Title: Startle induces early initiation of classically conditioned postural responses

Authors: Campbell AD\textsuperscript{1}, Chua R\textsuperscript{1}, Inglis JT\textsuperscript{1}, Carpenter MG\textsuperscript{1}.

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

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

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

Affiliations: \textsuperscript{1}School of Kinesiology, University of British Columbia

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Contact information: Carpenter MG (corresponding author):

Mailing address: Osborne Centre Unit I, 6108 Thunderbird Blvd, University of British Columbia, Vancouver, British Columbia, Canada, V6T 1Z3.

Email address: mark.carpenter@ubc.ca

Office phone: +1 (604) 822-8614

Fax: +1 (604) 822-9451

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Abstract

Startling acoustic stimuli (SAS) induce the early release of prepared motor responses. The current study used SAS, in conjunction with a classical conditioning paradigm, to examine advanced motor preparation of conditioned postural responses (PRs). After inducing generalized startle responses, standing posture was perturbed in 2 blocks of 15 Conditioning trials wherein each trial the onset of a non-startling auditory cue (i.e. a conditioned stimulus (CS)) preceded a leftward support-surface translation. Upon completing each block, a single trial was conducted. After block 1, a CS-Only trial was used to induce conditioned PRs in the absence of balance perturbations. After block 2, a post-Conditioning Startle trial that involved a CS subsequently followed by a SAS, was used to examine motor preparation of conditioned PRs. PRs were quantified in terms of centre of pressure displacements, ankle and hip kinematics as well as surface electromyography of proximal and distal bilateral muscle pairs. Results indicated that repeated experience with cued balance perturbations led to PR conditioning and, more importantly, motor preparation of PRs. Conditioning was evidenced in biomechanical and electromyographic responses observed in CS-Only trials as well as the progressive changes to evoked response parameters during repeated Conditioning trials. SAS presented in post-Conditioning Startle trials evoked early onsets of biomechanical and electromyographic responses while preserving relative response parameters that were each distinct from generalized startle responses. These results provide important insight into both the consequences of using cues in dynamic postural control studies and the neural mechanisms governing PRs.
Keywords

1. Motor preparation
2. Dynamic posturography
3. StartReact effect
4. Kinematics
5. Electromyography
6. Centre of pressure
Startle paradigms have been used to probe the central nervous system for evidence of advanced motor preparation during various voluntary tasks ranging from target-directed displacements of the elbow (Carlsen et al. 2004b), wrist (Valls-Solé et al. 1999), head (Siegmund et al. 2001, 2008; Oude Nijhuis et al. 2007), eye (Castellote et al. 2007) and ankle (Valls-Solé et al. 1999) to sit-to-stand (Queralt et al. 2008a) as well as obstacle avoidance during gait (Queralt et al. 2008b). In these paradigms, it has been shown that a startling acoustic stimulus (SAS) presented in combination with a cue can significantly shorten onset latencies of voluntary reactions while preserving movement parameters observed under non-startling conditions. Termed the StartReact effect (see Carlsen et al. 2011b for review), early onset latencies and preserved movement characteristics suggest that the SAS-induced movements were prepared in advance of movement execution (Carlsen et al. 2004b).

MacKinnon and colleagues (2007) have recently demonstrated that anticipatory postural adjustments, the postural response (PR) elicited prior to self-initiated movements (Bouisset and Zattara 1987; Massion 1992), may also be incorporated into the preparation of voluntary motor behaviours. Using a StartReact paradigm, MacKinnon et al. (2007) showed that the onset latencies of anticipatory postural adjustments to a self-initiated step could be significantly shortened with a SAS, while preserving many of their kinematic characteristics observed in control trials. From these results, it was concluded that the SAS triggered the early release of feedforward neural commands which included those responsible for initiating PRs that accompany voluntary movements (MacKinnon et al. 2007).
Evidence of advanced preparation during anticipatory postural adjustments raises the question as to whether motor preparation may also take place during other types of PRs, namely those that follow externally-generated balance perturbations (Nashner 1977, 1983; Horak and Nashner 1986; Allum and Honegger 1998; Carpenter et al. 1999). Readiness potentials observed in cortical activity that normally precedes the onset of cued, or self-initiated postural perturbations, support at least some level of preparation prior to initiating reactive PRs (Adkin et al. 2008; Jacobs et al. 2008; Mochizuki et al. 2010). Further evidence of PR motor preparation has emerged from studies that used cued balance perturbations in classical conditioning paradigms.

During classical conditioning, subjects are repeatedly exposed to trials in which a cue (i.e. a conditioned stimulus: CS) is subsequently and invariably followed by a perturbation of some kind (i.e. an unconditioned stimulus: US) that innately evokes a reflexive response (Kirsch et al. 2004). As a consequence of conditioning, onset latencies and amplitudes of evoked responses are known to decrease with repeated presentations of paired CS and US, and conditioned responses can also be observed to the CS alone after sufficient experience with repeated CS/US trials (Clark et al. 2002). These effects have previously been attributed to associative learning mechanisms (Woodruff-Pak and Disterhoft 2008) and possibly the emergence of a motor plan (Taub et al. 1965). Applying this conditioning technique to dynamic postural control, Kolb et al. (2002) coupled a non-startling auditory cue with a subsequent balance perturbation and observed similar changes to PR onset and amplitude measures. These changes suggested that, not only could reactive PRs be classically conditioned, but more importantly that conditioned responses may represent a motor response prepared in advance of the normal triggering (i.e. postural) stimulus (Taub et al. 1965; Kolb et al. 2002). Campbell et al.
(2009) replicated the results of previous PR conditioning work (Kolb et al. 2002, 2004) and further showed that 19-28 paired CS and US trials were sufficient to allow the CS alone to induce PRs in the absence of balance perturbations and associated stretch reflexes. These conditioned PRs involved complex motor sequences including excitation and inhibition of lower limb muscles that could be evoked immediately and 15mins after the original conditioning procedure and subsequent distractor trials (Campbell et al. 2009). These results suggested that conditioned PRs could be evoked by non-postural cues and retained in memory. Taken together, previous work suggests that the conditioned PRs observed in Campbell et al. (2009) may have been the consequence of motor preparation facilitated by cued external balance perturbations.

The aim of the current investigation was to extend the work of Campbell et al. (2009) by using a SAS as a probe to determine if conditioned PRs, evoked after repeated experiences with cued perturbations, could be prepared in advance of their initiation. The advantage of having used a classical conditioning paradigm to address this aim was that conditioned PRs induced by the CS alone would not be masked by stretch reflexes or biomechanical changes caused by perturbation-induced passive displacements of the body, thus allowing for a clearer examination of the prepared response. Two specific hypotheses were tested in this study. Based on prior work (Campbell et al. 2009), we hypothesized that, following a classical conditioning procedure, conditioned PRs could be evoked by an auditory cue in the absence of a balance perturbation. Second, we hypothesized that SAS would induce earlier absolute onsets of conditioned PRs while preserving other absolute and relative measures of response parameters.

Materials and Methods
All experimental procedures were approved by the ethics review board at the University of British Columbia. Seventeen subjects were recruited from the local university community and were individually briefed of all methods and data collection techniques prior to providing their informed consent to participate in the study. As will be described later, the total dataset was reduced to 12 subjects (19-32 years of age, 7 males, mean height and body mass ± 1SD: 1.76 ± 0.77m and 72.50 ± 8.42kg, respectively). All subjects were completely naïve to the experimental procedures prior to arriving at the laboratory.

Experimental setup

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A forceplate (#K00407, Bertec Corporation, USA) was used to sample ground reaction forces and moments along and around all axes, respectively, which were independently amplified (AM-6100, Bertec Corporation, USA) and individually A/D sampled at 1000Hz (Power1401, Cambridge Electronic Design, UK). These data were digitally lowpass filtered offline at 5Hz (Spike5, Cambridge Electronic Design, UK) and were used to calculate centre of pressure (COP) in the anterior-posterior and medio-lateral (M/L) directions for each trial (Matlab 7.0, The Mathworks Incorporated, USA).

Rigid bodies, comprised of 3 non-collinear infra-red light emitting diodes (iREDs), were affixed to the right shank, right thigh, trunk and the support-surface on which subjects stood. Raw 3-dimensional iRED displacements were sampled at 200Hz and saved on a trial-by-trial basis (Optotrak Certus, Northern Digital Incorporated, CAN). Prior to beginning the experimental protocol, subject-specific kinematic models were built in order to generate local
coordinate systems whose axes were aligned to the principal axes of segment rotation (Visual 3D, C-Motion, USA).

Raw marker positions were lowpass Butterworth filtered at 5Hz offline prior to applying the subject-specific kinematic models and calculating ankle and hip angular displacements in the frontal-plane (Visual3D, C-Motion, USA). The right ankle joint was defined as the angle between the right shank and the support-surface, whereas the hip joint was defined as the angle between the trunk and right thigh segments. Frontal-plane ankle and hip angular displacements were double differentiated to calculate their angular accelerations.

Surface electromyography (EMG)

EMG was recorded bilaterally from tibialis anterior (TA), soleus (SOL), gluteus medius (GM), external oblique (EO) and sternocleidomastoid (SCM). Two pre-gelled Ag/AgCl surface electrodes were placed ~2cm apart on recording areas that were shaved and cleaned with alcohol swabs. A single ground electrode was placed atop the acromion process of the right scapula. Raw EMG data were pre-amplified 500x, sampled at 3000Hz and band-pass filtered between 10-500Hz (Telemyo 2400R, Noraxon, USA) online, before being A/D converted at 1000Hz (Power1401, Cambridge Electronic Design, UK). These data were subsequently digitally high-pass filtered at 30Hz (Spike2, Cambridge Electronic Design, UK) offline in order to remove heart rate artifacts, baseline corrected then full-wave rectified.

Experimental procedures
Participants stood on the forceplate that was centred within, and flush with, the surface of a wooden stage (1.23m wide; 0.61m long) affixed to a translating sled (DR Stage, H2W Technologies Incorporated, USA). Throughout the experiment, subjects were asked to stand comfortably (stance width equal to 100% of their measured foot length), with their eyes open and gaze fixated on an eye-level target located approximately 2m away.

Quiet stance and pre-Conditioning Startle trials

Subjects were first asked to stand quietly for 60s while looking straight ahead at the target. A range of normal frontal-plane sway was calculated from this period as the mean ±1SD of the M/L moment. Once the 60s trial was completed, a subsequent 30s of quiet stance took place during which time 2 auditory stimuli were unexpectedly presented in randomized order (Figure 1) and separated by at least 20s. One stimulus was a non-startling tone (<80dB, 200ms duration), which later served as the CS, to ensure it did not evoke startle-like reflexes (Campbell et al. 2009) or any detectable movement in the frontal-plane. The other stimulus was a calibrated SAS (~120dB, 1000Hz, 40ms duration, ~1ms rise-time) (CR:231B Impulse sound level meter, Cirrus Research plc, UK) which was used to evoke a generalized startle response; termed the pre-Conditioning Startle trial (Figure 1). Both auditory stimuli originated from speakers located directly overhead of the participant.

Conditioning, CS-Only and post-Conditioning Startle trials
After a brief rest period, subjects experienced 2 blocks of 15 Conditioning trials. Each trial involved a leftward support-surface translation US (1m displacement, 0.25m/s velocity, 1.3m/s² acceleration) presented 300ms after the onset of the auditory CS (Figure 1). Any sound generated by the support-surface translation was determined to be <80dB (CR:231B Impulse sound level meter, Cirrus Research plc, UK). The temporal relationship between CS and US was consistent with trace conditioning paradigms whereby their relative timing produced a 100ms inter-stimulus interval (i.e. ‘trace’ interval) when neither stimulus was active (Christian and Thompson 2003; Woodruff-Pak and Disterhoft 2008). Trials were separated by a random fore-period lasting between 10s and 25s from the end of the previous trial marked by the return of the platform to its initial position. At the end of the first Conditioning block, a CS-Only trial was conducted whereby the CS was presented in the absence of the support-surface translation (Figure 1) in order to generate a conditioned PR (Kolb et al. 2002; Campbell et al. 2009). Following completion of the second Conditioning block, a single post-Conditioning Startle trial was conducted which involved a SAS presented 50ms after the onset of the CS in the absence of a support-surface translation (Figure 1). It was expected that, as a consequence of Conditioning, response onsets induced by CS-Only trials would approach and potentially precede the onset of the US (Woodruff-Pak and Disterhoft 2008; Campbell et al. 2009). Thus, in post-Conditioning Startle trials, the SAS was presented before the expected onset of the US to induce earlier onsets of responses triggered by the CS.

During all trials, an experimenter monitored the M/L moment of the forceplate in real-time. To limit the potential influences of anticipatory leaning on PRs (Diener et al. 1983; Tokuno et al. 2006), trials were manually triggered only when the M/L moment was within the
range of normal range of sway calculated during 60s of quiet stance. If a persistent lateral lean was observed, subjects were verbally coached back to resting positions.

Measures

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Thresholds for determining onsets of COP were established by calculating the mean ±1SD of angular accelerations from 500ms of data that immediately preceded the first stimulus within each trial. Onsets of COP displacements were then determined as the time when accelerations first surpassed and remained beyond threshold for at least 150ms. If onsets were detected, peak displacements were calculated as the greatest relative change from mean values calculated from 500ms of pre-stimulus data. Time-to-peak of COP displacements were calculated as the time from onset to the time of peak COP displacement.

Onsets, peak and time-to-peak of ankle and hip angular displacements were calculated by using the same methods as those applied to the kinetic dataset (see above).

EMG

Thresholds for determining EMG onsets were calculated as the mean +2SD of background EMG data recorded from a 500ms period that immediately preceded the start of each trial. Using a semi-automated algorithm, onsets of EMG activity were determined to be the time at which processed EMG signals first surpassed and remained above threshold for a minimum of 30ms while at no time dipping below for >3ms (Carpenter et al. 2008).
Onsets of PRs evoked during Conditioning, CS-Only and post-Conditioning Startle trials were accepted if they fell within a timeframe that began 90ms after CS onset and ended 220ms after US onset. The former is within range of reported mean onsets of practiced PRs in lower-limb muscles triggered by non-startling auditory tones (Nashner and Cordo 1981), whereas the latter is the reported mean of trunk muscle onsets triggered by frontal-plane support-surface translations (Carpenter et al. 2004). For pre-Conditioning Startle trials, generalized startle responses evoked in each muscle were accepted only if they were observed within a ±2SD range around previously reported mean onsets of muscle responses to SAS during stance (Oude Nijhuis et al. 2010). An onset of SCM activity observed within 90ms of a SAS was used as evidence of a startle effect (Forgaard et al. 2011).

Amplitudes of EMG responses were calculated as the integrated area of rectified EMG calculated 100ms after onset, minus resting activity from equivalent time periods prior to the trial. The analysis window was set to 100ms because it is a common timeframe for quantifying PR amplitudes (Carpenter et al. 2008; Campbell et al. 2009) while also being a period where sensory feedback has limited influence on triggered responses (Wadman et al. 1979). In trials where an onset was not detectable within a muscle, response amplitudes were not calculated.

Data Reduction

Two subjects did not produce conditioned PRs during CS-Only trials and therefore were removed from further analyses. From the 15 subjects remained for comparison between Conditioning and CS-Only responses, an additional 3 subjects were removed from analyses of post-Conditioning Startle effects because they did not have a detectable onset in at least 1 SCM
muscle within a 90ms period following the onset of the SAS. Therefore, a total of 12 subjects were included in analyses of CS-Only and post-Conditioning Startle trials.

Within the remaining 12 subjects, analysis of post-Conditioning Startle effects on EMG responses was limited to muscles that demonstrated a high probability of conditioning in the CS-Only trials. CS-Only responses were frequently observed in \( R_{GM} \) \((n=11)\), and \( R_{TA} \) \((n=10)\), to a lesser extent in \( L_{GM} \) \((n=7)\) and \( L_{TA} \) \((n=6)\) and rarely observed in other muscles. Therefore, subsequent analysis of CS-Only responses was focused on \( R_{GM} \) and \( R_{TA} \).

It was important to further examine EMG responses observed in \( R_{GM} \) and \( R_{TA} \) during post-Conditioning Startle trials to ensure that they were clearly distinguishable from generalized startle responses. In previous studies, SAS have been shown to simultaneously induce prepared responses as well as generalized startle responses (Siegmund et al. 2001) that are bilaterally symmetric (Landis et al. 1939). To ensure that our analyses were focused primarily on prepared responses, we removed post-Conditioning Startle trials from further analysis if the observed relative onset latencies of bilateral GM and TA activity did not exceed a mean ±2SD range of bilateral generalized startle response onsets calculated from pre-Conditioning Startle trials \((GM: 7±44ms; TA: 2±68ms)\). Based on these criteria, 10 of 11 subjects generated GM activity in post-Conditioning Startle trials that was distinguishable from generalized startle responses. Therefore, \( R_{GM} \) responses of remaining subjects were included in further analyses. In contrast, only 3 subjects had asymmetrical post-Conditioning Startle responses in TA that were distinguishable from generalized startle responses. As a result, no statistical analysis was performed on \( R_{TA} \) due to its small sample size.
Due to the bilaterally symmetric nature of generalized startle responses (Landis et al. 1939), it was expected that they would induce only minimal frontal-plane kinetic and kinematic displacements. Our expectations were confirmed as pre-Conditioning Startle trials induced only marginal frontal-plane displacements of the COP and body segments (Figure 2). Therefore, prepared responses and generalized startle responses were easily distinguishable for kinetic and kinematic data because they occurred in different planes of movement.

Analyses and Statistics

Onsets and amplitudes of COP and EMG responses from the last 5 trials of each Conditioning block were averaged and compared using pairwise $t$-tests to test for potential order effects. Since no effects of order were observed for any variable ($p > 0.05$) the responses from each conditioning block were pooled, and used to compare with CS-Only trials using pairwise $t$-tests. COP, ankle and hip kinematics as well as $r_{GM}$ measures were compared between CS-Only and post-Conditioning Startle trials using pairwise $t$-tests. In all cases, significance was set at probability values $\leq 0.05$ and significant trends were considered at $p$-values between $>0.05$ and $\leq 0.10$.

Results

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Conditioning compared to CS-Only trials
During Conditioning trials, the leftward support-surface translation initially induced ankle eversion, hip adduction and rightward displacements of the COP (Figure 2). This initial response was quickly followed by ankle inversion and hip abduction and leftward COP displacements (Figure 2). Over the course of the 15 Conditioning trials, onsets of COP displacements progressively decreased and plateaued by the end of the block (Figure 3), as would be expected during conditioned response acquisition (Woodruff-Pak and Disterhoft 2008).

CS-Only trials elicited early COP displacements to the right, followed by ankle inversion and hip abduction that collectively contributed to induce leftward whole-body sway (Figure 2). Note that the directions of these kinematic responses are counter to the direction of initial platform-induced movements observed during Conditioning trials. Onsets of COP displacements in CS-Only trials were not significantly different than those observed in Conditioning trials ($p=0.347$) (Figure 3), whereas peak COP displacements were found to be significantly attenuated in CS-Only compared to Conditioning trials ($t(11)=18.62, p<0.001$).

**CS-Only compared to post-Conditioning Startle trials**

CS-Only and post-Conditioning Startle trials both induced initial rightward COP displacement pattern (Figure 2) that was highly consistent across subjects (Figure 4). In both trials, initial rightward COP displacements were arrested and followed by leftward displacements that surpassed then oftentimes approached the starting position. Note that these responses differ markedly from the small, directionally non-specific, displacements associated with the generalized startle response (Figure 2 & 5). Mean onsets of M/L COP displacement were found to be significantly earlier (110ms earlier) in post-Conditioning Startle trials compared to CS-
Only trials ($t(11)=4.66, p=0.001$), whereas peak ($p=0.566$) of COP displacements were not different between CS-Only and post-Conditioning Startle trials (Figure 5 & Table 1). A significant trend for quicker time-to-peak COP displacements in post-Conditioning Startle trials was observed ($t(11)=2.07, p=0.062$).

Similar ankle and hip angular displacements were observed following CS-Only and post-Conditioning Startle trials, although the onsets of displacements were earlier in post-Conditioning Startle compared to CS-Only trials (Figures 5 & 6). These observations were confirmed statistically whereby the mean absolute onsets were significantly earlier in post-Conditioning Startle compared to CS-Only trials for the ankle (mean difference = 127ms; ($t(11)=4.01, p=0.002$)) and hip (mean difference = 87ms; ($t(11)=3.79, p=0.003$)) (Figure 5 & Table 1). The relative timing between onsets of ankle and hip displacements was not significantly different between conditions ($p=0.475$), with mean hip onsets preceding mean ankle onsets by 103±31ms and 83±29ms for CS-Only and post-Conditioning Startle trials, respectively. Peak amplitudes of ankle ($p=0.289$) and hip ($p=0.457$) angular displacements were not significantly different between conditions; however, time-to-peak angular displacements were significantly earlier during post-Conditioning Startle trials compared to CS-Only trials in the ankle ($t(11)=3.41, p=0.006$) with significant trends towards quicker time-to-peaks observed in the hip ($t(11)=1.97, p=0.074$) (Table 1).

**EMG**

*Conditioning compared to CS-Only trials*
Leftward support-surface translation elicited a pattern of muscle activity that included responses in EO, GM, SOL and TA muscles, primarily on the right side of the body (Figure 7). As shown in Figure 3, muscles that most frequently showed responses to CS-Only trials ($R_{TA}$ and $R_{GM}$) also demonstrated progressive decreases in onsets and amplitudes over the course of the Conditioning trials, providing further evidence that these muscles were conditioned (Woodruff-Pak and Disterhoft 2008). Mean absolute onsets of $R_{TA}$ and $R_{GM}$ responses in Conditioning trials were not significantly different from those observed in CS-Only trials ($R_{TA}$: $p=0.285$; $R_{GM}$: $p=0.263$) (Figure 3). Amplitudes of $R_{TA}$ were significantly attenuated in CS-Only trials compared to Conditioning trials ($t(9)=-3.19$, $p=0.011$) whereas no differences in amplitude were observed in $R_{GM}$ ($p=0.394$) (Figure 3).

The relative timing between $R_{GM}$ and $R_{TA}$ was not significantly different between conditions ($p=0.136$), with $R_{GM}$ preceding $R_{TA}$ onsets by an average of 70ms in Conditioning trials and 11ms in CS-Only trials.

CS-Only compared to post-Conditioning Startle trials

Figure 8 highlights in a representative subject the typical responses observed in SCM, GM and TA during pre- and post-Conditioning Startle trials. Post-Conditioning Startle trials evoked early and bilateral SCM with mean onsets of 59±6ms and 54±7ms for left and right SCM, respectively. These SCM response onsets were similar to those observed during generalized startle trials (Figure 8) and were within previously reported ranges of mean onsets of startle-induced SCM activity (Siegmund et al. 2001; MacKinnon et al. 2007; Oude Nijhuis et al. 2010).
Onset of RGM responses were significantly earlier ($t(9)=5.14$, $p=0.001$) in post-Conditioning Startle compared to CS-Only trials (average difference = 125 (±25ms). The amplitudes of RGM were significantly larger in post-Conditioning Startle compared to CS-Only trials ($t(9)=2.49$, $p=0.034$).

Discussion

The aim of the current investigation was to determine if conditioned PRs could be prepared in advance of balance perturbations. Two hypotheses were tested in this experiment. First, we hypothesized that classical conditioning would allow an auditory cue to evoke conditioned PRs in the absence of a balance perturbation. Second, we hypothesized that SAS would induce earlier absolute onsets of conditioned PRs compared to non-startle trials while maintaining relative patterns between joints and muscles. Our dataset confirmed that PRs were classically conditioned and that cues induced COP, angular displacements and EMG responses in the absence of balance perturbations. Moreover, our results have also demonstrated that the absolute onset latencies of conditioned PRs could be significantly reduced by SAS while maintaining their COP and multi-joint kinematic profiles, suggesting that PRs evoked by cued perturbations were prepared in advance of their execution.

Classical conditioning of PRs

The observed conditioned PRs met pre-established criteria for conditioned response acquisition; CS-Only trials evoked frontal-plane COP displacements and muscle responses in
RTA and rGM with similar latencies to responses evoked by Conditioning trials (Bouton and Moody 2004). Furthermore, COP and EMG measures of PR onset latencies decreased and eventually plateaued over the course of 15 Conditioning trials (Kolb et al. 1997, 2002; Woodruff-Pak and Disterhoft 2008; Kaulich et al. 2010). Conditioned PRs evoked by CS-Only trials involved corrective movements in the ankle, hip and COP that would be effective in protecting against falls induced by the particular postural perturbation used in this experiment. The kinematic strategy adopted by most subjects involved a combination of right hip abduction and right ankle inversion with the onsets of the former preceding those of the latter. This proximal to distal sequence of segment displacements has been observed by others during periods of instability that immediately follow lateral support-surface translations (Henry et al. 1998). During CS-Only trials, these angular displacements induced leftward sway of the body and therefore would have acted to prevent a fall caused by the applied balance perturbation. These results are consistent with the findings of Campbell et al. (2009) where appropriate muscle and biomechanical responses to a CS-Only trial were observed following classical conditioning of PRs to toes-up rotations.

**StartReact effect on cued PRs**

Our results suggest that conditioned PRs are among those motor behaviours that can be prepared in advance and evoked by SAS. While the behaviours typically investigated using the StartReact effect mostly include those under volitional control, there is some evidence that they may also include postural components coupled to voluntary behaviours. MacKinnon and colleagues (2007) have recently shown that anticipatory postural adjustments preceding
voluntary movements can also be prepared in advance of movement execution. Like MacKinnon et al. (2007), our findings also suggest that PRs can be prepared in advance of their execution. However, our work further suggests that to induce motor preparation of reactive PRs, they need not be combined with voluntary movement.

Implication of findings

Evidence of PR motor preparation may contribute to our understanding of the neural control of human balance and how cues may become integrated into dynamic postural control. Advanced motor preparation involves the interaction of various neural centres that our results suggest are also involved in preparing conditioned PRs. SAS was originally thought to influence prepared voluntary movement via interactions with brainstem structures; namely the reticular formation and descending reticulo-spinal tract (Valls-Solé et al. 1999). Recently, studies involving combinations of startle and transcortical magnetic stimulation techniques during reaction time tasks suggest that in fact a rapid cortical loop may also be involved in mediating the StartReact effect for prepared voluntary movements (Alibiglou and MacKinnon 2012; Carlsen et al. 2011a). It has been posited that similar brainstem (Jacobs and Horak 2007; Honeycutt et al. 2009, 2010) and cortical centres (Taube et al. 2006; Jacobs and Horak 2007; Adkin et al. 2008) may be involved in the regulation of dynamic postural control as well. Particularly with respect to the brainstem, researchers believe it may be a site containing representations of PR motor synergies (Jacobs and Horak 2007; Honeycutt et al. 2010). Given the potential overlaps in neural circuitry governing preparation of both voluntary movement and
postural control, as well as their susceptibility to onset latency facilitation by SAS, suggest that their underlying neural substrates are perhaps highly similar.

Utilizing cues to influence PRs is a common practice in dynamic posturography research. However, the current work has introduced an alternative explanation to the previously observed effects of cues on PRs. Previously, experimenters have utilized cued perturbations and noted changes compared to unexpected perturbations in EMG onset latencies and amplitudes or centre of pressure excursions that were later attributed to central set (Jacobs et al. 2008) or attention (Müller et al. 2004, 2007) effects. Alternatively, our findings suggest that introducing cues to postural tasks may cause changes to PR characteristics through mechanisms related to classical conditioning or motor preparation. It is unclear how substantial the effects of conditioning may have been on previous data as those experiments rarely if ever introduced CS-Only trials to rule out the potential for cues to act as conditioned stimuli. It is possible that the effect was negligible, as others have observed no changes to PR characteristics between cueing and not cueing perturbations (Diener et al. 1991). However, compared to Diener et al. (1991) where a 4-second inter-stimulus interval separated the cue from perturbation, those who have observed effects of precuing utilized inter-stimulus intervals more comparable to the 300ms used in the current investigation (500ms: McChesney et al. 1996; 325ms: Müller et al. 2007) which are well within the known timeframes of CS-US timing required for robust conditioned response acquisition (Schneiderman and Gormezano 1964; Smith et al. 1969; Freeman et al. 1993).

Future experiments should consider classical conditioning and motor preparation as potential contributing factors when cueing balance perturbations.
Limitations

Although we have provided evidence that supports motor preparation during dynamic postural control, we were unable to describe in detail the complete muscle response strategy. A broad array of muscles were examined in the hopes of producing an equally broad analysis of PR motor preparation, yet only in RGM, and to a limited extent RTA, could these effects be described. The question remains as to why only a small subset of muscles involved in the complete postural synergy produced conditioned responses during CS-Only trials? It has been well documented that support-surface balance perturbations, even when their parameters are held constant, do not evoke PRs with complete certainty (Henry et al. 1998; Carpenter et al. 2008). In terms of conditioning, inconsistencies in the relationships between conditioned and unconditioned stimuli are known to negatively affect associative learning which can delay the formation of conditioned responses (Gallistel and Gibbons 2000) and can ultimately limit the prevalence of CS-Only reactions. In the current experiment, the balance perturbation, despite having displacement parameters held constant from trial-to-trial, and also being invariably linked to the CS, evoked reactions in a highly variable array of muscles across subjects. Whether greater experience in the Conditioning protocol would have produced conditioned PRs in a broader array of muscles is unknown and is a point worth further inquiry. It is also unclear whether the variability of the CS-induced PR strategy is a normal consequence of using an US with inconsistent effects on the body. In comparison, such variability of conditioned response parameters is not seen in eye-blink conditioning where the effects of the US and the associated responses are limited specifically to the eye.

We have concluded that the SAS and related processes that govern the StartReact effect were the driving forces behind the observed decreases in various electrophysiological and
biomechanical descriptors of PR onsets. However, it is possible that the temporal overlap of the CS and SAS in post-Conditioning Startle trials could have allowed intersensory facilitation (Nickerson, 1973) or stimulus intensity effects (Carlsen et al. 2007) to affect the onset latencies of conditioned PRs in the manner we have related to the StartReact effect. Although the presence of either phenomenon is highly likely, we believe that it does not preclude our ability to suggest that the observed changes in response onsets were driven by the StartReact effect. Compared to CS-Only trials, the decreases in PR onsets observed in post-Conditioning Startle trials were well beyond the reported 20-50ms effect of intersensory facilitation (Nickerson, 1973) and the ~20ms decreases attributed to stimulus intensity effects (Carlsen et al. 2007). Even the summation of intersensory facilitation and stimulus intensity effects hardly approach the >100ms decreases in most markers used to characterize onset latencies of conditioned PRs evoked by post-Conditioning Startle. Furthermore, including trials where only early SCM activity was observed during post-Conditioning Startle trials supports our notion that stimulus intensity and intersensory facilitation acted only to facilitate the larger effect governed by StartReact.

Conclusions

We have demonstrated that a SAS can induce the rapid initiation of a PR conditioned to a cue and a lateral support-surface translation. In doing so, we have discovered a potential neural link between dynamic postural control and processes responsible for classical conditioning and motor preparation. It is our hope that future experiments aimed at understanding the human
postural system will expand on this proposal and consider its importance when incorporating
cues into dynamic postural control studies.
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Author contributions:

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

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

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


Figure captions

Figure 1

The top half includes schematics of each experimental trial type (CS-Only, pre-Conditioning Startle, Conditioning and post-Conditioning Startle) and the bottom half includes a timeline of the entire experimental session that began with 60s of ‘Quiet stance’ and ended with a post-Conditioning Startle trial. The text within the square brackets for each trial-type titles was used as labels within the timeline (i.e. ‘pre’ in timeline represents pre-Conditioning Startle trial).

Figure 2

For a single representative subject, posterior frontal-plane view of stick figures illustrating kinematics of right ankle, knee, hip and shoulder displacements during pre-Conditioning Startle, Conditioning, CS-Only and post-Conditioning Startle trials. Insets located below each stick figure represent the corresponding M/L and A/P COP displacements. Plots consist of 1.4s of data where each frame is separated by 50ms and the progression of time is depicted by color spectrum changes beginning as dark blue and ending in dark red. Medio-lateral marker positions were vertically aligned in the first frame of data and marker displacements were referenced to the location of the lateral malleolus marker (ankle) in all trials. For Conditioning trials, this meant that the perturbation-induced marker translations were removed to produce a platform-referenced displacement profile of body movements. Time ‘0’ denotes the conditioned stimulus (CS) onset, the solid vertical line denotes startling acoustic stimulus (SAS) onset and the vertical dashed line...
denotes balance perturbation onset. Note that all events on the timeline are not present in all trial types. See Figure 1 for details.

Figure 3

Group mean (±1SE) onsets and amplitudes of right tibialis anterior (TA) and right gluteus medius (GM) EMG activity and onsets of COP for each of the 15 trials during the first block of Conditioning. For onsets, time ‘zero’ represents the onset of the balance perturbation. Amplitudes of EMG are scaled to peak values achieved during maximum voluntary contractions. Logarithmic trend lines were applied to illustrate both the steady decrease in onset and amplitude measures over the course of Conditioning trials and that they eventually leveled off. Data from the last 5 Conditioning trials were averaged ( ) and compared statistically to the mean values calculated during CS-Only trials ( ) where ‘ns’ denotes a non-significant effect ($p>0.05$) and ‘**’ denotes a significant effect ($p\leq0.05$).

Figure 4

M/L COP displacements produced during CS-Only (thin black) and post-Conditioning Startle (post; thick black) trials for all 12 subjects (i.e. S01-S12). Arrows indicate onsets for each trial; hollow arrowheads for CS-Only trials and solid arrowheads for post-Conditioning Startle trials. Vertical hashed lines denote onsets of the CS in each trial.
Figure 5

Group mean (±1SE) displacements of the right hip, right ankle and M/L COP in post-Conditioning Startle (post; black), CS-Only (dark grey) and generalized startle responses observed during pre-Conditioning Startle trials (pre; light grey). The vertical dashed line represents the onset of the CS and the solid vertical line represents the onset of the startling acoustic stimulus (SAS). Note: the SAS was present only during ‘pre’ and ‘post’ trials.

Figure 6

Angular displacements of the right ankle (black) and right hip (grey) during both CS-Only (thin lines) trials and post-Conditioning Startle (post; thick lines) trials for all 12 subjects (i.e. S₀₁-S₁₂). Vertical dashed lines denote onset of the conditioned stimulus (CS) in each trial. Onsets of angular accelerations are indicated by solid arrows during post-Conditioning Startle trials and by hollow arrows during CS-Only trials.

Figure 7

Representative subject EMG data for right (black and positive) and left (grey and negative) sternocleidomastoid (SCM), external oblique (EO), gluteus medius (GM), soleus (SOL) and tibialis anterior (TA) during single Conditioning and CS-Only trials. EMG amplitudes are normalized to maximal amplitudes achieved during maximum voluntary contractions. Vertical dashed line represents the onset of the conditioned stimulus (CS On) and the vertical solid line denotes onset of the leftward support-surface translation (Platform On).
Figure 8

Representative subject bilateral (right: positive and black; left: negative and grey) EMG responses for sternocleidomastoid (SCM), gluteus medius (GM) and tibialis anterior (TA) during a pre-Conditioning Startle trial and post-Conditioning Startle trial. Arrows indicate calculated onsets of muscle responses. Pre-Conditioning Startle trials evoked generalized startle responses with bilaterally symmetric onsets of EMG responses in all muscles. In post-Conditioning Startle trials, asymmetric responses were observed in 10 of 11 subjects for GM responses and only in 3 subjects for TA.
SAS
pre-Conditioning Startle (pre)
Conditioning trial (CSUS)
post-Conditioning Startle (post)

Trial Types

<table>
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<tr>
<th>Time (s)</th>
<th>CS-Only</th>
<th>pre-Conditioning Startle (pre)</th>
<th>Conditioning trial (CSUS)</th>
<th>post-Conditioning Startle (post)</th>
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<tbody>
<tr>
<td>0</td>
<td>CS</td>
<td>SAS</td>
<td>US</td>
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Methods

Quiet stance

Experimental Blocks

Methods

Counter-balanced

Fig1
Fig 2
<table>
<thead>
<tr>
<th>Conditioning Trial #</th>
<th>Right TA</th>
<th>Right GM</th>
<th>COP</th>
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<tr>
<td>1 3 5 7 9 11 13 15</td>
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<tr>
<td>ns</td>
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<tr>
<td>Onset Latency (s)</td>
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<td>1 3 5 7 9 11 13 15</td>
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</table>
M/L Centre of Pressure

Fig 4

Time (s)

Displacement (cm)
Fig 5
Fig8
Table 1 – Group Mean ±1 Standard Error for onset, peak displacement and time-to-peak measures of COPx, ankle and hip displacements during CS-Only and post-Conditioning Startle (post) trials

<table>
<thead>
<tr>
<th></th>
<th>COPx</th>
<th></th>
<th>Ankle</th>
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<th>Hip</th>
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<tr>
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<td>CS-Only</td>
<td>post</td>
<td>CS-Only</td>
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<tr>
<td>Onset</td>
<td>215 ±18ms</td>
<td>104 ±13ms**</td>
<td>318 ±25ms</td>
<td>191 ±16ms**</td>
<td>237 ±15ms</td>
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<tr>
<td>Peak Displacement</td>
<td>-2.75 ±0.40cm</td>
<td>-3.06 ±0.57cm</td>
<td>-2.18 ±0.19deg</td>
<td>-1.71 ±0.45deg</td>
<td>2.55 ±0.30deg</td>
</tr>
<tr>
<td>Time-to-Peak</td>
<td>198 ±14ms</td>
<td>153 ±19ms#</td>
<td>608 ±43ms</td>
<td>433 ±53ms**</td>
<td>518 ±42ms</td>
</tr>
</tbody>
</table>

** denotes significant difference between 'CS-Only' and 'post'

# denotes trend towards difference between 'CS-Only' and 'post' (0.05 > p ≤ 0.10)