Startle induces early initiation of classically conditioned postural responses

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Campbell AD, Chua R, Inglis JT, Carpenter MG. Startle induces early initiation of classically conditioned postural responses. J Neurophysiol 108: 2946–2956, 2012. First published September 12, 2012; doi:10.1152/jn.01157.2011.—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 generalized startle responses were induced, standing posture was perturbed in 2 blocks of 15 Conditioning trials, where in each trial the onset of a nonstartling auditory cue [i.e., a conditioned stimulus (CS)] preceded a leftward support-surface translation. Upon completion of 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 center 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.

MacKinnon and colleagues (2007) have recently demonstrated that anticipatory postural adjustments, the postural response (PR) elicited before self-initiated movements (Bouisset and Zattara 1987; Massion 1992), may also be incorporated into the preparation of voluntary motor behaviors. 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 feed-forward 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 (Allum and Honegger 1998; Carpenter et al. 1999; Horak and Nashner 1986; Nashner 1977, 1983). 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 before 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 Disterhoff 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 nonstartling 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 (Kolb et al. 2002; Taub et al. 1965). 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 15 min after the original conditioning procedure and subsequent distractor trials (Campbell et al. 2009). These results suggested that conditioned PRs could be evoked by nonpostural 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 present investigation was to extend the work of Campbell et al. (2009) by using a SAS as a probe to determine whether 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 before providing their informed consent to participate in the study. As will be described later, the total dataset was reduced to 12 subjects (19–32 yr of age, 7 men, mean height and body mass ±1 SD: 1.76 ± 0.77 m and 72.50 ± 8.42 kg, respectively). All subjects were completely naive to the experimental procedures before arriving at the laboratory.

Experimental Setup

Kinetics and kinematics. A force plate (no. K00407, Bertec) was used to sample ground reaction forces and moments along and around all axes, respectively, which were independently amplified (AM-6100, Bertec) and individually A/D sampled at 1,000 Hz (Power1401, Cambridge Electronic Design). These data were digitally low-pass filtered offline at 5 Hz (Spike5, Cambridge Electronic Design) and were used to calculate center of pressure (COP) in the anterior-posterior and mediolateral (M/L) directions for each trial (Matlab 7.0, The Mathworks).

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

Raw marker positions were low-pass Butterworth filtered at 5 Hz offline prior to applying the subject-specific kinematic models and calculating ankle and hip angular displacements in the frontal-plane (Visual3D, C-Motion). 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. Electromyography (EMG) was recorded bilaterally from tibialis anterior (TA), soleus, gluteus medius (GM), external oblique, and sternocleidomastoid (SCM). Two pregelled Ag/AgCl surface electrodes were placed ~2 cm 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 preamplified ×500, sampled at 3,000 Hz, and band-pass filtered between 10 and 500 Hz (Telemyo 2400R, Noraxon) online, before being A/D converted at 1,000 Hz (Power1401, Cambridge Electronic Design). These data were subsequently digitally high-pass filtered at 30 Hz (Spike2, Cambridge Electronic Design) offline to remove heart rate artifacts, baseline corrected, and then full-wave rectified.

Experimental Procedures

Participants stood on the force plate that was centered within, and flush with, the surface of a wooden stage (1.23 m wide; 0.61 m long) affixed to a translating sled (DR Stage, H2W Technologies). 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 ~2 m away.

Quiet stance and pre-Conditioning Startle trials. Subjects were first asked to stand quietly for 60 s while looking straight ahead at the target. A range of normal frontal-plane sway was calculated from this period as the mean ±1 SD of the M/L moment. Once the 60-s trial was completed, a subsequent 30 s of quiet stance took place during which time two auditory stimuli were unexpectedly presented in randomized order (Fig. 1) and separated by at least 20 s. One stimulus was a nonstartling tone (<80 dB, 200-ms duration), which later...
served as the CS, to ensure it did not evoke startlelike reflexes (Campbell et al. 2009) or any detectable movement in the frontal-plane. The other stimulus was a calibrated SAS (~120 dB, 1,000 Hz, 40-ms duration, ~1-ms rise-time) (CR:231B Impulse sound level meter, Cirrus Research plc), which was used to evoke a generalized startle response; termed the pre-Conditioning Startle trial (Fig. 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 (1-m displacement, 0.25 m/s velocity, 1.3 m/s² acceleration) presented 300 ms after the onset of the auditory CS (Fig. 1). Any sound generated by the support-surface translation was determined to be <80 dB (CR:231B Impulse sound level meter, Cirrus Research plc). The temporal relationship between CS and US was consistent with trace conditioning paradigms, whereby their relative timing produced a 100-ms interstimulus 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 foreperiod lasting between 10 s and 25 s 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 (Fig. 1) to generate a conditioned PR (Campbell et al. 2009; Kolb et al. 2002). Following completion of the second Conditioning block, a single post-Conditioning Startle trial was conducted which involved a SAS presented 50 ms after the onset of the CS in the absence of a support-surface translation (Fig. 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 (Campbell et al. 2009; Woodruﬀ-Pak and Disterhoft 2008). 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 force plate 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 60 s of quiet stance. If a persistent lateral lean was observed, subjects were verbally coached back to resting positions.

**Measures**

**Kinetics and kinematics.** Thresholds for determining onsets of COP displacements were established by calculating the mean ± 1 SD of angular accelerations from 500 ms 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 150 ms. If onsets were detected, peak displacements were calculated as the greatest relative change from mean values calculated from 500 ms of prestimulus 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 ± 2 SD of background EMG data recorded from a 500-ms period that immediately preceded the start of each trial. Using a semiautomated 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 30 ms while at no time dipping below for >3 ms (Carpenter et al. 2008).

Onsets of PRs evoked during Conditioning, CS-Only, and post-Conditioning Startle trials were accepted if they fell within a time frame that began 90 ms after CS onset and ended 220 ms after US onset. The former is within range of reported mean onsets of practiced PRs in lower limb muscles triggered by nonstartling 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 ± 2 SD 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 90 ms 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 100 ms after onset, minus resting activity from equivalent time periods prior to the trial. The analysis window was set to 100 ms because it is a common time frame 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 that 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 one SCM muscle within a 90-ms 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 right GM (pGM; n = 11) and right TA (pTA; n = 10), to a lesser extent in left GM (lGM; n = 7) and left TA (lTA; n = 6) and rarely observed in other muscles. Therefore, subsequent analysis of CS-Only responses was focused on pGM and pTA.

It was important to further examine EMG responses observed in pGM and pTA 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 between bilateral GM muscles or bilateral TA muscle activity did not exceed a mean ± 2 SD range of bilateral generalized startle response onsets calculated from pre-Conditioning Startle trials (GM: 7 ± 44 ms; TA: 2 ± 68 ms). Based on these criteria, 10 of 11 subjects generated asymmetrical GM activity in post-Conditioning Startle trials that was distinguishable from generalized startle responses. Therefore, pGM responses of remaining subjects were included in further analyses. In contrast, only three 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 pTA 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 (Fig. 2). Therefore, prepared responses and generalized
Analysis and Statistics

Onsets and amplitudes of COP and EMG responses from the last five 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 sGM measures were compared between CS-Only and post-Conditioning Startle trials using pairwise t-tests. In all cases, significance was set at probability values ≤ 0.05, and significant trends were considered at P values between >0.05 and ≤0.10.

RESULTS

Kinetics and Kinematics

Conditioning compared with CS-Only trials. During Conditioning trials, the leftward support-surface translation initially induced ankle eversion, hip adduction, and rightward displacements of the COP (Fig. 2). This initial response was quickly followed by ankle inversion and hip abduction and leftward COP displacements (Fig. 2). Over the course of the 15 Conditioning trials, onsets of COP displacements progressively decreased and plateaued by the end of the block (Fig. 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 (Fig. 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) (Fig. 3), whereas peak COP displacements were found to be significantly attenuated in CS-Only compared with Conditioning trials [t(11) = 18.62, P < 0.001].

CS-Only compared with post-Conditioning Startle trials. CS-Only and post-Conditioning Startle trials both induced initial
rightward COP displacement pattern (Fig. 2) that was highly consistent across subjects (Fig. 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 nonspecific, displacements associated with the generalized startle response (Figs. 2 and 5). Mean onsets of M/L COP displacement were found to be significantly earlier (110 ms earlier) in post-Conditioning Startle trials compared with CS-Only trials \( t(11) = 4.66, P = 0.001 \), whereas peak (\( P = 0.566 \)) of COP displacements was not different between CS-Only and post-Conditioning Startle trials (Fig. 5 and Table 1). A significant trend for quicker time-to-peak COP displacements in post-Conditioning Startle trials was observed in the ankle \( t(11) = 3.41, P = 0.006 \) with significant trends toward quicker time-to-peaks observed in the hip \( t(11) = 1.97, P = 0.074 \) (Table 1).

**EMG**

*Conditioning compared with CS-Only trials.* Leftward support-surface translation elicited a pattern of muscle activity that included responses in external oblique, GM, soleus, and TA muscles, primarily on the right side of the body (Fig. 7). As shown in Fig. 3, muscles that most frequently showed responses to CS-Only trials (RTA and RGM) 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 (\( R TA: P = 0.285; R GM: P = 0.263 \) (Fig. 3). Amplitudes of \( R TA \) were significantly attenuated in CS-Only trials compared with Conditioning trials \( t(9) = 3.19, P = 0.011 \), whereas no differences in amplitude were observed in \( R GM (P = 0.394) \) (Fig. 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 70 ms in Conditioning trials and 11 ms in CS-Only trials.

*CS-Only compared with 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 ± 6 ms and 54 ± 7 ms for left and right SCM, respectively. These SCM response onsets were similar to those observed during gener-
alized startle trials (Fig. 8) and were within previously reported ranges of mean onsets of startle-induced SCM activity (MacKinnon et al. 2007; Oude Nijhuis et al. 2010; Siegmund et al. 2001).

Onset of RGM responses were significantly earlier \( t(9) = 5.14, P = 0.001 \) in post-Conditioning Startle compared with CS-Only trials (average difference \( t(9) = 125 \pm 25 \) ms). The amplitudes of RGM were significantly larger in post-Conditioning Startle compared with CS-Only trials \( t(9) = 2.49, P = 0.034 \).

**DISCUSSION**

The aim of the current investigation was to determine whether 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 with nonstartle 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 multijoint 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 preestablished criteria for conditioned response acquisition; CS-Only trials evoked frontal-plane COP displacements and muscle responses in \( R_{TA} \) and \( \gamma_{GM} \) 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 (Kaulich et al. 2010; Kolb et al. 1997, 2002; Woodruff-Pak and...

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Fig. 4. M/L COP displacements produced during CS-Only (thin black lines) and post-Conditioning Startle (post; thick black lines) 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 shaded lines denote onsets of the CS in each trial.

Fig. 5. Group mean (± 1 SE) displacements of the right hip, right ankle, and M/L COP (COPx) in post-Conditioning Startle (post; black lines), CS-Only (dark gray lines), and generalized startle responses observed during pre-Conditioning Startle trials (pre; light gray lines). The vertical dashed line represents the onset of the CS, and the solid vertical line represents the onset of the SAS. Note: the SAS was present only during “pre” and “post” trials.
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 behaviors that can be prepared in advance and evoked by

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**Table 1.** Group values 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>Ankle</th>
<th>Hip</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CS-Only</td>
<td>Post</td>
<td>CS-Only</td>
</tr>
<tr>
<td>Onset</td>
<td>215 ± 18 ms</td>
<td>104 ± 13 ms*</td>
<td>318 ± 25 ms</td>
</tr>
<tr>
<td>Peak displacement</td>
<td>−2.75 ± 0.40 cm</td>
<td>−3.06 ± 0.37 cm</td>
<td>−2.18 ± 0.19°</td>
</tr>
<tr>
<td>Time-to-peak</td>
<td>198 ± 14 ms</td>
<td>153 ± 19 ms#</td>
<td>608 ± 43 ms</td>
</tr>
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Values are means ± 1 SE. COPx, center of pressure displacement. CS, conditioned stimulus. *Significant difference between CS-Only and Post. #Trend toward difference between CS-Only and Post (0.05 > P ≤ 0.10).

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**Fig. 6.** Angular displacements of the right ankle (black lines) and right hip (gray lines) 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 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.
SAS. While the behaviors 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 behaviors. 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 centers that our results suggest are also involved in preparing conditioned PRs. SAS was originally thought to influence prepared voluntary movement via interactions with brain stem 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. 2012). It has been posited that similar brain stem (Honeycutt et al. 2009, 2010; Jacobs and Horak 2007) and cortical centers (Adkin et al. 2008; Jacobs and Horak 2007; Taube et al. 2006) may be involved in the regulation of dynamic postural control as well. Particularly with respect to the brain stem, researchers believe it may be a site containing representations of PR motor synergies (Honeycutt et al. 2010; Jacobs and Horak 2007). 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.

Fig. 7. Representative subject EMG data for right (black lines and positive) and left (gray lines and negative) sternocleidomastoid (SCM), external oblique (EO), GM, soleus (SOL), and 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 CS (CS On), and the vertical solid line denotes onset of the leftward support-surface translation (Platform On).
by SAS, this suggests 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 with unexpected perturbations in EMG onset latencies and amplitudes or COP 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). 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 from trial to trial, and also being invariably linked to the CS, evoked

![Fig. 8. Representative subject bilateral (right: positive and black lines; left: negative and gray lines) EMG responses for SCM, GM, and 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.](image-url)
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 with CS-Only trials, the decreases in PR onsets observed in post-Conditioning Startle trials were well beyond the reported 20- to 50-ms effect of intersensory facilitation (Nickerson 1973) and the ~20-ms decreases attributed to stimulus intensity effects (Carlsen et al. 2007). Even the summation of intersensory facilitation and stimulus intensity effects hardly approach the >100-ms 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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DISCLOSURES

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AUTHOR CONTRIBUTIONS

Author contributions: A.D.C. and M.G.C. conception and design of research; A.D.C. performed experiments; A.D.C. analyzed data; A.D.C., R.C., T.J.I., and M.G.C. interpreted results of experiments; A.D.C. prepared figures; A.D.C. drafted manuscript; A.D.C., R.C., T.J.I., and M.G.C. edited and revised manuscript; R.C., T.J.I., and M.G.C. approved final version of manuscript.

REFERENCES


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