First trial and StartReact effects induced by balance perturbations to upright stance

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First trial and StartReact effects induced by balance perturbations

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Abstract

Postural responses (PR) to a balance perturbation differ between the first and subsequent perturbations. One explanation for this first trial effect (FTE) is that perturbations act as startling stimuli, that initiate a generalized startle response (GSR) as well as the PR. Startling stimuli, such as startling acoustic stimuli (SAS), are known to elicit GSRs, as well as a StartReact effect, in which prepared movements are initiated earlier by a startling stimulus. In this study, a StartReact effect paradigm was used to determine if balance perturbations can also act as startle stimuli. Subjects completed two blocks of simple reaction time trials involving wrist extension to a visual imperative stimulus (IS). Each block included 15 CONTROL trials, that involved a warning cue and subsequent IS, followed by 10 repeated TEST trials, where either a SAS (TEST\textsubscript{SAS}) or a toes-up support-surface rotation (TEST\textsubscript{PERT}) was presented coincident with the IS. StartReact effects were observed during the first trial in both TEST\textsubscript{SAS} and TEST\textsubscript{PERT} conditions as evidenced by significantly earlier wrist movement and muscle onsets compared to CONTROL. Likewise, StartReact effects were observed in all repeated TEST\textsubscript{SAS} and TEST\textsubscript{PERT} trials. In contrast, GSR responses in SCM, and PRs were large in the first trial, but significantly attenuated over repeated presentation of the TEST\textsubscript{PERT} trials. Results suggest that balance perturbations can act as startling stimuli. Thus, FTEs are likely PRs which are super-imposed with a GSR, that is initially large, but habituates over time with repeated exposure to the startling influence of the balance perturbation.
Keywords:

1. First trial effect
2. Postural responses
3. Startle
4. StartReact effect
5. Reaction time

Glossary:

CATCH: catch trial
CONTROL: control trial
ECR: extensor carpi radialis
EMG: electromyography
EO: external oblique
FCR: flexor carpi radialis
FTE: first trial effect
IS: imperative stimulus
PRs: postural responses
RF: rectus femoris
SAS: starling acoustic stimuli
SCM: sternocleidomastoid
SOL: soleus
TA: tibialis anterior
WARN: warning cue
In a repeated sequence of trials involving discrete balance perturbations, amplitudes of postural responses (PRs) evoked by the first trial are significantly larger compared to subsequent trials involving the same postural stimulus (Keshner et al. 1987; Hansen et al. 1988; Bloem et al. 1998a; Chong et al. 1999; Oude Nijhuis et al. 2009, 2010). This observation has come to be known as the first trial effect (FTE) (Allum et al. 2011). Although it is common to observe FTEs in all types of balance perturbations to both sitting and standing postures (Blouin et al. 2006, 2007; Siegmund et al. 2008; Oude Nijhuis et al. 2009, 2010; Allum et al. 2011; Tang et al. 2012), the underlying mechanisms that contribute to the FTEs remain largely unknown.

One possible explanation for FTEs is that the first exposure to an unpredictable balance perturbation elicits a generalized startle response (GSR) that is superimposed on the basic postural response (Blouin et al. 2006; Siegmund et al. 2008; Nanhoe-Mahabier et al. 2012). GSRs are stereotyped patterns of bilateral flexion reactions that can be elicited by intense stimulation to visual, auditory or somatosensory systems (Bradley et al. 1990; Yeomans and Frankland 1995; Scott et al. 1999; Carlsen et al. 2011). Arguments in support of a GSR contribution to FTEs draw on similarities observed between GSRs and FTEs. For example, both GSRs and PRs are known to habituate rapidly after the first exposure to a series of repeated stimuli (Oude Nijhuis et al. 2010). Secondly, excitation of muscles normally associated with GSRs [i.e. masseter and sternocleidomastoid (SCM)] is frequently observed during the first response to a balance perturbation, but to a much lesser degree in subsequent perturbations (Oude Nijhuis et al. 2010). Finally, coherence between muscle responses in frequency bandwidths typically associated with GSRs has been observed in the first response to a seated perturbation, but not during subsequent balance responses (Blouin et al. 2006).
It is notable that most of the evidence in support of a GSR contribution to FTEs is indirect, and involves a number of significant limitations and assumptions. For example, SCM muscle activity onsets evoked by known startling stimuli [i.e. startling acoustic stimuli (SAS)] differ significantly from those induced by balance perturbations (Oude Nijhuis et al. 2010). Thus, it is plausible that perturbation-induced SCM activity may represent a response that is characteristically distinct from GSRs. Secondly, determining whether GSRs are evoked by balance perturbations is complicated by the fact that they develop in similar muscles and within similar timeframes to startle stimuli as PRs induced by balance perturbations. For example, GSRs evoked by known startling stimuli are oftentimes observed in muscles, such as tibialis anterior and soleus, within 100ms of startle stimulus onset (Brown et al. 1991), which is similar to PRs evoked in these muscles to support-surface balance perturbations (Nashner and Cordo, 1981). Consequently, GSRs induced by balance perturbations may superimpose onto PRs in certain muscles and potentially limit the ability to analyze, or to assess the presence of, one response independent of the other. Thirdly, direct support of GSR contributions to FTEs cannot be garnered by previous reports of muscle activity coherence as this statistical technique relies upon correlational measures and thus may not be used to imply causation (Siegmund et al. 2008). Finally, although it has been assumed that balance perturbations can stimulate sensory systems with sufficient intensity to elicit a GSR (Bisdorff et al. 1994; Commissaris et al. 2002), it has yet to be confirmed empirically whether balance perturbations can, in fact, act as a startling stimulus.

One approach to investigate whether a stimulus is capable of inducing GSRs is to determine if it can also induce the StartReact effect. The StartReact effect involves the involuntary release of a prepared motor response that occurs when a startle stimulus is paired with a “go” stimulus (i.e. an imperative stimulus (IS)) to initiate movement (see Carlsen et al.
Theoretically, any stimulus that can induce a GSR can also induce the StartReact effect; however, for its ease of implementation, SAS of ~120dB have been considered the standard for inducing both (Carlsen et al. 2011). SAS have been used to induce the StartReact effect in various motor behaviours that range from simple wrist extension (Valls-Solé et al. 1999) to anticipatory postural adjustments that precede step-initiation (MacKinnon et al. 2007) and conditioned PRs (Campbell et al. 2012). StartReact effects are focal, task-specific and thus are highly distinguishable from bilaterally symmetric, non-specific flexor muscle activity characterizing the GSR (Landis et al. 1939). StartReact effects also persist even in the presence of attenuated GSRs induced by pre-pulse inhibition (Valls-Solé et al. 2005), thus suggesting that they could be a relatively more robust indicator of the presence of startle stimuli than GSRs themselves.

Therefore, the main purpose of this experiment was to utilize a StartReact effect paradigm as a probe to determine if balance perturbations can act as startle stimuli. Assuming that balance perturbations can act as a startling stimuli, we hypothesized that balance perturbations would induce StartReact effects in a similar manner as SAS, when they are paired with a voluntary wrist extension reaction time task. Assuming that FTEs represent GSRs that habituate over the course of repeated trials, we hypothesized that evoked responses in SCM (a marker for GSRs) and postural muscles would be greatest in amplitude in the first trial and would decrease in subsequent trials.

Methodology

Twelve (5 males; 1 left-handed) healthy individuals (mean ±1SD age: 24.8 ±3.4 years, height: 1.71 ±0.07 m, weight: 65.90 ±9.40 kg) volunteered to participate in the study. All subjects
were entirely naïve of the experimental protocol prior to entering the laboratory and each of them were briefed on all experimental techniques and data collection procedures prior to providing their informed consent to participate. The research ethics board at the University of British Columbia approved all experimental procedures.

Experimental setup

Electromyography (EMG)

EMG data were sampled unilaterally from right tibialis anterior (TA), soleus (SOL), rectus femoris (RF), external oblique (EO) and from unilateral extensor carpi radialis (ECR) on the dominant hand, which was determined by self-report. SCM activity was recorded bilaterally. After cleaning and abrading the skin with alcohol swabs, 2 pre-gelled Ag/AgCl surface electrodes were placed ~2cm apart on each muscle belly. EMG records were pre-amplified 500x before being sampled at 3kHz (Telemyo 2400R, Noraxon, USA), band-pass filtered between 10 and 1500Hz and A/D converted at 1kHz (Power 1401, Cambridge Electronic Design, UK). DC offsets were removed from raw EMG data, which were then digitally high-pass filtered at 30Hz (Spike2, Cambridge Electronic Design, UK) to remove heart rate artifacts and then full-wave rectified.

Kinematics

Clusters of 3 non-collinear infra-red light emitting diodes (iREDS) were placed on each foot, shank, and thigh, as well as on the trunk of each subject. Individual iREDS were also affixed to the olecranon processes and heads of the ulnar bones. A digitizing probe was used to locate virtual markers on the lateral malleoli, fibular heads, greater trochanters, anterior superior
iliac spines and acromion processes (Visual3D, C-Motion, USA). Individual iRED and virtual marker coordinates were referenced to the global coordinate system where the X-axis was positive leftwards, Y-axis was positive forward and the Z-axis positive downwards. From these data, a 3 segment 2-dimensional kinematic model was developed to monitor body movements induced by balance perturbations in the sagittal-plane. The shank segment was defined between the lateral malleoli and fibular head markers; the trunk segment was defined between the greater trochanter and acromion markers; the upper-arm segment was defined between the olecranon and acromion markers. Virtual and iRED marker coordinates were sampled at 100Hz (Optotrak Certus, Northern Digital Incorporated, CAN) then low-pass Butterworth filtered offline at 5Hz (Visual3D, C-Motion, USA). Absolute 2-dimensional angular displacements of the shank, trunk and upper-arm were subsequently calculated offline in the sagittal-plane (Matlab 7.1, Mathworks, USA).

A single axis optical goniometer (S700 Joint Angle Shape Sensor™, Measurand Incorporated, CAN) with 1000Hz and 3.6° temporal and displacement resolution, respectively, was placed across the medial surface of the wrist of the dominant hand. The distal ends of the goniometer were firmly fixed to the medial surface of the palm and forearm, respectively. This orientation allowed for free, unimpeded flexion and extension of the wrist joint, while also aligning the goniometer’s point of greatest sensitivity near to the joint’s principle axis of rotation. Analog data from the goniometer were sampled at 1000Hz and digitally lowpass filtered offline at 5Hz (Spike2, Cambridge Electronic Design, UK) and converted to degrees.

Experimental procedures

Quiet stance
With a stance width equal to 100% of their measured foot length, subjects first stood quietly on a stage mounted to a rotating platform for approximately 60s with their arms relaxed at their sides while focusing on an eye-level target located ~2m away. During this time, a mean ±2SD of resting wrist position was calculated and used as a threshold for initiating subsequent reaction time trials. After 60s, 2 stimuli were presented spaced ~15s apart (with order counter balanced across participants). One stimulus was a red LED (200ms duration) located at the centre of the visual target, that would later function as an IS to initiate the reaction time task (see below). The other stimulus was an auditory cue (<80dB, two 50ms pulses separated by 50ms) that would later be used to warn subjects of an upcoming trial (WARN). The presentation of these stimuli during quiet stance served to verify that IS and WARN cues were non-startling.

Reaction time protocol

Subjects had 5 practice trials with the reaction time task. During each reaction time trial, subjects were first presented with the auditory warning cue (WARN) followed by the visual IS after a random 1.5-3.5s interval. After detecting the IS, subjects were instructed to fully extend the wrist as quickly as possible and then hold the extended position for ~0.5s before returning back to resting position. Wrist position was monitored in real-time and subjects were coached back to resting positions, if necessary, to within resting thresholds calculated during the quiet standing trial. At the beginning of practice trials, subjects were told to ‘react as quickly as possible’, which was reiterated at pre-defined 5-trial intervals throughout the experimental session.

After completing the practice trials, subjects performed 2 experimental blocks (SAS and PERT) that were each counterbalanced across participants. In each block, subjects first
performed 15 reaction time trials (CONTROL) (Figure 1). After CONTROL trials, subjects then
performed a series of 10 TEST trials. In the SAS block, TEST trials involved the WARN cue,
followed 1.5-3.5s by the IS presented simultaneously with a SAS (i.e. TEST\textsubscript{SAS}) (Figure 1). For
the PERT block, TEST trials (i.e. TEST\textsubscript{PERT}) involved the WARN cue followed 1.5-3.5s by the
IS presented simultaneously with a toes-up support-surface rotation ($12^\circ$, $120^\circ$/s, 100ms
duration) (Figure 1). Subjects were entirely unaware of when TEST trials were to begin and how
many would be in each block. Subjects were also informed that there would be instances
interspersed throughout each block where the WARN cue would not be followed by the IS
(CATCH trials) (Figure 1) and thus they should not react. During each block, a CATCH trial
was pseudo-randomly presented for every 5 CONTROL and TEST trials. Thus, 3 CATCH trials
were presented during the sequence of 15 CONTROL trials and a further 2 CATCH trials were
presented during the sequence of 10 TEST trials. Each CONTROL and TEST trial was
separated by a random inter-trial interval lasting 10-20s. Independent of performance, subjects
were reminded to react as quickly as possible to the IS at regular 5-trial intervals.

At the end of each experimental block, subjects were guided off of the platform and were
given a 5-minute rest period while seated. After which time, they stepped onto the platform to
receive a sequence of 5 Perturbation-Only trials, spaced 10-15s apart, that involved only the
support-surface rotation, which was not accompanied by the IS. During Perturbation-Only trials,
subjects were told that the IS would never be illuminated and thus they were no longer to
complete the reaction time task.

Dependent measures

Wrist kinematics
In all CONTROL and TEST trials, reaction times for wrist extension were calculated as the latencies between onsets of the IS and wrist extension. Mean and 2SD measures of resting wrist positions were determined from the goniometer for 500ms prior to the onset of the IS within each trial. Wrist extension onsets were determined in each reaction time trial as the time when goniometer displacements exceeded mean +2SD of resting amplitudes and remained supra-threshold for a minimum of 200ms. From onset, peak wrist displacements were determined as the displacement value achieved at full extension when movement had ceased (i.e. achieved zero velocity).

Wrist EMG

Onsets of EMG responses for ECR were calculated during each CONTROL and TEST trial. Thresholds were calculated as the mean +2SD of 500ms of background EMG levels prior to the start of each trial. Onsets were determined as the first time after IS onset that processed EMG signals surpassed and remained supra-threshold for at least 30ms while at no time dropping below threshold for >3ms (Carpenter et al., 2008).

SCM EMG

Onsets of SCM muscle activity were determined using the same algorithm applied to ECR (see above) and were similarly referenced to IS onset in each trial. Amplitudes were determined by subtracting 100ms integrals of pre-onset EMG signals from 100ms of post-onset EMG signals. This duration of analyses of response amplitudes was used because it is a period where sensory feedback has limited influence over triggered reactions (Wadman et al. 1979).
Postural responses

Both EMG and kinematic data quantified PRs evoked by support-surface rotations. For muscle activity related to PRs, absolute onsets and amplitudes were determined using the same algorithms applied to other records of EMG activity (see above). For the kinematic dataset, onsets of segment displacements were determined as the latency between perturbation onset and the time they surpassed a mean ±2SD threshold of resting positions calculated 500ms immediately before the onset of the IS and remained beyond threshold for 200ms. Peak displacements were also calculated as the greatest change achieved within 800ms of perturbation onset.

Statistical analyses

Reaction time

Wrist kinematic data for one subject had to be removed due to a technical issue. All dependent measures for wrist kinematics as well as for EMG of ECR for each block (SAS/PERT) were averaged across CONTROL trials and compared to the 1st and 10th TEST trials of each block using a 2 X 3 [Block (SAS, PERT) X Trial (CONTROL, 1st TEST, 10th TEST)] repeated measures ANOVA. Because SCM activity was neither expected nor observed during CONTROL trials, measures of its EMG activity were compared using a 2 X 2 [Block (SAS, PERT) X Trial (1st TEST, 10th TEST)] repeated measures ANOVA.

To examine FTEs on PRs, pre-planned t-tests were conducted to compare EMG and kinematic responses observed between the 1st and 10th TESTPERT trials. P-values were set at 0.05 for all statistical tests of main effects and interactions. For post hoc analyses, actual p-values were corrected for multiple comparisons using the Bonferonni method.
Results

Wrist EMG and kinematics

As shown in Figure 2, the presentation of the IS during the reaction time task was followed by ECR activity that was activated and sustained above background to initiate and maintain a maximum wrist extension. During CONTROL trials in SAS and PERT blocks, the IS was followed by initial activation of the ECR (mean onset 193ms and 196ms, respectively) and then by the onset of wrist extension (mean onset 238ms and 245ms, respectively) (Table 1). In response to TEST trials, when the IS was paired with a SAS (i.e. TESTSAS), earlier onsets of ECR and wrist extension were observed (Figure 2 & Table 1). Likewise, earlier onsets of ECR and wrist extension were observed when the IS was coupled with a balance perturbation (i.e. TESTPERT) (Figure 2 & Table 1). The effect of both the SAS and balance perturbations to elicit earlier onsets of ECR and wrist extension was similar during the 1st and 10th TEST trials. These observations were confirmed statistically where significant main effects of Trial were observed for absolute onsets of wrist extension ($F_{(2,20)}=35.65, p<0.001$) and ECR muscle activity ($F_{(2,22)}=97.15, p<0.001$) (Table 1). Post-hoc analyses confirmed that significantly earlier onsets were observed in both 1st TEST and 10th TEST trials compared to CONTROL trials ($p<0.05$) and also that no differences were observed between 1st and 10th TEST trials ($p>0.05$). No main effects of Block or Trial*Block interactions were significant for absolute onset latency measures of wrist extension or ECR ($p>0.05$) (see Table 1).

Neither main effects of Block and Trial, nor Block*Trial interactions were significant for peak wrist displacements ($p>0.05$) (Table 1), suggesting that the task of producing maximal wrist extension was consistently reproduced throughout the experiment.
The within-subject pattern of SCM responses over repeated TEST trials varied across subjects. However, there was a predominant trend across subjects of an increased prevalence of SCM responses in the 1st TEST trials compared to the 10th TEST trial (Figure 3A). In the 1st TEST trials of both SAS and PERT blocks, SCM responses were detected in 11 of 12 and 12 of 12 subjects, respectively (Figure 3A). However, by the 10th TEST trials, SCM responses were detected in only 5 of 12 subjects in SAS blocks and 8 of 12 subjects in PERT blocks (Figure 3A).

The decrease in the prevalence of SCM responses was accompanied by a significant decrease in the amplitude of SCM responses after the 1st TEST trial (Figure 3B/C). For subjects whose SCM activity exceeded the detection threshold in both the 1st and 10th trials of SAS and PERT blocks, the amplitudes were significantly reduced in the 10th compared to 1st trial (main effect of Trial: $F_{(1,4)}=53.44, p=0.002$), whereas, onset latencies were unaffected by Trial ($p>0.05$). Significant main effects of Block were observed for onset latencies of SCM ($F_{(1,4)}=24.26, p=0.008$) where earlier responses were observed in response to SAS compared to PERT blocks (Figure 3B/C & Table 1). There was no significant main effect of Block for SCM amplitude, nor Trial*Block interactions for either the amplitudes or onsets of SCM muscle activity ($p>0.05$).

Support-surface rotations presented during TESTPERT trials evoked characteristic EMG activity in various postural muscles (Figure 4A). Following the onset of balance perturbation, stretch reflexes observed in SOL occurred earlier than PRs in TA, RF and EO (Figure 4A).

Repeated experience with balance perturbations during TESTPERT trials did not significantly influence the onset latencies of any of the postural muscle activity examined ($p>0.05$) (Figure 4B). However, EMG amplitudes significantly decreased from the 1st to the 10th TESTPERT trial
in all muscles (SOL: $t(11)=3.41$, $p=0.006$; RF: $t(11)=2.51$, $p=0.029$; EO: $t(11)=2.38$, $p=0.037$) except TA ($p>0.05$) (Figure 4B). Toes-up support-surface tilts caused substantial angular displacements of the upper arm, trunk and shank segments (Figure 5). Onsets of shank, trunk or upper-arm angular displacements were not significantly influenced by Trial ($p>0.05$) (Figure 5). However, peak angular displacements of the trunk ($t(11)=3.65$, $p=0.004$) and upper-arm ($t(11)=4.24$, $p=0.002$) segments were significantly attenuated in the 10th TESTPERT trial compared to the 1st TESTPERT trial. Peak shank angular displacements were not significantly affected by Trial ($p>0.05$) (Figure 5).

Discussion

The main purpose of this experiment was to utilize a StartReact paradigm as a probe to determine if balance perturbations, like SAS, constitute startle stimuli. We hypothesized 1) that balance perturbations would induce StartReact effects in all reaction time trials in a similar manner as SAS, and 2) that evoked responses in SCM (a marker for GSR) and postural muscles would be greatest in amplitude in the first trial and would decrease in subsequent trials.

*Balance perturbations as startling stimuli*

Balance perturbations produce whole body displacements that induce signals in the vestibular, visual, and somatosensory systems (Peterka, 2002; Horak, 2006). Each of these sensory systems is capable of triggering a GSR independently. For example, GSRs have been elicited by stimulation of the vestibular system via unexpected vertical drops of the body (Gruner, 1989; Bisdorff et al. 1995), high intensity visual stimuli (Bradley et al. 1990), as well as tactile (Gokin and Karpukhina, 1985) and sural nerve stimulation (Delwaide and Crenna, 1984).
The convergence of multiple sources of sensory feedback, as would be expected in a balance perturbation, is known to further facilitate the triggering of GSRs (Yeomans et al. 2002), likely via multi-modal afferent connections with the caudal pontine reticular formation. More importantly, the multi-sensory signals generated by a balance perturbation could potentially reach the reticular formation with sufficiently short latencies to trigger a GSR, which can appear as early as 30-50ms post-startle in muscles of the face and neck (Brown et al. 1991). For example, vertical head accelerations are recorded as early as 20ms following a support-surface perturbation (Allum et al. 2008), while cortical evoked potentials in response to stretch of the triceps surae muscle (Davis et al. 2011) or visual stimuli (Di Russo et al. 2001) are observed with average latencies of 35ms and 55ms, respectively.

Through the novel application of a StartReact paradigm, the current study has provided the first empirical evidence in support of the hypothesis that balance perturbations can act as startling stimuli. First trial exposures to balance perturbations (i.e. TESTPERT) induced a StartReact effect, whereby earlier onsets of wrist extension and ECR activity were observed compared to CONTROL trials. The ability of a balance perturbation to elicit a StartReact effect was not limited to the first trial, as earlier onsets of wrist extension and ECR activity were still observed at the end of repeated TESTPERT trials compared to CONTROL trials. The StartReact effects elicited by the TESTPERT conditions during the first, and subsequent trials, were similar to the StartReact effects elicited by the SAS, suggesting that balance perturbations, like SAS, can act as a startling stimulus, as previously proposed (Bisdorff et al. 1994; Commissaris et al. 2002).

What are FTEs?
As hypothesized, there was an observed change in the PRs evoked by repeated balance perturbations. Specifically, the amplitudes of EO, RF and SOL were significantly decreased in the 10th compared to the 1st TESTPERT trial, while no changes to response onsets were observed for any of the muscles tested. These changes with repeated perturbations are consistent with those observed in prior studies of PR habituation where amplitudes of both EMG in postural muscles and kinematic displacements of body segments significantly decreased after the first balance perturbation (Oude Nijhuis et al. 2009, 2010), without a reported change in their onset latency (Keshner et al. 1987). The changes in PRs were paralleled by changes to activity in SCM, which is commonly recognized as a primary indicator of GSRs (Carlsen et al. 2011). SCM responses were large and frequent during the 1st TESTPERT trial, with only minimal SCM activity observed by the 10th TESTPERT trial. The decrease in GSR response in SCM activity over repeated perturbations cannot be explained by a decrease in the intensity of the startle stimulus itself, as all balance perturbations including the 1st and 10th, were shown to be capable of eliciting the same StartReact effect, and thus, represent the same intensity of startling stimuli. Therefore, the observations of decreased SCM responses over repeated balance perturbations, more likely reflects a habituation of a GSR response over repeated exposure to the same startle stimulus. These observations support the hypothesis that FTEs reflect PRs which are super-imposed with a GSR, that is initially large, but habituates with repeated exposure to the startling influence of the balance perturbation over time (Blouin et al. 2006). Interestingly, the proposed relationship between the habituation of GSR and PRs to repeated balance perturbations is also supported by clinical observations, with Parkinsonian patients displaying delayed habituation of PRs to postural perturbations (Nanhoe-Mahabier et al. 2012) as well as slower habituation of GSRs to repeated SAS stimuli (Nieuwenhuijzen et al. 2006).
Limitations

Although SCM activity is assumed to be a marker of a GSR, there is also the possibility that early SCM activity observed during TEST\textsubscript{PERT}, could reflect a PR to the early head accelerations induced by the postural perturbation (Blouin et al. 2006; Siegmund et al. 2008; Oude Nijhuis et al. 2009; Tang et al. 2012). While we recognize this as a potential limitation, there is ample evidence to suggest that SCM activity observed during toes-up rotations are more likely associated with the GSR as opposed to a PR. For example, in the current study, there were similar amplitudes of SCM activity evoked by balance perturbations and SAS trials during both the first and subsequent trials. Furthermore, during support-surface rotations, the timing and amplitude of SCM activity has been shown to be independent of direction of the perturbation during first trial (Tang et al. 2012), and habituated responses (Oude Nijhuis et al. 2009; Tang et al. 2012), despite differences in induced head accelerations (Carpenter et al. 1999), and directional dependent changes in other postural muscles (Carpenter et al. 1999, 2004, 2008). Similarly, Oude Nijhuis et al. (2010) observed SCM activity during support-surface rotations that was more similar in latency and pattern to other non-postural muscles (i.e. masseter), compared to muscles engaged in postural control. Thus, we would argue that early SCM activity observed during TEST\textsubscript{PERT} conditions is still a valid indicator of GSRs, in the current application.

Another limitation of the study is that the voluntary wrist extension task used to assess the StartReact effect could also have been confounded by a PR elicited in the wrist by the balance perturbation. As shown in Figure 6, the wrist responses to Perturbation-Only trials were minimal compared to the amplitude of wrist movement elicited during TEST\textsubscript{PERT} trials, although we recognize that this comparison may be limited by the potential order effect caused by having
the Perturbation-Only trials always performed after the SAS and PERT blocks. Furthermore, the relatively late onset of wrist movements during Perturbation-Only trials, suggests that any potential PR of the wrist would not be able to explain the very early initiation of the wrist response (StartReact effect) observed during TESTPERT trials (Figure 6). As an alternative, future studies could incorporate other voluntary tasks that are completely independent from the PR, including vocalizations (Chiu et al. 2011) or displacements of the tongue (Regnaux et al. 2005) and counter-balance the Perturbation-Only trials with others within the methodological design.

Finally, although StartReact effects were observed in TEST conditions, they were delayed compared to those evoked by SAS in other experiments. For example, ECR onsets were observed by Valls-Solé et al. (1999) and Carlsen et al. (2007) within 80ms of SAS onsets. Whereas in our study, ECR onsets occurred on average approximately 100ms after startle stimuli onset [mean range of 95ms and 110ms (Table 1)]. We believe these differing methodological procedures and analyses techniques may help explain differences between studies. Firstly, our experiment involved a simple reaction time task that was completed while subjects were standing upright, whereas other studies required subjects to remain seated during testing. The attentional resources required of bipedal stance may have been shifted away from the reaction time task and directed them towards the task of maintaining balance (Woollacott and Shumway-Cook, 2002), which could have a delaying effect on reaction time (Mangun and Buck, 1998). Secondly, our experimental analyses included all reaction time data regardless of the presence of SCM. Considering that Carlsen et al. (2007) observed significantly earlier reaction times during a StartReact paradigm in trials were SCM activity was observed compared to trials where startle stimuli were delivered but did not induce SCM activity, we recognize that the overall response
latencies we observed in TEST trials may have been slightly increased, on average, by analyzing
all StartReact trials, independent of SCM activity. However, the actual effect, if any, appears
minimal in our data, as there were no significant effects of Trial observed on the StartReact
latencies, despite a higher prevalence, and amplitude, of SCM responses in the 1st compared to
10th TEST trials.

Implications

Our findings highlight the potential for GSRs to influence FTEs and the subsequent time
course of PR habituation to repeated perturbation. The latter underscores a need to account for
this potentially confounding factor when comparing PRs across groups that may respond, and
habituate, differently to repeated startle stimuli, such as patients with Parkinson’s disease
(Nieuwenhuijzen et al. 2006), Steele-Richardson-Olszewski syndrome (Vidailhet et al. 1992),
hyperekplexia (Brown et al. 1991), progressive supranuclear palsy (Rothwell et al. 1994) and
dementia with Lewy bodies (Kofler et al. 2001). Likewise, it is important to recognize that
factors such as fear, can potentiate GSRs (Davis et al. 1993), and influence the rate of
habituation to repeated startle stimuli (Grillon et al. 1991), and thus may be an additional
confounding variable when comparing PRs in individuals with fear of falling, or
conditions/contexts in which fear and arousal may differ, or change over time.

Evidence supporting balance perturbations as potentially startling stimuli also provides
new perspectives on previous work that has incorporated voluntary movements and postural
perturbations to understand cognitive load and adaptation processes involved in balance control
(Redfern et al. 2002; Woollacott and Shumway-Cook 2002; Müller et al. 2004, 2007). Out of
these dual-task experiments has emerged the ‘posture first’ or ‘postural prioritization’ theories
which collectively suggest not only that cognitive resources are involved in mediating PRs, but also that their influences vary with time after perturbation. These conclusions are the result of observed changes to onset latencies of voluntary motor behaviours such that for a given time frame after perturbation, an increase in voluntary reaction time would reflect relatively greater cognitive demand placed on PRs and vice versa for decreases in reaction time. In light of our findings, decreases in reaction time of voluntary tasks performed in the presence of balance perturbations may instead be the result of StartReact effects. For reasons unrelated to startle, some dual-task experiments utilize choice-reaction time paradigms where more than 1 response alternative exists instead of the simple reaction time paradigm utilized in the current study. In motor control literature, movements produced during choice reaction time paradigms are inconsistently influenced by StartReact effects compared to the highly robust results observed when producing the same movement in simple reaction time paradigms (Carlsen et al. 2004, 2011). Thus, although the full extent of StartReact effects in dual-task scenarios remains unclear, the possibility for StartReact effects to interact with voluntary movements during dual-task paradigms alone warrants further examination.

The results of this study also have important implications for understanding the potential neural circuitry that contributes to GSRs and StartReact effects. Recent evidence has revealed that GSRs and StartReact effects are perhaps mediated by partially independent circuits (Valls-Solé et al. 2005; Alibiglou and MacKinnon 2012). For example, techniques such as pre-pulse and trans-cranial magnetic stimulation have been used to respectively influence either the SCM response or the StartReact effect in isolation (Valls-Solé et al. 2005; Alibiglou and MacKinnon 2012; Maslovat et al. 2012), suggesting that aspects of the GSR and StartReact circuits are at least partially independent. Our results provide additional evidence that supports the existence of
disassociated mechanisms. The significant differences between SCM onsets between TEST\textsubscript{PERT} and TEST\textsubscript{SAS} trials suggested that the timing of the GSR differed depending on stimulus type. However, significant delays of SCM onsets were not met with similar delays in StartReact effects evoked by perturbations and SAS. One would expect that if both GSRs and StartReact effects were mediated by the same mechanism, that the ~40ms delay in SCM onsets between TEST\textsubscript{PERT} and TEST\textsubscript{SAS} trials would have carried over into a similar delay in StartReact effects. That not being the case aligns with recent work suggesting that GSRs and StartReact effects are the end results of two partially disassociated neural mechanisms.

Conclusions and future directions

Our results have supported the notion that FTEs are mediated by a GSR induced by balance perturbations and have added further details regarding the persistent effects of startle stimuli induced by repeated balance perturbations. It appears that GSRs may not only to be a mediating factor in a single trial, but possibly in as many 10 repeated trials. Thus, the role and importance of GSRs in trials beyond the first must be considered. Future experiments in dynamic postural control must not ignore the possibility for GSRs or the startling nature of balance perturbations to influence their measures, especially if they are temporally based. Furthermore, considering GSRs as a natural consequence of postural instability may open new avenues of research into the neural mechanisms governing PRs and perhaps the relationship between PRs and clinical disorders, such as hyperekplexia and Parkinson’s disease, where abnormal responses to startle and postural instability are known to co-exist (Brown et al. 1991; Nanhoe-Mahabier et al. 2012).
Grants:

Funding for this project was provided by the Natural Sciences and Engineering Council of Canada (Campbell AD, Carpenter MG, Chua R, Inglis JT).

Author contributions:

Campbell AD: Principle investigator, concept development, data collection, analysis, manuscript development.

Squair JW: Data collection, analysis, manuscript development

Chua R, Inglis JT: Concept development, manuscript edits.

Carpenter MG: Supervising author, concept development, manuscript edits.
Figure legends

Figure 1
Graphic illustration of the stimuli involved in each trial type (CONTROL, TEST\textsubscript{SAS}, TEST\textsubscript{PERT} and CATCH). In CONTROL trials, a warning cue (WARN; 2 black rectangles) involving two 50ms auditory stimuli, preceded the onset of an imperative stimulus (IS; white-filled rectangle) by a random 1.5-3.5s fore-period. TEST\textsubscript{SAS} trials involved a WARN cue that preceded the simultaneous onset of the IS and a startling acoustic stimulus (SAS; tall rectangle). TEST\textsubscript{PERT} trials involved a WARN cue followed by the simultaneous onset of an IS and support-surface toes-up rotation (PERT; hatched line representing platform displacement). CATCH trials involved only the WARN cue and no subsequent stimuli.

Figure 2
Wrist angular displacements (black lines) and extensor carpi radialis (ECR; grey lines) responses during a single CONTROL trial as well as in the 1\textsuperscript{st} and 10\textsuperscript{th} TEST\textsubscript{SAS} and TEST\textsubscript{PERT} trials for a representative subject. Time ‘zero’ represents the onset of the imperative stimulus (IS) in each trial.

Figure 3
(A) Matrix of detected SCM responses for all subjects (S\textsubscript{01}-S\textsubscript{12}) across each TEST\textsubscript{SAS} and TEST\textsubscript{PERT} trial. Within each row, a black filled top portion indicates a detected SCM response in a given TEST\textsubscript{SAS} trial and a grey filled bottom portion indicates a detected SCM response in a
given TESTPERT trial. The last row indicates the sum total of detected responses across subjects within each TESTSAS (top) and TESTPERT (bottom) trial.

(B) SCM muscle responses evoked in CONTROL trials (solid grey) as well as in the 1\textsuperscript{st} (solid black) and 10\textsuperscript{th} (dashed black) TESTSAS and TESTPERT trials for a representative subject. Time ‘zero’ denotes the onset of the imperative stimulus, and the simultaneous onset of either the SAS or PERT stimulus depending on block.

(C) Plots depict group-wide averages (± 1SE) of SCM onset latencies and amplitudes within each TESTSAS and TESTPERT trials. Only those data colored black (i.e. first and last trials) were compared statistically. Note, black connector lines indicate significant differences between conditions.

Figure 4

(A) External oblique (EO), rectus femoris (RF), soleus (SOL) and tibialis anterior (TA) EMG responses evoked during the 1\textsuperscript{st} (black) and 10\textsuperscript{th} (grey) TESTPERT trials for a representative subject. Vertical black dashed line denotes the simultaneous onset of the balance perturbation and the imperative stimulus (IS) within each trial.

(B) Plots depicting the average (±1SE) onset and amplitude of EO, RF, SOL and TA during all TESTPERT trials. Note, only the 1\textsuperscript{st} and 10\textsuperscript{th} trials (i.e. data colored in black) were compared statistically. Black connector lines indicate significant differences between conditions.

Figure 5
(Left panel) Upper arm, trunk and shank absolute angular displacements evoked by the 1st (solid black) and 10th (dashed black) TESTPERT trials for a representative subject. Vertical dashed line denotes the simultaneous onset of the balance perturbation and imperative stimulus (IS).

(Right panel) Plots depicting the average (±1SE) onset and amplitude of upper arm, trunk and shank absolute angular displacements during all TESTPERT trials. Note, only the 1st and 10th trials (i.e. data colored in black) were compared statistically. Black connector lines indicate significant differences between conditions.

Figure 6

Group average (black lines) ±1SE (grey lines) of wrist angular displacement during the 1st TESTPERT trials (solid line) and the average of 5 Perturbation-Only trials (dashed line). Time ‘zero’ denotes simultaneous onset of imperative stimulus and perturbation in TESTPERT trials and the onset of the support-surface displacement during Perturbation-Only trials.


CONTROL trial | TEST$_{SAS}$ | TEST$_{PERT}$ | CATCH

WARN 1.5-3.5s IS

SAS

PERT

Fig1
Fig2
A. **SCM Responses**

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</table>

B. **SAS**

![SAS Waveform](image)

C. **PERT**

![PERT Waveform](image)
A. **PERT Block – TEST trials**

- **EO**
  - Amplitude (mV)
  - First Trial
  - Tenth Trial

- **RF**
  - Amplitude (mV)

- **SOL**
  - Amplitude (mV)

- **TA**
  - Amplitude (mV)

B. **Onset Latency**

- **EO**
  - Onset Latency (s)
- **RF**
  - Onset Latency (s)
- **SOL**
  - Onset Latency (s)
- **TA**
  - Onset Latency (s)

**Amplitude**

- **EO**
  - EMG Amplitude (mV)
- **RF**
  - EMG Amplitude (mV)
- **SOL**
  - EMG Amplitude (mV)
- **TA**
  - EMG Amplitude (mV)
Kinematics

Upper Arm
Trunk
Shank

PERT
IS

Onset Latency

Amplitude

Fig5
Fig 6
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<td>Last TEST</td>
<td>CONTROL</td>
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*“ns” denotes non-significant result for corresponding measure and level of ANOVA*

*each numerical cell value represents mean ± 1SE*