*-transformation so that slopes of the regressions could be compared across subjects. Given apparent relationships between acceleration scaling relationships and subject-averaged cortical response amplitudes, we additionally performed a univariate linear regression to assess the correlation between subject-averaged cortical response amplitudes and the slopes from the cortical response amplitude vs. perturbation acceleration regressions.

Effect of acceleration on condition-averaged cortical response latencies. To examine the effect of perturbation acceleration and direction on cortical response latency, we performed a balanced

mixed-model ANOVA (with acceleration and direction as within-subject factors, including possible acceleration × direction interaction, accounting for subject as a random effect) on cortical response latencies on condition averages. For comparison, we also performed the same ANOVA on the latency of peak perturbation acceleration in condition-averaged data. To assess the relationship between the peak latency of cortical responses and the peak latency of perturbation acceleration, we performed a univariate linear regression to assess the correlation between cortical response peak latency and perturbation acceleration peak latency.

Effect of trial number on cortical response amplitudes. To assess effects of trial number on cortical response amplitude, we performed a balanced repeated-measures, mixed-model ANOVA (with within-subject factors direction and acceleration repeated across trial blocks, including possible acceleration × trial block interaction, accounting for subject as a random effect) on single-trial cortical response amplitudes. We further quantified the effect of trial number by using a univariate linear regression to assess the correlation between z-transformed single-trial cortical response amplitudes and trial number across subjects, both within and across perturbation directions. This linear regression used trial numbers (*1–64*) instead of block numbers (*1–8*), which were used in the ANOVA. To account for the combination of multiple subjects in the univariate linear regression, we report the P value from a corresponding generalized linear model including subject and subject × trial number interaction terms (P values were identical within the precision reported). To further assess the distribution of the effect of trial number across subjects, we additionally performed linear regressions between single-trial cortical response amplitudes and trial number within each subject individually. We report the number of subjects showing a significant correlation between cortical response amplitude and trial number (at $\alpha = 0.05$) and the mean and standard deviation of the significant R^2 values. The regressions between cortical response amplitude and trial number within individuals used data without prior z-transformation so that slopes of the regressions could be compared across subjects. Given apparent relationships between changes in cortical response amplitudes across trials and subject-averaged cortical response amplitudes, we additionally performed a univariate linear regression to assess the correlation between subject-averaged cortical response amplitudes and the slopes from the cortical response amplitude vs. trial number regressions. Because not all subjects showed significant dependencies of cortical response amplitudes on trial number or perturbation acceleration, we performed Fisher's exact test of independence to test for association between dependence of cortical response amplitudes on perturbation acceleration and dependence of cortical response amplitudes on trial number.

Effect of acceleration on muscle response amplitudes. To examine the effect of perturbation acceleration and direction on muscle response amplitudes, we performed a balanced mixed-model ANOVA (with acceleration and direction as within-subject factors, including possible acceleration × direction interaction, accounting for subject as a random effect) on muscle response amplitudes (independently for TA-EMG, MG-EMG, and SC-EMG) on condition averages. We further quantified the effects of acceleration by using univariate linear regressions to assess correlations between z-transformed single-trial muscle response amplitudes and peak acceleration measured on single trials across subjects, both within and across perturbation directions for each muscle. Again, the z-transformation was performed before these regressions to remove between-subjects variance, in order to quantify the within-subject effects of acceleration. To account for the combination of multiple subjects in the univariate linear regression, we report the P value from a corresponding generalized linear model including subject and subject × acceleration interaction terms (P values were identical within the precision reported). To further assess the distributions of the acceleration effects across subjects, we additionally performed linear regressions between single-trial muscle response amplitudes and perturbation acceleration within each subject and each muscle individually. We report the number of subjects showing significant correlations between muscle response amplitudes and perturbation acceleration (at $\alpha = 0.05$) and the mean and standard deviation of the significant R^2 values.

Effect of trial number on muscle response amplitudes. To assess effects of trial number on muscle response amplitudes, we performed balanced repeated-measures, mixed-model ANOVAs (with within-subject factors direction and acceleration repeated across trial blocks, including possible acceleration × trial block interaction, accounting for subject as a random effect) on single-trial muscle response amplitudes. We further quantified the effects of trial number by using univariate linear regressions to assess the correlations between z-transformed single-trial muscle response amplitudes and trial number across subjects, both within and across perturbation directions for each muscle. To account for the combination of multiple subjects in the univariate linear regression, we report the P value from a corresponding generalized linear model including subject and subject × trial number interaction terms (no adjustments to P values crossed the significance threshold of $\alpha = 0.05$). To further assess the distribution of the effects of trial number across subjects, we additionally performed linear regressions between single-trial muscle response amplitudes and trial number within each subject and each muscle individually. We report the number of subjects showing significant correlations between muscle response amplitudes and trial number (at $\alpha = 0.05$) and the mean and standard deviation of the significant R^2 values. Because not all subjects showed significant dependencies of muscle response amplitudes with trial number or perturbation acceleration for each muscle, we additionally performed Fisher's exact test of independence to test for association between dependence of response amplitudes for each muscle on perturbation acceleration and dependence of response amplitudes for the same muscle on trial number.

Associations between cortical and muscle response amplitudes. We used univariate linear regressions to assess correlations between z-transformed cortical response amplitudes and z-transformed simultaneous (100–200 ms) or subsequent (200–300 ms) muscle response amplitudes across all subjects, both within and across perturbation directions, for each muscle and each time bin independently. To account for the combination of multiple subjects in the univariate linear regression, we report the P value from a corresponding generalized linear model including subject and subject × response amplitude interaction terms (no adjustments to P values crossed the significance threshold of $\alpha = 0.05$). To further assess the distribution of correlations between muscle and cortical response amplitudes across subjects, we additionally repeated these linear regressions within each subject individually. We report the number of subjects showing significant correlations between muscle and cortical response amplitudes (at $\alpha = 0.05$) and the mean and standard deviation of the significant R^2 values. To assess whether acceleration dependence of cortical response amplitudes was associated with acceleration dependence of simultaneous muscle response amplitudes, we performed Fisher's exact test of independence to test for association between acceleration dependence of cortical response amplitudes and acceleration dependence of simultaneous response amplitudes for each muscle. To assess whether dependence of cortical response amplitudes on trial number was associated with dependence of simultaneous muscle response amplitudes on trial number, we additionally performed Fisher's exact test of independence to test for association between dependence of cortical response amplitudes on trial number and dependence of simultaneous response amplitudes for each muscle on trial number.

RESULTS

Summary

Overall, our results revealed that cortical and muscle responses increased weakly in amplitude with increasing perturbation acceleration in condition-averaged and single-trial data.

Cortical and muscle responses also decreased weakly in amplitude throughout the duration of the experiment across single-trial data. Muscle and cortical responses were only weakly correlated with each other across single trials. Furthermore, increasing cortical response amplitudes with perturbation acceleration within an individual did not predict whether or not the individual would also show larger balance-correcting muscle responses or startle-related muscle responses with acceleration. In contrast, reduction in cortical response amplitude throughout the duration of the experiment was significantly associated with a reduction in startle-related muscle activity across subjects but was not associated with a reduction in balance-correcting muscle activity.

Behavioral Responses and Body Motion

All participants were able to recover balance without assistance but were not always able to resist stepping responses to perturbations. Despite instructions to recover balance without taking a step, subjects stepped on 243 of 1,024 perturbations (24% of trials), with individuals ranging from 0 to 56 steps out of 64 perturbations. Stepping trials were not excluded from analysis because steps occurred later than the window of analysis, consistent with prior findings (Chvatal et al. 2011). Accordingly, by the end of our primary window of analysis (100–200 ms) we observed foot displacements due to platform translation to be larger than 5 cm, whereas head displacements were much less than 0.5 cm (Fig. 2). For these time windows analyzed, signal-to-noise ratios of cortical and muscle responses on single trials were about half those of condition averages (Fig. 3) and were sufficient for single-trial analyses.

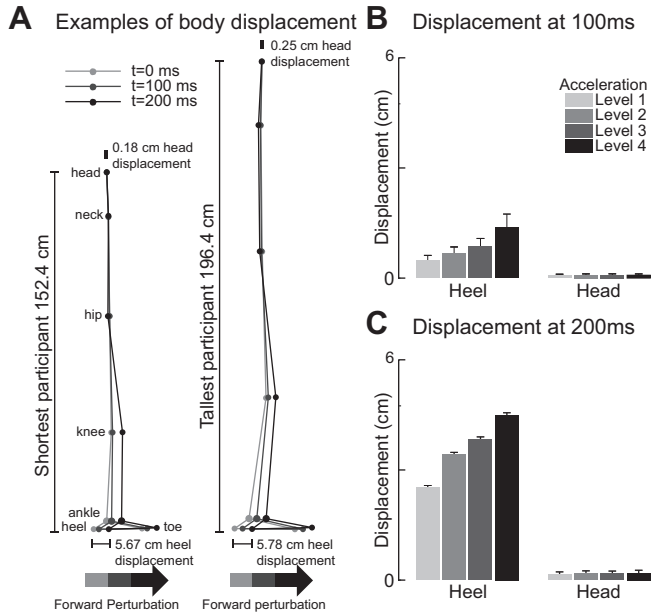

Fig. 2. Subject kinematics during primary window of analysis. *A*: examples of body displacement for the shortest (152.4 cm, female, *left*) and tallest (196.4 cm, male, *right*) participants in the first exposure to the largest forward perturbation at perturbation onset (0 ms), 100 ms, and 200 ms after perturbation onset. The average absolute head and heel displacements for these individuals across perturbations at the highest acceleration at 200 ms are indicated in text by the head and heel markers. *B*: mean and standard deviation of heel and head displacements at 100 ms after perturbation onset across subjects ($n = 15$, 9 women, 6 men; 1 subject was excluded because of miscalibration). *C*: the same measurements at 200 ms.

Stepping responses were more frequent in forward perturbations and at higher accelerations. ANOVA revealed significant effects of perturbation direction [$F(1,108) = 76.5$, $P < 0.0001$] and acceleration level [$F(3,108) = 5.3$, $P = 0.002$] on step frequency. Post hoc Tukey tests revealed that stepping responses were more frequent in forward perturbations [36% of trials (SD 31)] compared with backward perturbations [12% (SD 19), $P < 0.05$]. Stepping responses were more frequent at the highest acceleration level compared with all other levels ($P < 0.05$), with no other significant differences in step frequency between acceleration levels. Additionally, the number of stepping responses across subjects was inversely correlated with subject height ($P = 0.01$, $R^2 = 0.34$), with shorter subjects taking compensatory steps more frequently.

Cortical Response Amplitudes Varied with Subject Height

Subject height was the only factor explaining large differences in cortical balance N1 amplitude between subjects (Fig. 4). Subject-averaged cortical responses were combined across directions because cortical balance N1 [Fig. 4, *A* and *B*; 56 μV (SD 23), 153 ms (SD 9)] did not differ between forward [57 μV (SD 24), 153 ms (SD 10)] and backward [56 μV (SD 23), 153 ms (SD 11)] directions in peak amplitude ($P = 0.6$, paired *t*-test) or latency ($P = 0.8$). N1 amplitudes were inversely correlated with subject height, with larger amplitudes in shorter subjects (Fig. 4*C*; $P = 0.002$). The difference in N1 amplitude between subjects could not be explained by differences in weight (Fig. 4*D*; $P = 0.3$) or differences in actual (recorded) perturbation acceleration ($P = 0.4$). N1 amplitude was not significantly correlated with age (Fig. 4*E*; $P = 0.5$) or number of steps taken (Fig. 4*F*; $P = 0.04$) at significance level $\alpha = 0.0125$. N1 latency did not show significant correlation with any of these measures (all $P > 0.0125$).

Cortical Response Amplitudes Increased with Perturbation Acceleration in Condition Averages and Single Trials

Cortical responses increased in amplitude with perturbation acceleration in condition averages (Fig. 5) and on single trials (Fig. 6*A*) and did not differ between perturbation directions (Fig. 5). ANOVA revealed a significant effect of acceleration [$F(3,105) = 19.7$, $P < 0.0001$] but not direction ($P = 0.7$) on the cortical balance N1 peak amplitude in condition averages. Acceleration × direction interaction effects were not significant ($P = 0.5$). A post hoc Tukey test revealed a significant increase in cortical balance N1 peak amplitude in all comparisons with increasing acceleration (Fig. 5*D*; $P < 0.05$), except for the comparison between acceleration *levels 1* and *2*. Single-trial *z*-scored N1 amplitudes were positively correlated with peak perturbation acceleration recorded on single trials (Fig. 6*A*; $P < 0.0001$). Combining data across directions within individuals, 12 of 16 individuals showed significant positive correlations between single-trial N1 amplitudes and peak acceleration ($P < 0.05$), with $R^2 = 0.14$ (SD 0.06) across directions [forward: $R^2 = 0.19$ (SD 0.13); backward: $R^2 = 0.20$ (SD 0.09)]. The slopes of significant acceleration scaling relationships were positively correlated with subject-averaged N1 amplitudes ($n = 12$, $P = 0.04$, $R^2 = 0.30$), such that stronger acceleration scaling relationships were observed in subjects with larger N1 amplitudes. A similar correlation is obtained

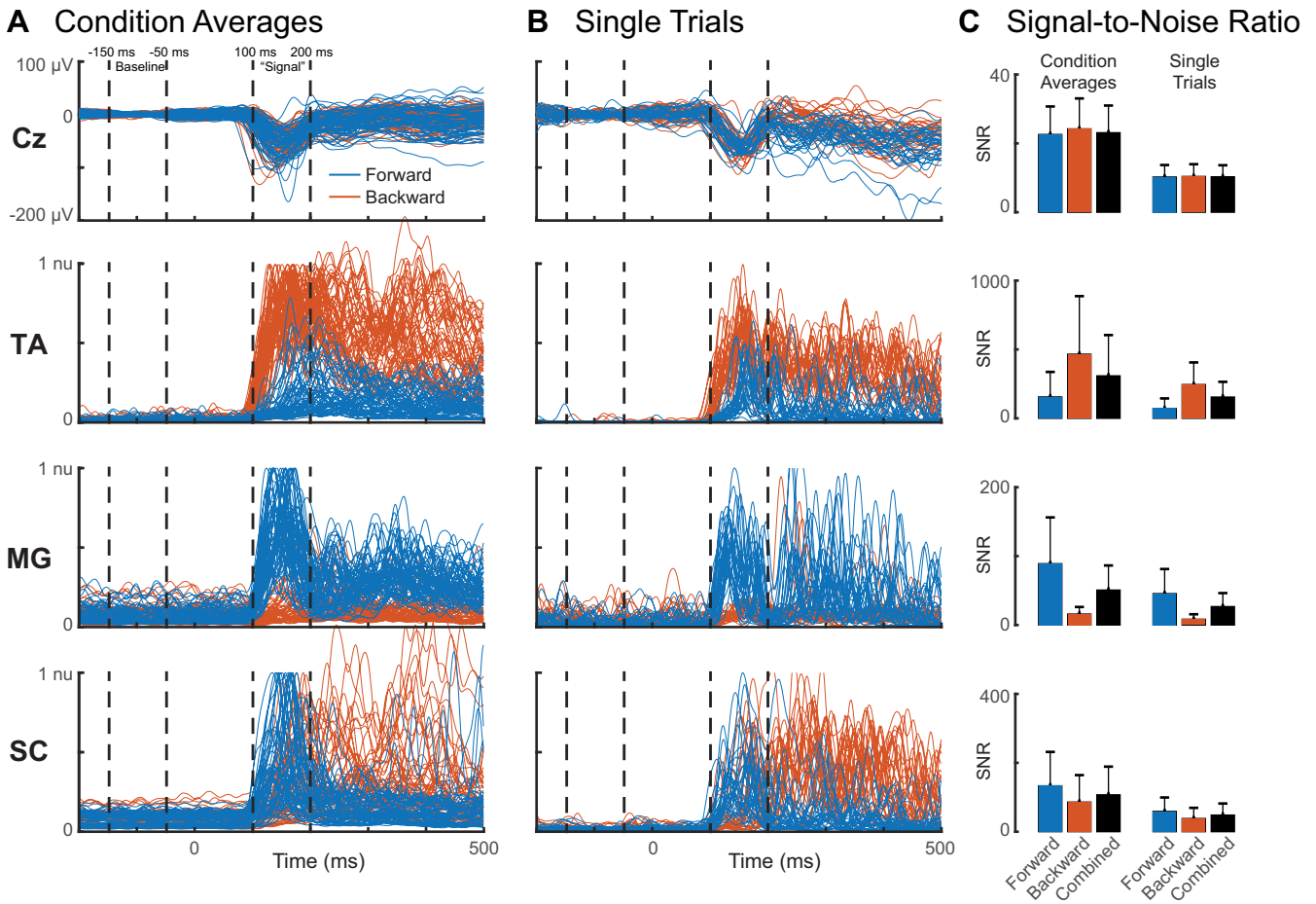

Fig. 3. Signal-to-noise ratio (SNR) of condition-averaged and single-trial data. *A*: condition-averaged responses for all conditions (4 accelerations × 2 directions) and all subjects (n = 16, 9 women, 7 men). Responses to forward perturbations are in blue, and responses to backward perturbations are in red. *B*: single-trial responses in a representative subject (female) across all (64) trials. *C*: SNR for condition averages (*left*) and single trials (*right*) for forward perturbations, backward perturbations, and both directions combined (n = 16, 9 women, 7 men). MG, medial gastrocnemius; SC, sternocleidomastoid; TA, tibialis anterior.

when including the slopes from nonsignificant acceleration scaling relationships (n = 16, P = 0.002, R^2 = 0.48).

Cortical Response Latencies Were Shorter for Larger Perturbations

Cortical response latency on condition averages decreased with increasing perturbation acceleration and did not differ between perturbation directions. ANOVA revealed a significant effect of acceleration [$F(3,105) = 34.4$, $P < 0.0001$] but not direction ($P = 0.8$) on cortical balance N1 peak latency in condition averages. Acceleration × direction interaction effects were not significant ($P = 0.3$). A post hoc Tukey test revealed a significant decrease in peak latency with each comparison of increasing acceleration ($P < 0.05$) except for the comparison between acceleration *levels 2* and *3*. We also note that the perturbations in the different acceleration conditions also varied in acceleration peak latency [Fig. 1; $F(3,105) = 636.7$, $P < 0.0001$], with significant differences in acceleration peak latency between all comparisons of acceleration levels ($P < 0.05$). The highest acceleration (*level 4*) had the shortest peak latency, followed by acceleration *level 3*, then *level 1*, with the longest latency at acceleration *level 2*. Accordingly, cortical balance N1 peak latency was positively correlated with the latency of peak perturbation acceleration in condition-averaged data ($P < 0.0001$, $R^2 = 0.19$), with greater variation in acceleration peak latency than cortical balance N1 peak latency.

Cortical Response Amplitudes Decreased Across Trials

Single-trial cortical responses decreased in amplitude across trial blocks in both directions (Fig. 6*B* and Fig. 7). ANOVA revealed a significant effect of trial block [$F(7,105) = 14.1$, $P < 0.0001$] on the cortical balance N1 peak amplitude on single trials. Post hoc Tukey tests revealed that the cortical balance N1 was significantly larger in the first trial block compared with *blocks 3–8* ($P < 0.05$). Other significant comparisons are indicated in Fig. 7*D*. Effects of acceleration were consistent with those reported for condition averages above. Acceleration × block interaction effects were not significant ($P = 0.6$). Single-trial z-scored N1 amplitudes were inversely correlated with trial number (Fig. 6*B*; $P < 0.0001$). Combining data across directions within individuals, 10 of 16 individuals showed significant negative correlations between single-trial N1 amplitudes and trial number ($P < 0.05$), with $R^2 = 0.18$ (SD 0.10) across directions [forward: $R^2 = 0.19$ (SD 0.07); backward: $R^2 = 0.28$ (SD 0.13)]. The slopes of significant reductions in N1 amplitude across single trials were inversely correlated with subject-averaged N1 amplitudes (n =

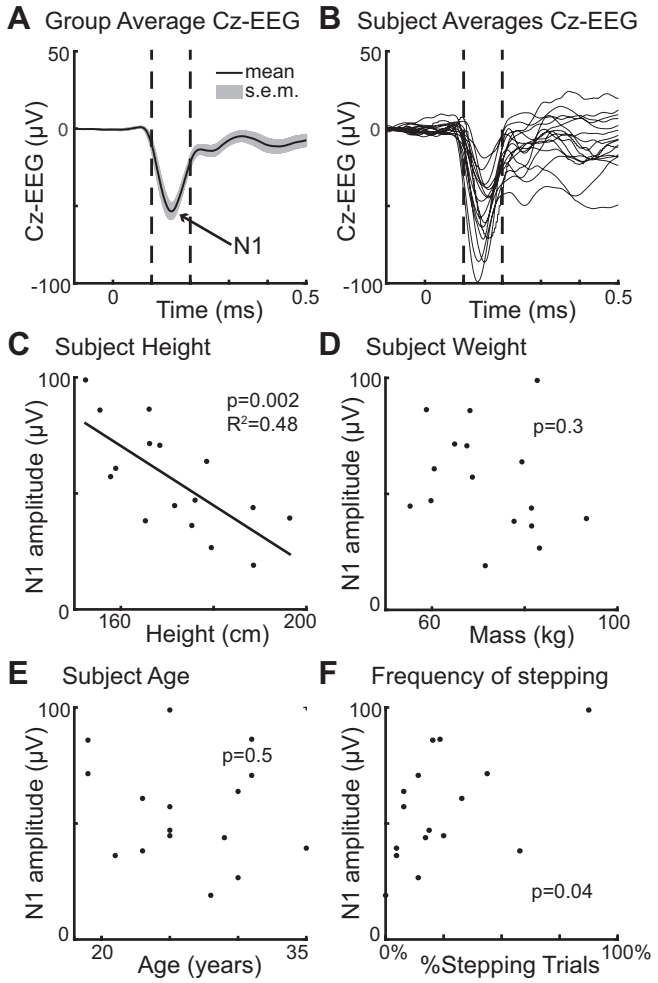

Fig. 4. N1 amplitude across subjects is related to subject height. *A*: in the grand average of Cz-EEG across all subjects, an N1 peak of 54 μV occurred 152 ms after perturbation onset. Standard error of the mean is shown in gray. *B*: in Cz-EEG averages within subjects, cortical balance N1 peaks of 56 μV (SD 23) occurred 153 ms (SD 9) after perturbation onset. *C–F*: N1 amplitude was inversely correlated with subject height (*C*, $P = 0.002$) but did not show significant associations with subject weight (*D*, $P = 0.3$), subject age (*E*, $P = 0.5$), or the proportion of trials in which subjects were unable to resist stepping responses (*F*, $P = 0.04$) at significance level $\alpha = 0.0125$. In all panels, $n = 16$ (9 women, 7 men).

10, $P = 0.03$, $R^2 = 0.64$), such that individuals with larger subject-averaged N1 amplitudes showed greater reductions in single-trial N1 amplitude across trials. A similar correlation is obtained when including the slopes from nonsignificant reduction in N1 amplitude across trials ($n = 16$, $P = 0.03$, $R^2 = 0.25$). The individuals who showed significant correlations between N1 amplitude and trial number were statistically independent of those who showed significant correlations between N1 amplitude and perturbation acceleration (Fisher's exact test of independence, $P = 0.1$).

Muscle Response Amplitudes Increased with Acceleration and Varied by Perturbation Direction in Condition Averages and Single Trials

Muscle responses increased in amplitude with perturbation acceleration in condition averages (Fig. 5) and on single trials (Fig. 6*B*). ANOVA revealed significant effects of acceleration on the initial burst of muscle activity in TA-EMG [$F(3,105) = 17.4$, $P < 0.0001$], MG-EMG [$F(3,105) = 28.4$, $P < 0.0001$], and SC-EMG [$F(3,105) = 58.6$, $P < 0.0001$] in condition averages. Post hoc Tukey tests revealed that TA-EMG and MG-EMG were significantly larger at the highest acceleration compared with all other levels and higher at acceleration *level 3* compared with *level 2* (Fig. 5*D*; $P < 0.05$), and SC-EMG was significantly larger for all comparisons of increasing acceleration ($P < 0.05$) except for the comparison between acceleration *levels 1* and *2*. Single-trial *z*-scored muscle response amplitudes were positively correlated with recorded peak acceleration in both directions for TA-EMG (Fig. 6*A*; $P < 0.0001$), MG-EMG (forward: $P = 0.0002$; backward: $P < 0.0001$; combined: $P < 0.0001$), and SC-EMG ($P < 0.0001$).

Muscle responses also varied by direction. ANOVA revealed significant effects of perturbation direction on TA-EMG [$F(1,105) = 1{,}670.2$, $P < 0.0001$], MG-EMG [$F(1,105) = 1{,}139.8$, $P < 0.0001$], and SC-EMG [$F(1,105) = 20.4$, $P < 0.0001$]. Post hoc Tukey tests revealed that TA-EMG was larger in forward perturbations ($P < 0.05$) whereas MG-EMG and SC-EMG were larger in backward perturbations ($P < 0.05$). An acceleration × direction interaction effect was found for MG-EMG [$F(3,105) = 14.9$, $P < 0.0001$] but not TA-EMG ($P = 0.9$) or SC-EMG ($P = 0.09$). The acceleration × direction interaction for MG-EMG was characterized by stronger acceleration scaling in backward perturbations (Fig. 6*A*).

Acceleration scaling of single-trial muscle response amplitudes was observed within most individuals. Twelve of 16 individuals showed significant positive correlations between single-trial *z*-scored TA-EMG amplitudes and peak acceleration in at least one direction ($P < 0.05$), with $R^2 = 0.24$ (SD 0.12) in forward perturbations and $R^2 = 0.29$ (SD 0.12) in backward perturbations. Thirteen of 16 individuals showed significant positive correlations between single-trial *z*-scored MG-EMG amplitudes and peak acceleration in at least one direction ($P < 0.05$), with $R^2 = 0.28$ (SD 0.15) in forward perturbations and $R^2 = 0.27$ (SD 0.15) in backward perturbations. Combining data across directions within individuals, 15 of 16 individuals showed significant positive correlations between single-trial *z*-scored SC-EMG amplitudes and peak acceleration ($P < 0.05$), with $R^2 = 0.18$ (SD 0.12) across directions [forward: $R^2 = 0.33$ (SD 0.13); backward: $R^2 = 0.27$ (SD 0.11)].

Muscle Response Amplitudes Decreased Across Trials

Single-trial TA-EMG and SC-EMG muscle responses decreased in amplitude across trial blocks in both directions, whereas MG-EMG only decreased across forward perturbations (Fig. 7). ANOVA revealed a significant effect of trial block on TA-EMG [$F(7,105) = 10.2$, $P < 0.0001$] and SC-EMG [$F(7,105) = 22.0$, $P < 0.0001$] but not MG-EMG ($P = 0.1$). Post hoc Tukey tests revealed that both TA-EMG and SC-EMG were larger in the first trial block compared with all other blocks ($P < 0.05$). Other significant post hoc comparisons are indicated in Fig. 7*D*. Effects of acceleration and direction were consistent with those reported for condition averages above. Acceleration × block interaction effects were not significant for TA-EMG ($P = 0.99$), MG-EMG ($P = 0.2$), or SC-EMG ($P = 0.06$). Single-trial *z*-scored muscle response amplitudes were inversely correlated with trial number in both

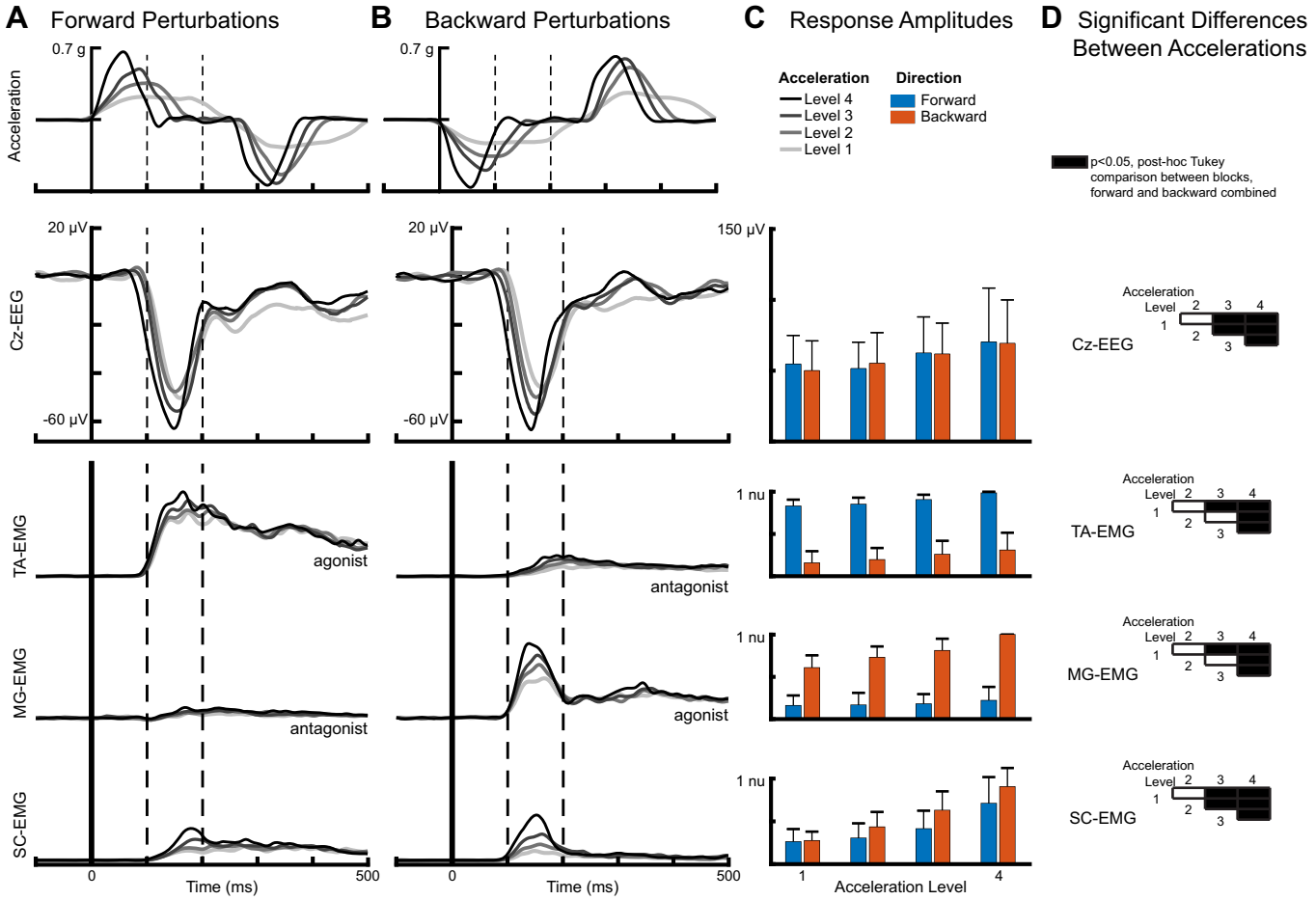

Fig. 5. Grand averages of cortical and muscle responses by acceleration. *A*: averaged responses to forward perturbations plotted by acceleration level. Darker lines indicate larger accelerations. Peak platform acceleration is reached in the first 100 ms, and response amplitudes are quantified in the following 100 ms (indicated by dashed vertical lines). *B*: averaged responses to backward perturbations plotted by acceleration level. *C*: mean and standard deviation of response amplitudes on condition averages by direction and by acceleration level. Responses to forward perturbations are in blue, and responses to backward perturbations are in red. *D*: significant post hoc comparisons (Tukey, $P < 0.05$) between acceleration levels are indicated by black spaces on the comparison grid. In all panels, $n = 16$ (9 women, 7 men). MG, medial gastrocnemius; SC, sternocleidomastoid; TA, tibialis anterior.

directions for TA-EMG (Fig. 6*B*; forward: $P = 0.002$; backward: $P < 0.0001$; combined: $P = 0.001$) and SC-EMG ($P < 0.0001$). MG-EMG amplitudes were inversely correlated with trial number only across perturbations in the backward direction (forward: $P = 0.2$; backward: $P = 0.002$; combined: $P = 0.3$).

Reduction in single-trial muscle response amplitude on a trial-by-trial basis within individuals was most apparent for non-balance-correcting muscle activity. Eleven of 16 individuals showed significant inverse correlations between single-trial z-scored SC-EMG amplitudes and trial number ($P < 0.05$), with $R^2 = 0.11$ (SD 0.09) across directions [forward: $R^2 = 0.17$ (SD 0.07); backward: $R^2 = 0.21$ (SD 0.10)]. Ten of 16 individuals showed reductions of single-trial z-scored antagonist TA-EMG amplitudes with trial number across backward perturbations [$P < 0.05$, $R^2 = 0.26$ (SD 0.09)], whereas only 3 of 16 individuals showed reductions of single-trial z-scored agonist TA-EMG amplitudes with trial number across forward perturbations [$P < 0.05$, $R^2 = 0.18$ (SD 0.06)]. Single-trial z-scored MG-EMG amplitudes reduced with trial number in 4 of 16 individuals in backward perturbations [$P < 0.05$, $R^2 = 0.22$ (SD 0.10)] and 3 of 16 individuals in forward perturbations [$P < 0.05$, $R^2 = 0.25$ (SD 0.19)]. No significant associations were found between individuals who showed correlations between muscle response amplitudes and perturbation acceleration and individuals who showed correlations between muscle response amplitudes and trial number in either direction (Fisher's exact test of independence, all $P > 0.05$).

Associations Between Cortical and Muscle Response Amplitudes Were Very Weak

Single-trial cortical response amplitudes were weakly correlated to simultaneous muscle response amplitudes (Fig. 8*A*) but showed relatively stronger correlations with startle-related muscle responses than with balance-correcting muscle responses. Single-trial z-transformed cortical balance N1 amplitudes were weakly correlated to simultaneous z-transformed EMG activity in both directions in TA-EMG (Fig. 8*A*; forward: $P = 0.0002$; backward: $P < 0.0001$; combined: $P = 0.001$) and SC-EMG ($P < 0.0001$) but were only correlated to simultaneous z-transformed MG-EMG in backward perturbations (forward: $P = 0.2$; backward: $P < 0.0001$; combined: $P < 0.0001$). Combining single-trial data across perturbation directions within individuals, 11 of 16 individuals showed significant correlations between single-trial cortical balance N1

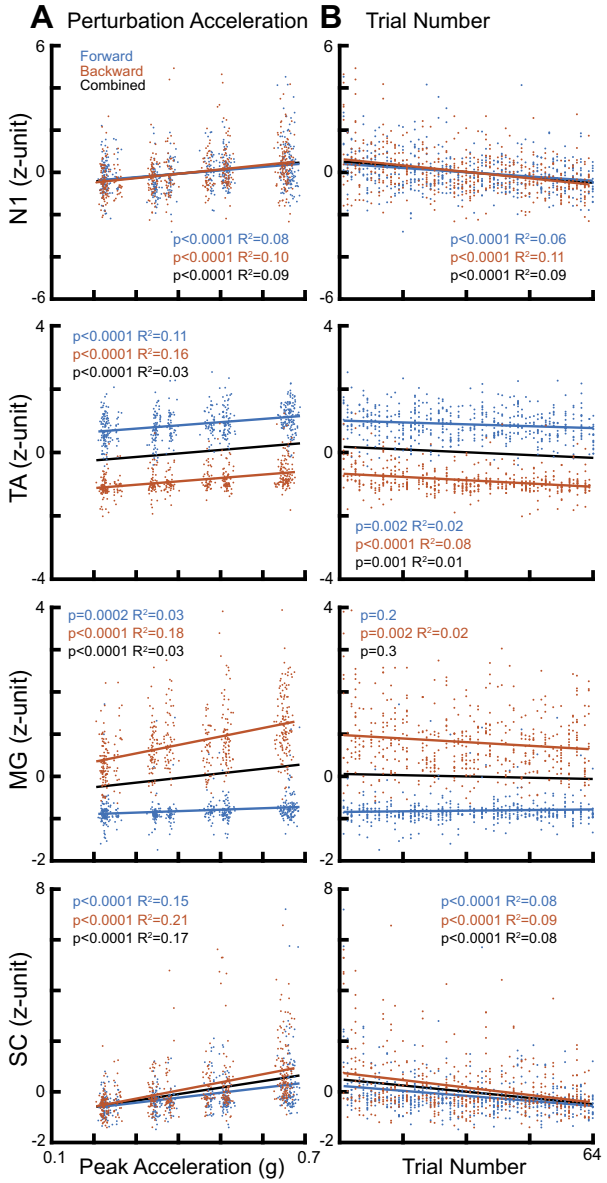

Fig. 6. *A*: *z*-scored single-trial response amplitudes plotted against peak acceleration measured on single trials. Data and regression lines from forward perturbations are in blue, and data and regression lines from backward perturbations are in red. Regression lines including data across both perturbation directions are in black. *P* values and adjusted R^2 values of the regression lines follow the same color coding. *B*: *z*-scored single-trial response amplitudes plotted against trial number. In all panels, $n = 16$ (9 women, 7 men). MG, medial gastrocnemius; SC, sternocleidomastoid; TA, tibialis anterior.

amplitude and simultaneous SC-EMG ($P < 0.05$), with $R^2 = 0.40$ (SD 0.15) [forward: $R^2 = 0.36$ (SD 0.21); backward: $R^2 = 0.50$ (SD 0.20)]. Nine of 16 individuals showed significant correlations between single-trial cortical balance N1 amplitude and simultaneous antagonist TA-EMG in backward perturbations [$P < 0.05$, $R^2 = 0.36$ (SD 0.18)], whereas only 3 of 16 individuals showed significant correlations between single-trial N1 amplitude and simultaneous agonist TA-EMG in forward perturbations [$P < 0.05$, $R^2 = 0.17$ (SD 0.10)]. Only 3 of 16 individuals showed significant correlations between single-trial cortical balance N1 amplitude and simultaneous agonist MG-EMG in backward perturbations [$P < 0.05$, $R^2 = 0.26$ (SD 0.16)], whereas 7 of 16 individuals showed significant correlations between single-trial N1 amplitude and simultaneous antagonist MG-EMG in forward perturbations [$P < 0.05$, $R^2 = 0.14$ (SD 0.04)].

Correlations between single-trial cortical response amplitude and perturbation acceleration were not predictive of correlations between muscle response amplitude and perturbation acceleration. Individuals who showed significant correlations between perturbation acceleration and single-trial cortical balance N1 amplitude were statistically independent of individuals who showed significant correlations between perturbation acceleration and simultaneous single-trial TA-EMG (Fisher's exact test of independence, forward: $P = 0.2$; backward: $P = 0.4$), MG-EMG (forward: $P = 0.3$; backward: $P = 0.1$), and SC-EMG ($P = 0.3$ across directions).

Reduction in single-trial cortical response amplitude with trial number was associated with a reduction in startle-related muscle activity but was not associated with a reduction in balance-correcting muscle activity. There was a significant association between individuals who showed significant correlations between trial number and cortical balance N1 and individuals who showed significant correlations between trial number and SC-EMG (Fisher's exact test of independence, $P = 0.03$ across directions). Specifically, individuals who showed a reduction (or lack of reduction) in cortical response amplitude across trials were more likely to show a similar reduction (or lack of reduction) in SC-EMG amplitude across trials. In contrast, individuals who showed significant correlations between trial number and single-trial cortical balance N1 amplitude were statistically independent of individuals who showed significant correlations between trial number and simultaneous TA-EMG (forward: $P = 0.5$; backward: $P = 0.3$) or MG-EMG (forward: $P = 0.3$; backward: $P = 0.4$).

Single-trial cortical response amplitudes were also weakly correlated to subsequent muscle response amplitudes (Fig. 8*B*). Single-trial *z*-transformed cortical balance N1 amplitudes were weakly correlated to subsequent (200–300 ms) *z*-transformed EMG activity in both directions in TA-EMG (Fig. 8*B*; $P < 0.0001$), MG-EMG (forward: $P = 0.002$; backward: $P = 0.0001$; combined: $P < 0.0001$), and SC-EMG ($P < 0.0001$). Combining single-trial data across perturbation directions within individuals, 7 of 16 individuals showed significant correlations between cortical balance N1 amplitude and subsequent (200–300 ms) SC-EMG ($P < 0.05$), with $R^2 = 0.23$ (SD 0.10) across directions [forward: $R^2 = 0.25$ (SD 0.15); backward: $R^2 = 0.25$ (SD 0.16)]. Nine of 16 individuals showed significant correlations between single-trial cortical balance N1 amplitude and subsequent antagonist TA-EMG in backward perturbations [$P < 0.05$, $R^2 = 0.35$ (SD 0.18)], whereas only 4 of 16 individuals showed significant correlations between N1 amplitude and subsequent agonist TA-EMG in forward perturbations [$P < 0.05$, $R^2 = 0.14$ (SD 0.04)]. Only 4 of 16 individuals showed significant correlations between cortical balance N1 amplitude and subsequent agonist MG-EMG in backward perturbations [$P < 0.05$, $R^2 = 0.15$ (SD 0.04)], and only 2 of 16 individuals showed significant correlations between N1 amplitude and subsequent antagonist MG-EMG in forward perturbations [$P < 0.05$, $R^2 = 0.32$ (SD 0.20)].

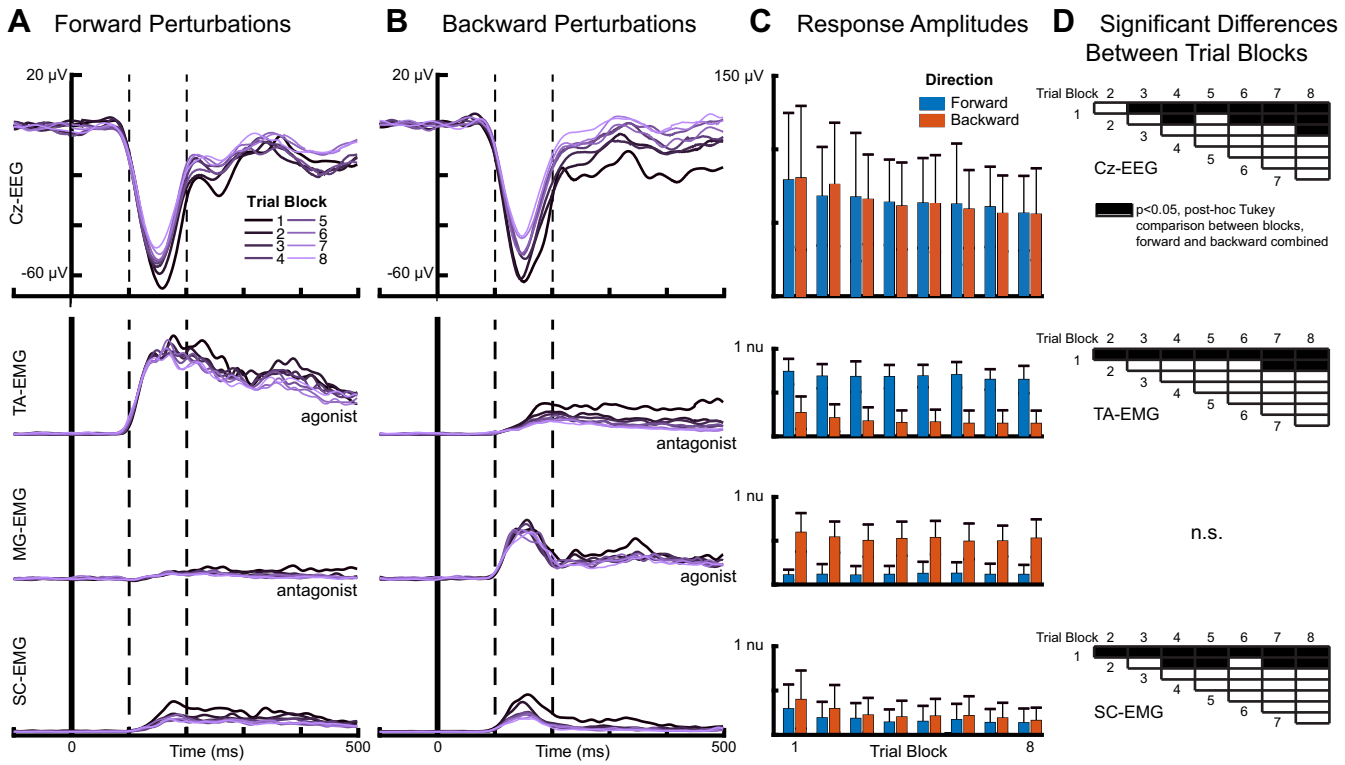

Fig. 7. Grand averages of cortical and muscle responses by trial block. *A*: averaged responses to forward perturbations plotted by trial block. Darker shades of purple indicate earlier trial blocks. *B*: averaged responses to backward perturbations plotted by trial block. *C*: mean and standard deviation of response amplitudes on single trials by direction and by trial block. Responses to forward perturbations are in blue, and responses to backward perturbations are in red. *D*: significant post hoc comparisons (Tukey, $P < 0.05$) between trial blocks are indicated by black spaces on the comparison grid. In all panels, $n = 16$ (9 women, 7 men). MG, medial gastrocnemius; SC, sternocleidomastoid; TA, tibialis anterior.

DISCUSSION

Our results suggest that cortical and muscle responses to balance perturbation are elicited by common sensory inputs but their amplitudes are modulated by independent mechanisms. Although cortical and muscle responses each scaled weakly with perturbation acceleration, consistent with prior studies concluding shared sensory drive (Berger et al. 1987; Dietz et al. 1984b, 1985a, 1985b; Staines et al. 2001), acceleration scaling was not apparent in all individuals. Moreover, scaling of cortical response amplitude to sensory input and attenuation of cortical responses across trials were dissociated from the amplitude of balance-correcting muscle responses on a trial-by-trial basis within subjects. In contrast, attenuation of cortical responses was associated with attenuation of startle responses, consistent with a reduction in perceived threat (Adkin et al. 2008). Moreover, individuals with larger N1 amplitudes had greater attenuation across trials, suggesting that these individuals initially perceived perturbations as more threatening. Dissociations in modulation of cortical and balance-correcting muscle response amplitudes are likely due to differences between spinal and supraspinal sensory gating (Berger et al. 1990; Staines et al. 2000). Accordingly, cortical responses in balance have been shown to be influenced by perceived threat (Adkin et al. 2008; Mochizuki et al. 2010), attention (Little and Woollacott 2015; Quant et al. 2004b), and predictability (Adkin et al. 2006, 2008; Mochizuki et al. 2008, 2010), which may vary between subjects and across trials.

Our averaged data were in agreement with prior studies showing cortical response scaling with perturbation amplitude (Mochizuki et al. 2010) and suggesting shared sensory inputs to cortical and muscle responses (Berger et al. 1987; Dietz et al. 1984b, 1985a, 1985b; Staines et al. 2001). However, we demonstrated a trial-by-trial dissociation between cortical and muscle response scaling to sensory input within subjects. Studies in the 1980s relied on averaging as many as 100 trials (Dietz et al. 1984b, 1985a, 1985b), which may have been necessary because of motor artifacts in perturbed walking as well as the lower signal-to-noise ratio of older EEG technology. Recent studies using postural perturbations average across 15–60 trials per condition (Adkin et al. 2006, 2008; Mochizuki et al. 2008, 2009, 2010; Quant et al. 2004a, 2004b). However, Quintern and colleagues (1985) and Mierau and colleagues (2015) both used single-trial analyses to demonstrate a dissociation between cortical and balance-correcting muscle responses as we do here. The feasibility of single-trial analysis depends in part on advances in EEG technology, including the use of active electrodes, but may also depend on details of the experimental design. In particular, applying perturbations at the feet induces smaller and later motion of the head compared with the rest of the body (Fig. 2). Additionally, we only delivered perturbations during periods of low background EEG activity, which we monitored during the experiment.

Our results reinforce the idea that the cortical balance N1 is not yoked in amplitude to the initial brain stem-mediated corrective muscle activity (Mierau et al. 2015; Quintern et al. 1985) that arises from sensory inputs from perturbation (Lockhart and Ting 2007; Welch and Ting 2008, 2009). This was particularly evident in individuals who exhibited scaling to

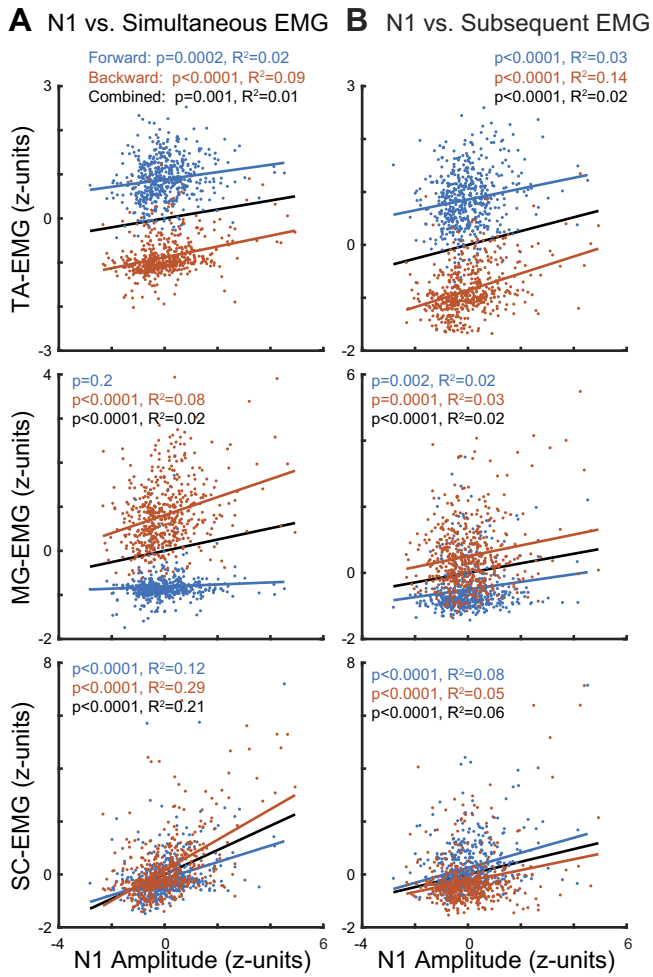

Fig. 8. Correlations between cortical and muscle response amplitudes were weak. Linear regressions between z-transformed cortical balance N1 amplitudes and simultaneous (A; 100–200 ms) and subsequent (B; 200–300 ms) z-transformed muscle response amplitudes. Data and regression lines from forward perturbations are in blue, and data and regression lines from backward perturbations are in red. Regression lines including data across both perturbation directions are in black. P values and adjusted R^2 values of the regression lines follow the same color coding. In all panels, $n = 16$ (9 women, 7 men). MG, medial gastrocnemius; SC, sternocleidomastoid; TA, tibialis anterior.

acceleration in only muscle or cortical responses, but not both, and was supported by the very weak correlations between single-trial muscle and cortical response amplitudes. Nevertheless, the N1 response is time-locked to the perturbation and likely triggered by the sensory input that modulates balance-correcting muscle responses. This is supported by the comparable time delay for both muscle and cortical responses in a patient with slow peripheral conduction velocities (Dietz et al. 1985a). Our perturbation effects on the balance N1 are in line with, but smaller than, those reported by Mochizuki et al. (2010), who used larger differences in perturbation amplitudes but could not dissociate the effects of perturbation acceleration, peak velocity, total displacement, or perturbation duration. Although we did not previously define individuals as either "scalers" or "nonscalers" in terms of their muscle responses due to perturbation acceleration, we did observe a range of sensitivities to perturbation acceleration across individuals (Welch and Ting 2008, 2009). Similarly, a prior study using seated perturbations also showed cortical response scaling with acceleration in two of three subjects (Staines et al. 2001), where the lack of scaling in one subject was suggested to be due to a ceiling effect in response amplitude. However, our study shows greater acceleration scaling of cortical responses in individuals with larger cortical response amplitudes. Therefore, rather than disappearing because of saturation, acceleration scaling may be an additional component of the cortical responses that can be reduced or absent in some individuals. Although we cannot rule out the possibility that a wider range of accelerations might have revealed scaling in more individuals, some subjects could intrinsically be nonresponders, or the lack of scaling in some individuals could be due to more transient factors, such as differences in attention or threat assessment.

Attenuation of cortical responses was associated with attenuation of startle responses rather than attenuation of balance-correcting muscle responses. Attenuation of cortical balance N1 amplitude has previously been dissociated from balance-correcting muscle responses across perturbations that were predictable in direction and amplitude (Mierau et al. 2015; Quintern et al. 1985). Because our perturbation directions and amplitudes were randomized, we did not expect muscle or cortical responses to decrease across trials (Horak and Diener 1994; Horak and Nashner 1986; Welch and Ting 2008, 2009, 2014). However, replicating the dissociation between attenuations of cortical balance N1 and balance-correcting muscle responses suggests that the decrease in cortical response amplitude is not related to changes in sensory activation or adaptation of the corrective muscle responses. Instead, we found that the attenuation of cortical responses was associated with attenuation of SC muscle activity, representative of the startle reflex (Nonnekes et al. 2015), suggesting that attenuation of cortical responses was related to a reduction in perceived threat with experience (Adkin et al. 2008; Mochizuki et al. 2010). Although the SC muscle may also be activated as part of the balance-correcting motor response, both startling acoustic stimuli and postural perturbations activate the SC muscle in coordination with the masseter muscle of the jaw, which does not contribute to postural correction. These neck and jaw muscles habituate on similar timescales, both faster and to a greater extent than habituation of primary agonist muscle activations in the leg (Oude Nijhuis et al. 2010). Thus contributions of balance-correcting activations of the SC muscle are likely smaller than contributions of startle-reflex activity. Although our perturbations were unpredictable in timing, amplitude, and direction, we cannot exclude the possibility that other features of the perturbation became more predictable with experience, which could have further influenced cortical response amplitudes across trials (Adkin et al. 2006).

Our data suggest that differences in cortical response amplitudes between subjects were related to differences in subject height and perceived threat, but there are likely other factors that we did not measure. The effect of subject height reflects our failure to match perturbation magnitudes to body size, but height only explains half of the variation among subjects. Specifically, taller subjects experienced proportionally smaller perturbations relative to their body size, consistent with their smaller cortical responses. Perturbations were relatively more difficult for shorter subjects, who had higher rates of stepping responses. The effect of height could not be attributed to differences in subject weight or perturbation acceleration, as

the same variability in peak perturbation acceleration was observed in all individuals and in both perturbation directions. Although age has been previously shown to influence the cortical balance N1 amplitude (Duckrow et al. 1999), we did not find a relationship between N1 amplitude and age, likely because of our relatively narrow age distribution [26 yr (SD 5), range 19–35 yr]. Individuals showing greater attenuation of cortical responses across trials also displayed larger cortical response amplitudes overall, suggesting that they initially perceived greater threat. Although larger cortical responses were also associated with higher rates of stepping and greater increases in amplitude with perturbation acceleration, the lack of a relationship between increasing cortical responses with acceleration and decreasing cortical responses across trials, and the lack of a relationship between either of these effects and the rate of stepping, suggests that these were independent factors contributing to differences in cortical response amplitudes between subjects. Additionally, some subjects may intrinsically produce larger cortical responses to perturbation. Indeed, evoked cortical responses in cognitive tasks have a strong genetic component. One example is the influence of dopamine-related genetic polymorphisms on the cortical error-related and feedback-related negativities in cognitive assessments (Ullsperger 2010). Although we are not aware of any genetic variants of the cortical balance N1, it is possible that such influences exist.

It is also possible that differences in cortical response amplitudes and modulation between subjects and across trials reflect more transient factors, such as attention. Although we did not directly measure attention in the present study, subjects anecdotally reported being more nervous and alert at the beginning of the experiment, becoming more comfortable and less attentive as they realized they were not likely to fall, which is consistent with previously reported reductions in electrodermal responses to repeated perturbations (Sibley et al. 2008). As such, previously reported decreases in the cortical balance N1 amplitude with reduced attention (Little and Woollacott 2015; Quant et al. 2004b) may contribute to the reduction in cortical response amplitudes observed across trials in the present study and could explain why individuals who scaled cortical responses with perturbation acceleration showed larger-amplitude cortical responses. Additionally, it is possible that the specific focus of attention could have influenced whether an individual's cortical responses tracked perturbation acceleration. Indeed, asking subjects to pay attention to either perturbation velocity or perturbation magnitude in order to rank successive perturbations with respect to either feature influenced afferent activity in a manner that enhanced discrimination of the task-relevant feature at the expense of task-irrelevant information (Ribot-Ciscar et al. 2009). Furthermore, focusing attention toward cutaneous or proprioceptive stimuli based on task goals has been demonstrated to selectively facilitate SEPs to task-relevant information while suppressing task-irrelevant information (Staines et al. 2000) via attentional mechanisms in a brain network involving prefrontal cortex (Staines et al. 2002). Although SEPs are distinct from the cortical balance N1, occurring over somatosensory cortex in response to stimulation of somatosensory nerves, this example demonstrates a more complex influence of attention on ascending sensory information to the cortex, which could possibly extend to the cortical balance N1.

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DISCLOSURES

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

AUTHOR CONTRIBUTIONS

A.M.P. and L.H.T. conceived and designed research; A.M.P. performed experiments; A.M.P. analyzed data; A.M.P., G.H., and L.H.T. interpreted results of experiments; A.M.P. prepared figures; A.M.P. drafted manuscript; A.M.P., G.H., and L.H.T. edited and revised manuscript; A.M.P., G.H., and L.H.T. approved final version of manuscript.

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