INSIGHTS INTO HUMAN DYNAMIC BALANCE CONTROL: POSTURAL RESPONSE INITIATION EXPLORED THROUGH CLASSICAL CONDITIONING AND STARTLE

by

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Abstract

As a scientific discipline, dynamic posturography aims to understand the neurological and biomechanical mechanisms that contribute to postural stability and corrective postural responses (PRs). The main focus of this thesis was to better understand the neurophysiology of corrective PRs that prevent falls that emerge from external forces applied to the body by balance perturbations. In a sequence of 4 studies, this thesis utilized novel applications of established techniques (classical conditioning and startle paradigms) to address questions regarding the role of sensory feedback in PRs initiation and the nature of PRs that are evoked by balance perturbations.

The first of 4 experiments tested the link between sensory feedback derived by balance perturbations and PR initiation by attempting to trigger PRs using auditory cues that, prior to classical conditioning provided no relevant information pertaining to balance perturbations or postural stability. The second study examined the extent to which conditioned PRs may exist as prepared motor behaviours that could be initiated by startling acoustic stimuli in the absence of balance perturbations. The third study attempted to extend the previous findings of PR motor preparation into a more ecologically valid scenario involving unexpected balance perturbations. The fourth and final study in this thesis examined whether startle responses could contribute to first-trial effects observed on PRs evoked by the first in a repeated sequence of balance perturbations.

Individually, each study provided highly novel contributions to the field of dynamic posturography. However, when taken together, they provide novel insight into both the mechanisms involved in PR initiation and the understanding of reactions evoked by balance perturbations.

Preface

All of the work presented henceforth was conducted in the Neural Control of Posture and Movement Laboratory at the University of British Columbia, Point Grey campus. All projects and associated methods were approved by the University of British Columbia's Research Ethics Board [certificate #H06-04047].

A version of Chapter 2 has been published [Campbell AD, Dakin CJ, Carpenter MG. Postural responses explored through classical conditioning. Neurosci 164:986-997, 2009]. I was the lead investigator, responsible for all major areas of concept formation, data collection and analysis, as well as manuscript composition. Dakin CJ was involved in the early stages of concept formation and contributed to manuscript edits. Carpenter MG was the supervisory author on this project and was involved throughout the project in concept formation and manuscript composition.

A version of Chapter 3 has been published in the Journal of Neurophysiology [Campbell AD, Chua R, Inglis JT, Carpenter MG. Startle induces early initiation of conditioned postural responses. J Neurophysiol doi: 10.1152/jn.01157.2011]. I was the lead investigator, responsible for all major areas of concept formation, data collection and analysis, as well as the majority of manuscript composition. Chua R and Inglis JT were involved in the early stages of concept formation and contributed to manuscript edits. Carpenter MG was the supervisory author on this project and was involved throughout the project in concept formation and manuscript edits.

I was the lead investigator for the projects located in Chapters 4 and 5 where I was responsible for all major areas of concept formation, data collection and analysis, as well as the majority of manuscript composition. Chua R and Inglis JT were involved in the early stages of concept formation and contributed to manuscript edits. Squair JW contributed to data collection and manuscript edits. Carpenter MG was the supervisory author on this project and was involved throughout the project in concept formation and manuscript edits.

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List of Abbreviations

A/D: Analog-to-digital

ANOVA: Analysis of variance

A/P: Anterior-posterior

BoS: Base of support

COM: Centre of mass

COP: Centre of pressure

CNS: Central nervous system

CR: Conditioned response

CS: Conditioned stimulus

dB: Decibels

EMG: Electromyography

EO: External oblique

ECR: Extensor carpi radialis

FCR: Flexor carpi radialis

FTEs: First trial effects

GM: Gluteus medius

iRED: infra-red light emitting diode

IS: Imperative stimulus

LMN: Lower motoneuron

M/L: Medio-lateral

PR: Postural response

RF: Rectus femoris

SAS: Startling acoustic stimulus

SCM: Sternocleidomastoid

SD: Standard deviation

SE: Standard error

SOA: Stimulus onset asynchrony

SOL: Soleus

TA: Tibialis anterior

UR: Unconditioned response

US: Unconditioned stimulus

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Dedication

To those whose guidance, wisdom and love saw me through the emotional odyssey of graduate school, I dedicate this thesis to you.

Chapter 1: Literature Review

1.1 Introduction

The scientific investigation of standing balance addresses questions of how upright posture is maintained and falls are prevented. The importance of understanding balance rests on the significant detrimental impacts of falls. From a standing position, falls lead to high-speed impacts with the ground and related injuries including fractures to the hip, soft tissue contusions and head injuries (Tinetti and Williams, 1997). Those that experience fall-related injuries suffer emotional consequences related to fear of future falls, which leads to avoidance of activity (Kosorok et al., 1992) and reduced quality of life (Zijlstra et al., 2006). Falls are a leading cause of admittance to long-term care facilities in elderly individuals (Tinetti and Williams, 1997), which places an enormous financial strain on healthcare systems and on families (Stevens et al., 2006). Thus, the high financial costs as well as the negative emotional and societal impacts associated with falls makes understanding dynamic postural control and its basic neurophysiology of critical importance.

Healthy, young humans are generally well adept at maintaining bipedal stance and preventing falls. However, the ease with which humans achieve and maintain postural stability is at odds with the sheer complexity of the task. Coupled with the inherent mechanical instability of bipedal stance itself (Winter, 1995), humans experience balance perturbations from a multitude of external forces that can arise during everyday tasks. In each situation, rapid detection of destabilizing forces and equally rapid initiation of highly specialized sequences of muscle activity are required to quickly counter the applied forces. Termed postural responses (PRs), these motor responses are the neurophysiological consequence of balance perturbations and offer the first lines of defense against preventing falls.

1.1.1 Postural responses

Stable bipedal stance is achieved by maintaining the vertical projections of the body's centre of mass (CoM) within the base of support (BoS) defined by the outer borders of the feet. Shortly following the onset of a balance disturbance, body segments that become displaced may cause the body's CoM to become repositioned relative to the BoS. If the

postural perturbation is of sufficient amplitude as to deviate the CoM towards the borders of the BoS, appropriate sequences of muscle responses are required to either arrest the induced body sway (Winter, 1995) or to adjust the BoS using compensatory stepping or grasping (Maki et al., 2000) that ensure the CoM stays within the BoS. Muscular activity (either excitatory or inhibitory) that facilitates a rapid reorientation of body segments to regain stability is considered a PR. Whether a muscle becomes engaged in the PR depends heavily on the way in which balance was perturbed. However, those muscles that become engaged do so in a highly stereotyped fashion whose relative timing and amplitudes between muscles are specific to the nature of the postural perturbation (Nashner, 1977, 1983; Henry et al., 1998; Carpenter et al., 1999) the context in which postural perturbations were delivered (Horak and Nashner, 1986; Carpenter et al., 2004) and previous experience (Keshner et al., 1987).

1.1.2 PR initiation

Studying how PRs are initiated in the real world is nearly impossible to accomplish. Real world perturbations that lead to falls or near falls are unpredictable events; thus, PRs that occur in the real world are equally unpredictable and challenging to capture with scientific equipment. In the laboratory setting, however, researchers are afforded tight control over environmental variables and use of scientific equipment that makes it possible to examine PRs as well as the circumstances that lead individuals to initiate appropriate PRs.

The techniques used to induce PRs in the laboratory setting involve applying destabilizing forces that disturb relationship between the CoM and the BoS. For example, translations or rotations of the support-surface are commonly used to induce PRs via rapid linear or angular displacements of the BoS, respectively (Henry et al., 1998; Bloem et al., 2002). Other commonly used techniques, such as a tether-release from a leaning position (Mackey and Robinovitch, 2006) or proximal segment pushes/pulls (Adkin et al., 2008) induce postural instability by perturbing the upper-body directly. Each method for disturbing balance imposes a unique set of destabilizing forces on the body and thus causes a unique set of physical movements that if left uncorrected, would lead to a fall.

When stable stance has been compromised, rapid onsets of PRs are required to prevent falls and related injuries. A PR, no matter how well coordinated or modulated,

cannot prevent a fall unless it is initiated with short latency following a balance perturbation. In healthy individuals, successful maintenance of stance is accomplished by PRs observed in an array of lower-limb, trunk, neck and shoulder muscles that typically occur within 80-200ms of a perturbation to balance (Diener et al., 1988; Allum and Honegger, 1997; Allum and Pfaltz, 1985; Keshner et al., 1987; Carpenter et al., 1999, 2004). This highly rapid timeframe has been verified by many researchers using various forms of balance perturbing stimuli and has been used to define PRs under a class of movement that is too slow to be stretch reflex-like and too fast to be voluntary (Nashner and Cordo, 1981).

1.1.3 Multi-sensory experiences induced by balance perturbations

Before PRs are initiated, destabilizing forces applied to the body induce multi-sensory feedback that provides our nervous system with specific information pertaining to body movement and how the environment has changed as a result of balance perturbations. Because balance perturbations can be of an almost infinite variety, the sensory consequences that originate from them are equally as diverse.

Consider a situation while standing where the support-surface is rapidly rotated in the toes-up direction. At the very instant that the support-surface is displaced, changes in the configuration of the body quickly emerge; ankle dorsi-flexion that occurs in tandem with changes to the support-surface are shortly followed by coupled knee extension and hip flexion (Carpenter et al., 1999) as well as vertical accelerations of the head (Allum and Pfaltz, 1985). Three sensory systems in particular (vestibular, visual and proprioceptive) are able to transduce the specific physical consequences of balance perturbations into a redundant array of neural signals that provide almost immediate feedback to the CNS regarding postural stability. A major focus of research in the neurophysiology of human dynamic postural control has centred on understanding how each of these sensory signals, in isolation or in combination, are used to influence PR initiation and modulate PR amplitudes.

1.2 Part I: Sensory contributions to PR initiation

1.2.1 Individual sensory contributions to PR initiation

Vestibular contributions to PR initiation

The vestibular apparatus is a bilateral sensory organ located within a bony labyrinth of the inner ears that is comprised of fluid-filled otoliths and semi-circular canals (Kandel et al., 2004). When head position is displaced, hair cells (i.e. cilia) imbedded in an active membrane become mechanically strained, which allows for head rotations and translations to be transduced into neural signals used to interpret head and body movements (MacPherson et al., 2007) and body position relative to vertical (Kandel et al., 2004).

Direct electrical stimulation to the vestibular system is known to induce rapid changes in lower-limb reflexes and whole-body sway (Britton et al., 1993; Dakin et al., 2007). Movements induced by vestibular stimulation are the result of the afferent connections to efferent motor pathways (i.e. the vestibulo-spinal tract) at the vestibular nuclei in the brainstem (Kandel et al., 2004). These efferent pathways innervate axial and appendicular muscles throughout the body (Britton et al., 1993) and thus form the link between vestibular stimulation and related motor outputs.

Coupled with the ability to induce movement via vestibular stimulation, evidence of head accelerations experienced at short latency following balance perturbations (Allum and Pfaltz, 1985; Runge et al., 1998; Carpenter et al., 1999) suggests that signals originating from the vestibular system could provide the initial neural impulse used to trigger PRs. The role of the vestibular system in PR initiation has been tested mostly using bilateral vestibular loss (BVL) patients (whose vestibular afferents have been rendered entirely incapable of encoding changes in head position) in balance perturbing paradigms. PRs evoked in various muscles show a variable sensitivity to the presence of vestibular inputs. In some situations, subtle delays in PRs have been observed in BVL patients compared to controls (Allum and Pfaltz, 1985; Keshner et al., 1987) whereas others report similar onset latencies between groups (Runge et al., 1998). Separate from changes in onset times, BVL patients are consistently able to evoke PRs in at least a few muscles (Allum and Pfaltz, 1985; Keshner et al., 1987; Horak et al., 1990; Allum et al., 1994; Allum and Honegger, 1998; Runge et al.,

1998), which suggests that the vestibular system may not be solely responsible for PR initiation. Further support for a lack of vestibular involvement in PR initiation is provided by work using healthy subjects where experimenters attempt to recreate PRs observed after support-surface perturbations by providing isolated perturbations to the head during quiet stance (Horak et al., 1994). The results demonstrated that, although isolated head displacements could induce motor responses in proximal and distal muscles, the amplitudes of the evoked responses and the pattern across muscles were dissimilar compared to those evoked by whole-body balance perturbations.

Visual contributions to PR initiation

The visual system is comprised of the eyes, retina and optic nerve. The retina is a photosensitive organ, which transduces light passing through the eyes into neural signals that jointly travel along the optic nerve towards the occipital lobe and ocular centres in the brainstem where they are interpreted to provide a sense of verticality and movement (Kandel et al., 2004). In the absence of any physical perturbations, input into the visual system via visual scene displacements can induce postural realignment and whole-body sway through a process known as vection (Lestienne et al., 1977). Vection is the perception of self-motion that is caused by a conflict between changes to the visual scene that does not align with inputs from other sensory systems (Lestienne et al., 1977). When induced during quiet stance, this sensory conflict is known to cause muscle contractions and whole-body sway. Various forms of visual scene displacements are known to induce motor responses in postural muscles (Lee and Lishman, 1975; Lestienne et al., 1977; Nashner and Berthoz, 1978; Keshner et al., 2004). As such, many believed that the visual field changes caused by balance perturbations might contribute to PR initiation. Although the visual system may be capable of inducing vection-related movement, the available evidence suggests that the visual system alone does not trigger PRs. The motor responses that are typically observed after rapid displacements of the visual scene occur as early as 1.2s (Lestienne et al., 1977) which is well outside of known PR onset latencies (Nashner and Cordo, 1981). Furthermore, the availability of visual information altered by simple eye closure, pathology or otherwise does not have a significant influence over PR onset latencies (Allum and Pfaltz, 1985; Manchester et al., 1989; Sundermier and Woollacott, 1998; Carpenter et al., 1999; Nataka

and Yabe, 2001). Even when vestibular loss subjects receive balance perturbations in the absence of vision, PR onset latencies are generally unaffected (Allum et al., 1995).

Proprioceptive contributions to PR initiation

Proprioception is the sense provided by muscle spindles, golgi tendon organs and cutaneous receptors imbedded within muscles, joints and skin, respectively (Kandel et al., 2004). Proprioceptors collectively provide feedback pertaining to changes in muscle length, muscle force as well as skin deformation that describes the movement of segments and joints and the sense of body position (Kandel et al., 2004).

When stimulated, proprioceptors are capable of inducing muscle contractions and changes to reflex loop physiology. Muscle spindles are length-sensitive proprioceptors that can induce rapid muscle contraction via afferent connections with alpha motor neurons in the spinal cord (Kandel et al., 2004). Golgi tendon organs are load-sensitive receptors embedded within the musculo-tendinous junction that are known to alter phase-dependent reflex pathways (Kandel et al., 2004). Cutaneous receptors are imbedded within the skin of the human body and collectively code for various forces and deformation patterns (Johnson, 2001). Direct electrical stimulation to cutaneous afferents (Zehr and Kido, 2001) or vibratory stimulation of the receptor itself (Kavounoudias et al., 2001) can induce a host of motor and reflex-tuning responses in muscles of the upper- and lower-body.

Balance perturbations of any type cause appreciable segment displacements that stretches and loads muscles while straining the skin located around joints and on the foot sole (Magnusson et al., 1990). Because proprioceptors are located throughout the body, they provide high-resolution information regarding the widespread physical effects of balance perturbations. Also because of their widespread locations, it has been problematic for researchers to examine their isolated effects and thus examine the totality of their role in balance correction. Consequently, the contributions of proprioceptors to PR initiation have mostly been examined in the lower limbs.

The inability for subject I.W. to maintain bipedal stance perhaps provides the best example of the important role for proprioception in dynamic postural control (Cole and Sedgwick, 1992). Subject I.W. has a large-scale proprioceptive deficit caused by pathology to large diameter afferents that emanate from muscle spindles and cutaneous

mechanoreceptors. Thus, I.W.'s sense of limb position and movement was dramatically reduced and, in the immediate term following the onset of the pathology, rendered him entirely incapable of standing. Due to the widespread sensory deficit, this particular case cannot provide details pertaining to the specific aspects of proprioception that most influence PR initiation. However, cases of proprioceptive loss isolated to the leg have provided a useful model from which to understand the influence of proprioception in PR initiation. In the absence of proprioceptive sense at, and distal to, the ankle joint, perturbations to upright stance induce PRs with normal timing (Bloem et al., 2002). However, when proprioception is eliminated from the entire leg, PRs are delayed in some muscles, yet they still occur within 200ms of perturbation onset (Bloem et al., 2002) and thus are within range of an otherwise 'healthy' PR. These data suggest that collective afferent feedback generated by muscle spindles, golgi-tendon organs or cutaneous receptors of the leg are not critical to initiate PRs. These observations have led researchers to postulate that the proprioceptive signal most critical to PR initiation may reside within more proximal hip and trunk areas (Allum et al., 1995; Bloem et al., 2002). However, direct tests of this proposal have yet to be conducted.

Other experiments that have influenced individual aspects of the proprioceptive system further argue that it is not critically involved in triggering PRs. Using perturbations where ankle joint angles are nulled or enhanced show no apparent disparity in PR timing (Allum and Honegger, 1997; Bloem et al., 2002) suggesting that stretch and load receptors in muscles that span the ankle joint likely do not affect PR initiation. In addition, PRs evoked while partially submerged in water are normally timed despite a reduction in load applied to muscles of the leg and pressure applied to the foot sole (Dietz et al., 1989). Delays in PR onsets are also unaffected by cutaneous afferent cooling or anesthesia localized to the lower-leg or the foot sole which temporarily reduces the reliability of cutaneous feedback (Do et al., 1994; Perry et al., 2000).

Other sensory contributions to PR initiation

When balance perturbations displace the body, it is thought that receptors known as graviceptors located in the thorax can detect changes in vertical orientation and thus could potentially contribute to realignment responses (Mittelstaedt et al., 1996). To date,

graviceptors have not been extensively investigated and thus their role in PR initiation is mostly unknown.

1.2.2 Integrated sensory contributions to PR initiation

Researchers have used many independent techniques to isolate the potential influences of sensory feedback in order to understand the factors that contribute to PR initiation. The ability to still initiate PRs while using subjects with pathological sensory deficits and protocols that limit the extent of sensory feedback suggest that there is no single source of feedback responsible for triggering PRs. The common conclusion from work that unsuccessfully delayed or abolished PRs is that remaining systems could be more heavily involved in PR initiation (Allum et al., 1995). The 'sensory organization test' has provided a means to assess, the relative contributions of different sources of sensory feedback to PR initiation in healthy subjects. Through an iterative process by which visual, proprioceptive and vestibular contributions to PR can be manipulated, results from sensory organization tests suggest that perhaps a hierarchy exists wherein the absence of 'high-level' sensory feedback that is preferentially used to trigger PR, lower level aspects could be used if necessary (Nashner et al., 1982). This theory may help explain why, in previous studies, PRs continue to be initiated despite significant deficits to normal sensory function.

1.2.3 General interpretation of sensory contributions to PR initiation

The information provided to the CNS by sensory feedback is critical to the initiation of PRs. However, the contributions of each system and how they are integrated into the processes of PR initiation have yet to be determined. Although different in many respects, prior investigations into PR initiation have consistently introduced balance perturbations as a means of evoking PRs. As later sections will describe, it may be possible to use alternative means to trigger PRs that do not involve physically displacing the body. Investigating PRs in this manner would allow for the apparent link between perturbation-induced sensory feedback and PR initiation to be examined in a manner not common in dynamic posturography research.

1.3 Part II: What is triggered by balance perturbations?

The diverse arrays of PRs that emerge following destabilization have led to various theories regarding their neural origin and how they are represented within the nervous system. The theories that attempt to explain 'what' PRs are, have significantly changed over time, where the earliest theories described PRs as a sequence of stretch reflexes whereas more contemporary theories describe PRs as postural synergies stored centrally and are selected and triggered by sensory input.

1.3.1 Stretch-induced reflexes

Originally, due to the timing of perturbation-evoked PRs, it was conceived that they were simply a set of 'functional stretch reflexes' induced by a muscle stretch induced by balance perturbations (Nashner, 1977) that propagated through the body in a distal to proximal fashion that began in the muscles spanning the ankle joint and progressed rostrally to the muscles of the upper-leg. Although this concept provided a framework from which to begin to understand PRs, more recent studies suggest that to consider PRs as stretch reflexes is a dramatic oversimplification. One aspect of PRs that cannot be explained by stretch reflexes is the upper-body muscle activity that occurs near the onset of lower-limb stretch reflexes caused by support-surface balance perturbations (Keshner et al., 1988). During support-surface perturbations, the forces that lead to stretching of muscle originate distally and propagate proximally in an orderly fashion across segments. For the observation of upper-body responses to be the consequence of stretch to proximal muscles, concomitant stretch of upper and lower-body muscles would be required, which cannot occur when using support-surface perturbations. Another argument against functional stretch reflexes is that while PRs are always considered assistive in regaining stability, stretch reflexes induced by perturbations do not always generate forces that act to re-stabilize the body. In fact, during support-surface rotations, stretch reflexes that emerge in the lower limb actually further destabilize posture (Diener et al., 1983, 1984; Carpenter et al., 1999). The most convincing evidence against PRs being a series of stretch reflexes is in the fact that PRs can even be observed in muscles that are unable to produce a stretch reflex response because of pathologies (Bloem et al., 2002) or experimental techniques that restrict muscle lengthening (Allum and Honegger, 1997; Bloem et al., 2002).

PRs have also been described by another type of stretch-induced response termed long-latency reflexes. The sequence of muscle responses that follow rapid displacements of the support-surface have been categorized based on their timing as either short-, medium- or long-latency reflexes (Diener et al., 1984, 1985). When evoked by support-surface toes-up rotation, the first 2 responses are invariably present in lengthened muscle and represent a motor response driven by spindle feedback to spinal centres (Schieppati and Nardone, 1997). Long latency reflexes, although commonly observed in stretched muscles, are also observed in antagonists to stretched muscles during quiet stance (Allum and Büdingen, 1979; Diener et al., 1983, 1984) and thus they can serve in postural correction. Their relatively long latency compared to other stretch-induced components is thought to emerge from a relatively longer conduction pathway that instead of terminating in the spinal cord, projects towards supra-spinal and cortical centres (Deuschl et al., 1989). Evidence of cortical involvement during dynamic balance tasks (Taube et al., 2006; Jacobs and Horak, 2007; Adkin et al., 2008; Mochizuki et al., 2008) perhaps support existence of the same trans-cortical conduction pathway characteristic of other known long-latency reflexes that emerge from stretch of individual segments, such as the thumb, fingers, etc. (Deuchl et al., 1988). However, it is known that stretch reflexes are not a critical prerequisite to observing PRs in a particular muscle (Bloem et al., 2002). Also, context-specific PRs can be evoked in decerebrate animal preparations (Honeycutt et al., 2009) where trans-cortical pathways have been abolished. Therefore, it does not seem plausible that stretch or long-loop reflexes can explain the comprehensive nature of PRs evoked by balance perturbations.

1.3.2 Postural strategies/synergies

When examined as a whole, the PRs that emerge throughout the body can be seen as a highly organized sequence of muscle contractions. Thus, instead of describing each muscle response as an individual entity (like a stretch reflex), researchers have described the collection of responses observed across muscles under common headings such as postural ‘strategies’ or ‘synergies’. Strategies describe the net biomechanical outcome of a collection of muscle responses that serve a particular purpose or global objective (Runge et al., 1999). For example, the ‘ankle strategy’ or ‘hip strategy’ (Horak and Nashner, 1986) would describe a sequence of muscle contractions that mediate movement about the ankle or hip

joint, respectively. Postural synergies are also a collection of muscle responses, however, in contrast to strategies, have no apparent task-goal (like controlling ankle joint movement) but interact and combine with other synergies to prevent falls in different contexts (Ting and McKay, 2007).

Regardless of terminology, these proposed theories of PRs helps to rationalize how the nervous system manages the complex process of dynamic postural control. Each type of balance perturbation requires a highly specific sequence of muscle contractions to occur at very short latency to prevent falls. If the temporal and spatial characteristics for each muscle were controlled independently, the computational demand to complete even the simplest of required movements would be staggering. Synergies organize individual movement elements into a reduced set of units and thus, alleviate the burden of control by lessening the involvement of comprehensive central processing (Ting and McKay, 2007).

Evidence suggests that central factors may be involved in mediating the initiation of postural synergies. Evidence of motor-related cortical responses that precede balance perturbations (Jacobs and Horak, 2007; Mochizuki et al., 2008), the involvement of descending cortico-spinal tracts in postural correction (Taube et al., 2006) and the altered coordination of postural muscle activity in decerebrate animal preparations (Honeycutt et al., 2009) collectively suggests that the cortex may be involved in initiating postural synergies. Further indirect support a cortical role in postural synergy initiation emerges from known relationships between PR characteristics and psychological factors such as cognition (Maki and McIlroy, 2007), emotion (Carpenter et al., 2004), attention (Redfern et al., 2001) and central set (Horak et al., 1989). Evidence of subcortical contributions to postural synergies has emerged from data of patterned reticular neuron activity that relates to PRs evoked by different types of support-surface perturbations in the standing cat (Stapley et al., 2009) and by the preservation of at least rudimentary postural control strategies in decorticate animal preparations (Honeycutt et al., 2009). It has further been suggested that neural networks that reside within the spinal cord, likened to central pattern generators that regulate the sequencing and amplitude of muscle responses during gait, could reflect the neural representation of postural synergies (Dietz, 1992; Forssberg and Hirschfeld, 1994; Allum et al., 1998).

Despite their appeal, questions remain that ultimately challenge the existence of postural synergies. One question is whether or not the observed sequences of PRs that follow perturbation are represented as structured elements within the nervous system. Early descriptions of perturbation-evoked responses emerged only from qualitative interpretations of muscle activity and biomechanical variables (Horak and Nashner, 1986). It has since been suggested that the existence of postural synergies may be an artifact of using highly sophisticated analytical processes whose purpose is to identify patterns (Ting and McKay, 2007) and thus they may not be represented neurologically. Consequently, it remains a possibility that the sequence of muscle responses that follow balance perturbations are not governed by postural synergies.

1.3.3 Remaining questions about the nature of PRs

Neuromechanical delays between sensory input and motor output results in PRs being evoked while the body is in a state of movement. Because of this, PRs are produced in the presence of other responses or movements that could potentially alter their physiology after they have been triggered. Assessment of PRs is rarely, if ever, conducted in a manner that allows for its isolated assessment in the absence of other factors that may alter its physiological or biomechanical properties. Thus, attempts to classify PR onsets and amplitudes as being evidence of a certain type of response is significantly confounded by the current methods used to evoke PRs.

Examples of the issues facing analysis of PRs are numerous. One major issue results from the responses, other than PRs, that are evoked by balance perturbations. To induce postural instability requires rapid and extensive displacement of body segments, which oftentimes lengthens muscles to the extent that stretch reflexes are induced (Carpenter et al., 1999). Although the onsets of stretch reflexes occur relatively early compared to PRs, the induced muscle activity may extend into the timeframe of when PRs occur. Thus, when stretch reflexes and PRs are evoked in the same muscle (as is the case during support-surface translations), their temporal relationship precludes accurate assessment of PR timing and amplitude characteristics. However, alternative methods of destabilizing balance that involve rotating the support-surface have allowed for functional separation of stretch reflexes and PRs between heteronymous muscles and, thus, for their assessment in isolation

(Carpenter et al., 1999). Although it is possible to induce PRs and stretch reflexes in different muscles, it does not preclude the possibility for stretch reflexes to influence the physiology of PRs via spinal mechanisms; such as reciprocal inhibition.

Another major limitation of using current techniques is the difficulty with which to identify the specific biomechanical consequences of PRs that occur when standing balance is perturbed. The perturbing forces applied to the body put the body into motion before PRs can be initiated. Thus, rather than being able to determine the movements that PRs produce, the biomechanical outcome of PRs can only be described in terms of the passive movement they were able to terminate.

A third issue that arises during dynamic postural control studies result from using repeated balance perturbations to induce PRs. Multiple exposures to balance perturbations are known to induce changes in evoked PRs (Keshner et al., 1987; Blouin et al., 2006; Oude Nijhuis et el., 2009, 2010) when they are presented serially or even in random directions. These changes are considered to be the result of a number of factors including habituation (Keshner et al., 1987) and first-trial effects (Oude Nijhuis et al., 2010) that may be mediated by startle responses (Blouin et al., 2006).

1.4 Major aim and scope of thesis

This thesis involves 4 distinct experiments that aim collectively to understand how sensory feedback is used to trigger PRs and to determine the nature of PRs triggered by balance perturbations. In the first experiment, a classical conditioning paradigm was used to challenge the widely held notion that balance-relevant sensory feedback is required to initiate PRs. In the second and third experiments, classical conditioning in conjunction with auditory startle stimuli were used to examine the extent to which PRs may exist as centrally programmed motor behaviours that could be released in the absence of balance perturbations. The focus of the fourth study was to determine if PRs could be distinguished from first-trial effects and other time-dependent factors that hinder assessment of triggered PRs.

1.5 Part III: Methods

Although various paradigms have been employed to understand the neurophysiology of PRs, the standard amongst most studies has been to assess the PRs using physical perturbations to balance. Many different techniques have been used to evoke PRs in the laboratory setting; however, the most common and most well understood techniques have involved either rotations or the translations of the support-surface.

1.5.1 Balance perturbations and PRs

With the BoS tightly coupled to movements of the support-surface, changing the position of the support-surface has an immediate effect on postural stability. Although both rotations and translations evoke PRs, they affect balance in highly distinct ways and thus evoke PRs with equally distinct characteristics. However, the very specific manner in which each method actually disturbs balance has allowed for unique aspects of PRs to be examined that could not have been accomplished by interpreting results from using only one type of perturbation in isolation of the other.

1.5.2 Support-surface rotations

When the axis of rotation is aligned to the principal axes of rotation, mechanical support-surface rotations can produce a near pure perturbation to the ankle joint in either of the sagittal, frontal or oblique planes (Carpenter et al., 1999). Although not the most ecologically valid approach, surface rotations have proven extremely useful in examining how PRs and other perturbation-induced responses are organized throughout the body. For example, a sagittal-plane toes-up rotation induces instability by rapidly displacing the orientation of the support-surface that, without intervention would lead to a fall in the posterior direction. Further contributing to the destabilizing nature of toes-up rotations is the plantar flexor torques caused by bilateral stretch reflexes of the plantar flexor muscles of the lower legs (Diener et al., 1983, 1984). To counter these destabilizing forces, PRs are rapidly deployed in numerous muscles that act collectively to drive the CoM forward. Muscles located on the anterior side of the body are well positioned to move body segments, and therefore the whole body CoM, in the appropriate direction. Following a toes-up rotation, PRs are observed bilaterally in lower-limb, trunk and upper-body muscles (Carpenter et al.,

1999) that collectively dorsi-flex the ankles, extend the knee, flex the trunk and neck and displace the upper limbs anteriorly, repositioning the CoM and regaining postural stability.

1.5.3 Support-surface translations

Support-surface translations provide an ecologically valid approach from which to examine the human postural system (De Graaf and Van Weperen, 1997). Humans experience horizontal displacements of the support-surface in many ways during everyday life such as while standing on an accelerating subway or a bus. Antero-posterior (A/P) and medio-lateral (M/L) linear translations of the support-surface have consequently been used in the laboratory to model these challenges to the postural system. Again, because the location of the BoS is tightly linked to the floor, a translation of the support-surface in any direction effectively displaces the BoS. Due to the inertial properties of the body during stance, instability occurs because displacements of proximal body segments induced by support-surface translations tend to lag behind those situated more closely to the ground. This delayed sequence of lower- and upper-body displacements leads to a related displacement of the whole body CoM relative to the BoS which, without intervention, leads to falls in the direction opposite to that of the support-surface.

Support-surface translations that occur in the frontal plane cause lateral displacements of BoS that bias the vertical projection of the CoM towards the leg on the side opposite to the movement of the platform and unloads the leg on the same side. The result of this differential loading caused by the perturbation is an array of postural muscle activity that is bilaterally asymmetric in timing and amplitude (Henry et al., 1998). Immediately upon the onset of a lateral support-surface translation, the loaded limb must rapidly and progressively increase extensor joint torques about the ankle, knee and hip to resist increasing downward vertical forces. The unloaded leg and hip extend as well in an attempt to maintain contact with the surface. The extension of both lower-limbs causes bilateral EMG responses in extensor muscles (i.e. SOL, GAS and RF) to occur almost simultaneously (Henry et al., 1998) which makes onset latency in certain muscles a poor marker for the asymmetric responses triggered by lateral translations. However, significant differences exist in the onsets of superficial and deep abdominal musculature during frontal-plane translations (Carpenter et al., 2008). Amplitudes of the evoked responses in muscles of the leg and trunk

are markedly asymmetrical between bilateral muscle groups (Henry et al., 1998; Carpenter et al., 2008).

1.5.4 Support-surface rotations versus translations

Rotations and translations have been used to disturb balance because they each allow for very specific analysis of the responses evoked by postural perturbations and the adaptations that occur over the course of repeated exposures to the same postural stimulus. Although both aspects can be investigated using only one form of surface displacements, using both has developed a richer understanding of these mechanisms.

A natural consequence to balance perturbations is the initiation of both early stretch reflexes and later developing PRs. Although the onset latencies of both responses are very different (i.e. 30-50ms for stretch reflexes compared to 80-120ms for PRs), the burst duration of stretch reflexes are long enough to infringe upon the timeframe of PR onsets, which if produced in the same muscles, would prevent a full and unimpeded analysis of both responses. This is exactly the case when using surface translations to evoke PRs. For example, when experiencing a backward translation, muscles located on the posterior surface of the body that experience an initial stretch (i.e. soleus) are the same muscles that are involved in regaining balance by producing PRs. Triggering PRs in muscles that are initially excited above tonic background levels makes it difficult to determine exactly when stretch reflex activity ends and PRs begin thereby complicating analysis of amplitudes and preventing accurate indications of onsets. As a result, when using surface translations, researchers have been forced to calculate PR amplitudes by using fixed windows of time relative to postural stimulus onsets rather than making amplitude calculations relative to PR onsets (Diener et al. 1988; Carpenter et al. 1999). In contrast, surface rotations are unique in that they completely circumvent the aforementioned issues by functionally separating stretch reflexes and PRs into antagonistic muscle pairs thereby allowing for both responses to be analyzed independent of the other. With the earliest portions of the PR left unmasked by stretch reflexes, the mechanisms related to PR initiation and amplitude modulation could be more directly investigated.

1.6 Two alternative techniques for examining PRs

As useful as perturbations are in triggering PRs, they also contribute confounds that may further limit understanding of dynamic postural control. Two alternative methods, classical conditioning and startle paradigms (see next section), are proposed in the current thesis as novel techniques to assess the neural control of dynamic balance. Each method allows for movement to be induced by stimuli that do not make physical contact with the body and thus may circumvent the confounding issues attributable to forces induced by balance perturbations.

1.6.1 Classical conditioning

It has been common practice in dynamic postural control studies to present cues prior to balance perturbations for the purposes of providing subjects with advanced information about perturbation timing, direction or magnitude (Nashner and Cordo, 1981; Horak et al., 1989; McChesney et al., 1996; Adkin et al., 2008). Another paradigm that also incorporates the use of cues prior to movement onset is classical conditioning. Classical conditioning is most commonly used to examine associative learning and related neural mechanisms associated with simple motor behaviours (i.e. eye-blink) (Thompson, 1990; Lavond et al., 1993). Briefly, during classical conditioning paradigms, cues (termed conditioned stimuli; CS) are presented prior to the onset of another stimulus (termed the unconditioned stimulus; US) that innately evokes a reaction. The result of CS and US coupling are a host of changes to evoked responses that are described below and reflect learning and adaptation of neural mechanisms.

Trace conditioning paradigms

Classical conditioning can be performed in various ways that are dictated by the temporal relationships between the CS and the US. One example is a paradigm known as trace conditioning where an inter-stimulus 'trace' interval exists between CS offset and US onset (Christian and Thompson, 2003). Other paradigms exist which alter the timing between the CS and US such that they co-terminate (i.e. delay conditioning) (Clark and Squire, 1998). Regardless of the temporal relationship, when a CS precedes a US, changes

to the evoked responses occur which are used as evidence of the adaptability of the neural networks.

The eye-blink response system has been the model from which the majority of classical conditioning literature has emerged. Typically, conditioning of an eye-blink response involves a corneal air-puff (i.e. a US) which innately evokes an eye-blink response (i.e. an unconditioned response; UR) that is preceded by an auditory cue (i.e. a CS) (Clark and Squire, 1998). Indicators used to identify successful conditioning are a change to 1) response onsets that become progressively earlier to more closely approximate the onset of the US and 2) amplitudes that progressively decrease over repeated trials (Woodruff-Pak and Disterhoft, 2008). With sufficient exposure to paired CS/US trials, changes to onset latencies and amplitudes become asymptotic and plateau at values that are highly specific to the predictive value of the CS both in terms of its relative timing with the US (Schneiderman and Gormezano, 1964), but also of US parameters (Freeman et al., 1993). For example, if the US is a corneal air puff, paired CS/US trials will cause onsets of eye-blinks (motor responses) to occur progressively earlier. Earlier activation of this specific motor response is an adaptation that specifically aids in limiting the impact of the US.

Another independent indication of successful conditioning is the emergence of motor responses in trials where the CS is presented in the absence of the US (i.e. CS-Only trials) (Bouton and Moody, 2004). Prior to conditioning, the CS is checked to ensure that it does not produce discernible responses on its own. It is only through repeated coupling with the US that CS-Only trials come to evoke conditioned motor responses whose characteristics are highly specific to the nature of the US and the timing between CS and US.

Comparison of the responses evoked by the US alone before conditioning and the CS alone after conditioning allows for insight into what aspects of the response can be conditioned. More importantly, movements evoked with CS-Only trials are not affected by physical perturbations to the body, which therefore allows for movement to be assessed in the absence of stretch reflexes and/or passive displacements of the body.

Classical conditioning and dynamic postural control

Classical conditioning paradigms have the potential to be highly useful in understanding PR initiation and related neurophysiological mechanisms. Certain theories

suggest that a direct relationship exists between sensory feedback and PR initiation (see Part I). Classical conditioning could possibly be used to examine the link between sensory input and PR initiation. Comparisons of PRs evoked by perturbations with conditioned PRs evoked by CS-Only trials could be an examination of the link between sensory feedback and PRs and also possibly provide an avenue for inducing PRs (using CS-Only trials) in the absence of balance perturbations.

1.6.2 Startle paradigms

Auditory startle paradigms, generalized startle responses and motor preparation

Intense and unexpected stimuli have been shown to induce generalized startle responses characterized by whole body bilateral flexion reactions (Landis et al., 1939) that begin in the face and neck musculature within ~30ms of stimulus onset (Brown et al., 1991a,b) and propagate distally to eventually terminate in the muscles of the lower-limb (Brown et al., 1991a,b). It is believed that generalized startle reactions are evolutionarily conserved responses that aim to shield the body from external threats (Yeomans and Frankland, 1996). These reactions have been evoked using a variety of sensory modalities; however, intense auditory stimuli (i.e. startling acoustic stimuli; SAS) of ~40ms duration and ~120dB intensity have been the most widely used in experimental settings (Carlsen et al., 2011b). The specific timing and top-down propagation of SAS-induced generalized startle responses observed in humans, combined with animal work, have led to the conclusion that generalized startle responses originate in the brainstem (Davis, 1984; Yeomans and Frankland, 1996), specifically the reticular formation, and propagates through the body via reticulo-spinal pathways (Yeomans and Frankland, 1996) to terminate on spinal motor neurons.

Not only have auditory startle paradigms been used to investigate the mechanisms involved in producing generalized startle responses, they have also been used to examine the neurophysiology of motor preparation. Cognitive perspectives of motor control have typically proposed a general 3-stage information-processing model that describes how an incoming stimulus is transformed into a particular response. These 3 stages include 1) stimulus identification, 2) response selection and 3) response programming (Donders, 1869;

Schmidt and Lee, 2011). In the complete absence of warning or advanced information pertaining to task demands, these 3 stages are completed in the aforementioned order where the time dedicated to each phase is dependent on the requirement of cognitive resources (Klapp, 1995). However, in situations in which the response required after a particular stimulus is known in advance (such as in simple reaction time paradigms), this theoretical framework predicts that 'response selection' and 'response programming' can take place prior to the delivery of the stimulus. Thus, a specific motor response can be prepared in advance and all that is required after the onset of the stimulus is to identify the stimulus and initiate the pre-programmed response (Klapp, 1995).

In laboratory experiments, auditory startle paradigms have been used to validate the notion of advanced preparation of motor responses. For example, when simple movements of the wrist or elbow are to occur after the onset of an imperative stimulus (IS) in a simple reaction time paradigm, SAS presented at the instant of IS onsets significantly reduce reaction time compared to control (Valls-Solé et al., 1999; Carlsen et al., 2011a,b for reviews). Termed the StartReact effect, relative response parameters are generally unaffected despite their early onset, which has been used as evidence of advanced motor preparation (Carlsen et al., 2011a,b). As would be predicted by a 3-stage information-processing model, SAS does not shorten movement onsets in choice-reaction time paradigms where response selection and programming cannot take place prior to the IS (Carlsen et al., 2011a,b).

Neurophysiology of StartReact effects

Using SAS, motor preparation has been observed in a wide variety of situations ranging from the simplest being wrist and elbow extension (Valls-Solé et al., 1999; Carlsen et al., 2004a) to the more complex being rise-to-toes (Valle-Solé et al., 1999), sit-to-stand (Queralt et al., 2008a), obstacle avoidance during gait (Queralt et al., 2008b) and anticipatory postural adjustment prior to stepping (MacKinnon et al., 2007). Despite the differences in task and the muscles involved, these movements share in the ability to be prepared and evoked by SAS. Not all movements are similarly affected by SAS. For example, finger movement onsets tend not to be significantly altered by SAS in simple reaction time tasks compared to arm extension movements of the same limb (Carlsen et al., 2009). This

discrepancy in susceptibility to SAS has been attributed, not to a lack of motor preparation for particular behaviours, but to a lack of access of certain motor circuits to those which govern StartReact effects (Carlsen et al., 2009).

In a pioneering study which first used SAS to examine StartReact effects for various motor tasks, even the most delayed onset latencies of SAS-induced responses were markedly earlier than the most rapid onsets of the same motor tasks produced voluntarily in the absence of SAS (Valls-Solé et al., 1999). These results were interpreted as evidence for 2 distinct neural pathways; one that governs voluntary action and another that facilitates StartReact effects. Given the timing of SAS-induced responses, length of pathways and known conduction velocities, Valls-Solé and colleagues (1999) proposed that StartReact effects were mediated by subcortical structures; namely the reticular formation and the descending reticulo-spinal efferent pathway. This rudimentary circuit has since been expanded upon to include at present day, a comprehensive neural network that includes subcortical and possibly cortical loops (Alibiglou and MacKinnon, 2012).

Startle and dynamic postural control

Incorporating startle paradigms into the examination of dynamic postural control has the potential to widely influence our understanding of how PRs are organized within the nervous system and the mechanisms that lead to their initiation. As is the case during simple reaction time tasks and classical conditioning paradigms, cues are oftentimes used in balance research to specifically forewarn subjects of upcoming postural stimulus parameters such as timing, direction and magnitude. In these instances, SAS could determine if these instructional sets and the changes to PRs observed by their presence could be result of motor preparation. Furthermore, SAS are also capable of evoking responses that otherwise may require physical perturbations to induce. Thus, prepared responses evoked by SAS could be analyzed in the absence of stretch reflexes and other biomechanical changes that typically occur following physical perturbations. Finally, having been identified as a potential component of the responses evoked by balance perturbations, startle responses and the stimuli used to evoke them could provide interesting perspectives into, and potentially expand upon, current understanding of PRs.

Chapter 2: Postural responses explored through classical conditioning

2.1 Introduction

Unexpected postural disturbances elicit stretch reflexes in muscles that are passively lengthened by the initial postural stimulus, followed by postural responses (PRs) in muscles recruited throughout the body to maintain equilibrium and regain postural stability. PRs consist of two distinct components. The first component (oftentimes labeled automatic postural reflexes (Nashner and Cordo, 1981), balance correcting responses (Carpenter et al., 1999) or long-latency reflexes (Diener et al., 1983)) is initiated 80–120ms post-perturbation (Nashner and Cordo, 1981), and thus has a latency that is too late to be considered part of a spinal stretch reflex and too early to be under volitional control (Diener et al., 1984). The second component of the PR constitutes voluntary stabilizing reactions that are initiated with latencies of 350–750ms (Carpenter et al., 1999). Together these responses are combined across muscles and joints to form synergies that are specific to the direction and type of postural disturbance (Carpenter et al., 1999), and are modulated with respect to the magnitude (Diener et al., 1984), velocity (Diener et al., 1983, 1984) and context (Carpenter et al., 2004) in which the postural perturbation is presented.

Although the involvement of sensory inputs in modulating PR amplitudes has been well established, the role of sensory inputs in triggering PRs is currently unknown. Classical theories assume that visual, vestibular and somatosensory systems detect and inform the central nervous system (CNS) of the initial postural disturbance, which then triggers an appropriate postural synergy via spinal or trans-cortical reflex pathways (see Jacobs and Horak, 2007 for review). However, recent studies have failed to provide convincing evidence to support the role of peripheral sensory systems in triggering PRs. For example, no significant differences in the onset latencies of PRs have been reported in studies comparing healthy controls to patients with selective bilateral vestibular (Allum and Pfaltz, 1985) or lower-leg proprioceptive loss (Bloem et al., 2002). Furthermore, PR onset latencies have been shown to be unchanged when either lower-leg cutaneous (Do et al., 1994; Perry et al., 2000) or visual (Sundermier and Woollacott, 1998; Nakata and Yabe, 2001) inputs were absent at the time of postural perturbations. These results suggest that either: (a) sources of somatosensory information from areas other than the lower-leg may contribute to PR

initiation or (b) information from any one of these sensory systems is not essential for triggering PRs.

One possible way of determining if sensory information about an unexpected postural disturbance is necessary to trigger PRs would be to use a classical conditioning paradigm. Classical conditioning is a unique research tool that can be used to determine if a specific motor response can be triggered by stimuli not normally used by the CNS to elicit a particular behavior. Through repetitive time-locked coupling of a neutral conditioned stimulus (CS) with an unconditioned stimulus (US) that innately evokes an unconditioned response (UR), the CS alone will, over time, be capable of evoking a conditioned response (the former UR) in the absence of the US (Clark et al., 2002). The conditioned response, when triggered by the CS alone, would therefore occur in the absence of the sensory information that would otherwise normally evoke the UR.

The purpose of the current study was to utilize a classical conditioning paradigm to determine whether PRs could be triggered in the absence of the sensory cues normally generated by postural perturbations. It was hypothesized that after the repetitive coupling of an auditory cue with a postural perturbation, the CNS would be able to trigger PRs to the auditory cue alone in the absence of the sensory feedback normally generated by a postural perturbation. Since balance perturbations would be absent at this time, the generation of muscular activity profiles with similar characteristics to those elicited by postural perturbations would verify that an adaptable neural mechanism could learn to incorporate other forms of sensory information, not typically associated with balance perturbations, into the processes involved in triggering PRs.

2.2 Experimental procedures

Twenty-one healthy young individuals volunteered for Experiment #1 (8 males; 19-27 years of age; mean height and body mass ± 1SD: 1.71 ± 0.11m and 68.71 ± 14.85kg, respectively) and six healthy young individuals volunteered for Experiment #2 (4 males; 21-26 years of age; mean height and body mass ± 1SD: 1.77 ± 0.01m and 77.65 ± 2.16kg, respectively). All subjects gave informed and voluntary consent to participate in these studies. The University of British Columbia Clinical Research Ethics Board approved all experimental procedures that were conducted in accordance with the Declaration of Helsinki.

2.2.1 Experiment #1

Feet-in-place PRs were evoked via toes-up support-surface tilts (angular velocity: 120^{o}/s; angular displacement: 12^{o} from a horizontal reference) in two counter-balanced blocks of trials (a 'Control' block and a 'Conditioning' block). Relatively large magnitudes of platform displacements were used in order to ensure that participants would generate PRs even in the presence of the response habituation that occurs following multiple balance perturbations (Keshner et al., 1987) and classical conditioning procedures (Kolb et al., 2002). Eleven participants received the Conditioning block first (Group #1) while the remaining 10 participants experienced the Control block first (Group #2). During the Conditioning block, a 200ms auditory 'Cue' (<70dB) was followed a 100ms inter-stimulus 'trace' interval. The trace interval was subsequently followed by the onset of the platform movement (Figure 2.1). Having a trace interval separate the auditory cue and the support-surface movement is central to trace conditioning protocols (Christian and Thompson, 2003; Woodruff-Pak and Disterhoft, 2008). Since the balance perturbation followed the onset of the auditory CS by 300msecs, the expected onsets of PRs would occur approximately 400ms after CS onset which eliminated the potential for audio-spinal reflexes, acoustic startle or anticipatory responses to be masked by PRs. Conditioning trials were separated by a random fore-period of 10-55s and were repeated until the onsets of postural muscles and the variability of these onsets were reduced compared to first 10 trials as verified by online monitoring of these measures. To meet the aforementioned criteria, participants received a range of 19-28 Conditioning trials. During the Control block, participants responded to 10 unexpected toes-up support-surface tilts separated by a random 10-55s fore-period. Following both Conditioning and Control blocks, single unexpected auditory tones were presented without accompanying postural perturbations; termed Cue-Only$_{Conditioning}$ and Cue-Only$_{Control}$ trials, respectively (Figure 2.1). If memory traces coupling the auditory and postural stimuli were developed, Cue-Only$_{Conditioning}$ trials were expected to elicit conditioned PRs in the absence of postural perturbations. Counter-balancing the order of Conditioning and Control blocks allowed Cue-Only$_{Control}$ trials to serve 2 purposes. When the Conditioning block preceded the Control block (Group #1), Cue-Only$_{Control}$ trials probed the potential carry-over effects of the initial conditioning procedure. Due to the time needed to complete a 5min rest period between blocks and subsequent 10 Control trials, Cue-Only$_{Conditioning}$ and Cue-Only$_{Control}$ trial

were separated by approximately 15min. Conversely, when the Control block preceded the Conditioning block (Group #2), Cue-Only$_{Control}$ trials were used to determine whether the auditory cue alone could initiate audio-spinal reflexes or acoustic startle responses.

2.2.2 Experiment #2

Experiment #2 was designed to examine the extinction of conditioned PRs. All subjects who participated in Experiment #2 were entirely naïve to the current experimental procedures and did not participate in Experiment #1. Subjects were asked to assume a comfortable standing position on the tilting platform with arms relaxed at their sides with eyes fixated straight ahead on a target located 4m away. The extinction procedure involved 3 blocks of trials. The first block consisted of 5 trials wherein each trial, a single auditory tone (i.e. Tone-Only) was randomly presented 10-55s from each other in order to determine the neutrality of the acoustic stimulus being used. Being non-startling and having no prior experience with the acoustic stimulus, it was expected that participants would not physically react to the supposed neutral auditory stimulus. The tone used in the extinction protocol was identical to the CS used in Experiment #1 and was shown in pilot work to not evoke detectable electrophysiological responses. The second block involved the conditioning of PRs. In each trial of conditioning, an auditory tone preceded a toes-up support-surface tilt. The characteristics of the 2 stimuli and the timing between them were identical to those used in Experiment #1. Participants experienced 25 conditioning trials that were randomly presented 10-55s from one another. Based on the results of pilot work, it was expected that 25 conditioning trials would be enough to foster the development of a robust association between the auditory cue and the platform tilt. The third and final block involved the extinction phase of Experiment #2 whereby a second series of 5 Tone-Only trials was presented in the absence of balance perturbations. If participants had been conditioned to produce PRs to the auditory cue (i.e. the CS) we expected that the first of the 5 Tone-Only trials in this final block would evoke conditioned PRs with high probability. In subsequent Tone-Only trials, we expected that conditioned PRs would be much less probable, thus indicating extinction of the conditioned PRs.

2.3 Analyses and measures

2.3.1 Experiment #1

Surface electromyography (EMG) was used in all subjects to record muscle activity unilaterally from soleus (SOL), tibialis anterior (TA) and rectus femoris (RF) on the right leg. EMG data were collected at 3000Hz and amplified 500x, bandpass filtered between 10 and 500Hz (Telemyo 2400R, Noraxon, USA) and A/D sampled at 1000Hz (Power 1401, Cambridge Electronic Design, UK). Offline, these data were then full-wave rectified and low pass filtered at 100Hz (Spike2, Cambridge Electronic Design, UK). Within each trial, EMG onsets were identified as the first time when muscle activity surpassed a mean plus 2 standard deviations measure of background activity and remained supra-threshold for a minimum of 20ms (Spike2, Cambridge Electronic Design, UK). Mean background EMG activity was analyzed within a 300msec period prior to the first stimulus in each trial (i.e. the platform tilt in Control trials and the auditory stimulus in Conditioning, Cue-Only$_{Conditioning}$ and Cue-Only$_{Control}$ trials). EMG onsets were visually inspected online to ensure that the semi-automated algorithm reliably calculated accurate EMG onsets. Trials in which substantial anticipatory activity was noticed before the first auditory (i.e. in Conditioning and Cue-Only trials) or postural (i.e. in Control trials) stimulus, were removed from analysis. One subject in Group #1 consistently produced significant anticipatory activity prior to Cue presentations, therefore their data were removed from further experimental analyses. EMG amplitudes were calculated on the remaining data via trapezoid integration of the filtered waveforms for 100ms following EMG onsets and were referenced to an equivalent period of integrated background activity located immediately prior to the first stimulus in each respective trial.

Kinematic data were collected only in the first study using infra-red light emitting diodes placed on the lateral malleolus and the head of the fibula on the right leg (Optotrak Certus, Northern Digital Inc., CAN). Raw co-ordinate data were sampled at 200Hz and low pass filtered offline at 5Hz prior to calculating 2-dimensional absolute angular displacements of the shank in the sagittal plane for each trial (MATLAB 7.0, Mathworks, USA). Acceleration profiles are commonly used to characterize body segment movements following balance perturbations (Allum and Pfaltz, 1985; Carpenter et al., 2001; Bloem et al., 2002),

therefore, shank angular acceleration profiles were generated by double differentiating the shank angular displacements. The first peak negative acceleration was used to indicate postural corrections initiated in the direction opposite to the toes-up support-surface tilts. Onset latencies of the first negative shank accelerations were determined for each trial with respect to the onsets of the initial platform acceleration. Peak and time-to-peak of the negative shank accelerations were also calculated and referenced to their values at the onsets of negative shank accelerations (Spike2, Cambridge Electronic Design, UK).

2.3.2 Experiment #2

EMG data were collected at 3000Hz and amplified 500x, bandpass filtered between 10 and 500Hz (Telemyo 2400R, Noraxon, USA) and A/D sampled at 1000Hz (Power 1401, Cambridge Electronic Design, UK) from all muscles (i.e. TA, RF and SOL) used in Experiment #1. These data were also full-wave rectified and low pass filtered at 100Hz (Spike2, Cambridge Electronic Design, UK). The algorithms used to determine the onset latencies of TA and RF were the same as those in Experiment #1. However, in these cases, the algorithm's purpose was not to detail the specific latency of the muscular response, but rather it was used to determine if a response was present within the defined window of time following the expected onset of the balance perturbation during the first and last blocks of 5 Tone-Only trials. A similar algorithm was used to mark periods of SOL inhibition. In this particular application, a mean of 300ms of background EMG was determined and inhibition was considered to occur when SOL EMG activity fell below 1 standard deviation of the mean background for at least 20ms.

2.4 Statistics

2.4.1 Experiment #1

To minimize the potential impact of variability due to PR habituation (Keshner et al., 1987), averages of all EMG and kinematic measures were calculated from the last 5 Control and Conditioning trials from each subject. Student's paired *t*-tests were used to compare all dependent measures between 1) Control and Conditioning trials, 2) Conditioning and Cue-Only$_{Conditioning}$ trials and 3) Cue-Only$_{Conditioning}$ and Cue-Only$_{Control}$ trials. Statistical

comparisons of Cue-Only$_{Control}$ trials were limited to those participants who produced conditioned PRs to both Cue-Only$_{Conditioning}$ and Cue-Only$_{Control}$ trials. Pearson correlations were independently run on EMG and kinematic dependent measures to investigate the relationships between responses evoked in Conditioning and Cue-Only$_{Conditioning}$ trials. Onset latencies of EMG and kinematic data were referenced to the onset of the platform movements in Control and Conditioning trials. In Cue-Only$_{Conditioning}$ and Cue-Only$_{Control}$ trials when there were no support-surface displacements, onset latencies in these trials were referenced to the expected onset of the platform movement (i.e. 300ms after the Cue onset). Significance levels were adjusted to $p \leq 0.017$ for all paired *t*-tests using the Bonferroni method to correct for multiple pairwise comparisons.

2.4.2 Experiment #2

The probabilities of TA and RF excitation and SOL inhibition were examined in the first and the last blocks of 5 Tone-Only trials to determine the frequency with which the neutral auditory cue generated detectable muscular responses and whether that changed following conditioning and subsequent extinction trials. These data were compiled in order to calculate the percentage of subjects who generated detectable muscular responses to the 5 auditory cues presented before and immediately following the conditioning block of trials.

2.5 Results

2.5.1 Control vs. Conditioning trials

Consistent with previous reports (Diener et al., 1983; Carpenter et al., 1999), Control trials that involved unexpected toes-up support-surface tilts elicited early stretch reflexes in SOL, followed by PRs in TA and RF to counter the initial backward angular rotations of the shank (Figure 2.1). Mean onset latencies for SOL stretch reflexes and PRs in TA and RF (Table 2.1) were consistent with previous reports of EMG onsets in these muscles following toes-up support-surface tilts (Nashner and Cordo, 1981; Carpenter et al., 1999). SOL stretch reflex onset latencies were not significantly different between Control and Conditioning blocks ($t(19)=0.695$, $p=0.495$). However, significantly earlier EMG onset latencies were observed during Conditioning compared to Control trials in both TA ($t(19)=7.12$, $p<0.000$)

and RF ($t(19)=4.07$, $p=0.001$). Amplitudes of SOL, TA and RF were found to be significantly reduced within Conditioning compared to Control trials: SOL: $t(19)=8.99$, $p<0.000$; TA: $t(19)=7.73$, $p<0.000$; RF: $t(19)=4.41$, $p<0.000$. Shank segments rotated in the same direction in Conditioning and Control trials (Figure 2.1), however, negative shank angular acceleration onsets occurred significantly earlier ($t(19)=3.95$, $p=0.001$) and peak negative accelerations were significantly reduced ($t(19)=3.46$, $p=0.003$) during Conditioning blocks. Relative to the onset of the first negative shank angular acceleration, the time-to-peak of negative acceleration profiles were not significantly different between Conditioning and Control blocks ($t(19)=0.21$, $p=0.834$).

2.5.2 Conditioning trials vs. Cue-Only$_{Conditioning}$ trials

Despite the absence of a postural perturbation, presentations of Cue-Only$_{Conditioning}$ trials that immediately followed Conditioning blocks elicited similar patterns of muscular responses in TA and RF as in Conditioning trials (Figure 2.1). All 20 subjects produced observable PRs in TA and 18 of the 20 subjects produced PRs in both TA and RF during the Cue-Only$_{Conditioning}$ trials (Figure 2.2). Compared to Conditioning trials, Cue-Only$_{Conditioning}$ trials elicited significantly earlier onsets in TA ($t(19)=5.91$, $p<0.000$) while no differences were observed in RF onsets ($t(17)=1.82$, $p=0.086$). The onset latencies in TA were significantly correlated between Conditioning and Cue-Only$_{Conditioning}$ trials ($r=0.663$, $p=0.001$) whereas correlations of RF onsets only trended towards significance ($r=0.434$, $p=0.072$). There was a significant reduction in mean TA and RF response amplitudes in Cue-Only$_{Conditioning}$ compared to Conditioning trials: ($t(19)=6.62$, $p<0.000$ and $t(17)=5.21$, $p<0.000$, respectively). Stretch reflexes present in SOL during Control and Conditioning trials were not elicited in Cue-Only$_{Conditioning}$ trials. Rather, in 8 of 20 possible instances, Cue-Only$_{Conditioning}$ trials elicited tonic SOL inhibition coincident with time periods in which stretch reflexes would appear during ankle dorsi-flexing platform rotations (Figure 2.3).

Although the amplitudes of the muscular responses triggered by Cue-Only$_{Conditioning}$ trials were markedly reduced compared to those generated following a postural disturbance, they were large enough to cause kinematic angular displacements of the shank. Common peaks were identified in the acceleration profiles of Conditioning and Cue-Only$_{Conditioning}$ trials despite the fact that the shank initially rotated in opposing directions (Figure 2.4). In

the absence of the platform tilt and SOL stretch reflexes, the initial positive accelerations were not generated in Cue-Only$_{Conditioning}$ trials. However, peaks of negative accelerations were common between Conditioning and Cue-Only$_{Conditioning}$ trials (Figure 2.4). The onsets of the negative accelerations occurred significantly earlier in Cue-Only$_{Conditioning}$ trials compared to those that followed the platform movement in Conditioning trials: ($t(19)=9.04$, $p<0.000$). Furthermore, the peak negative accelerations achieved were significantly reduced in amplitude ($t(19)=4.80$, $p<0.000$) and delayed in time to reach peak amplitude ($t(19)=8.25$, $p<0.000$) in Cue-Only$_{Conditioning}$ trials compared to Conditioning trials. Measures of onset latency ($r=0.507$, $p=0.022$), peak ($r=0.647$, $p=0.002$) and time-to-peak ($r=0.839$, $p<0.000$) of negative shank accelerations were all significantly correlated between Conditioning and Cue-Only$_{Conditioning}$ trials.

2.5.3 Carry-over effects vs. audio-spinal reflexes and acoustic startle responses

As noted earlier, participants were parsed into 2 groups; each receiving a counter-balanced order of Conditioning and Control blocks. Participants in Group #1 ($n=10$) experienced the Conditioning block before the Control block of trials, therefore, the Cue-Only$_{Control}$ trials experienced by members of Group #1 were meant to analyze the carry-over effects of the initial Conditioning block. Six of the possible 10 participants in Group #1 generated EMG activity in TA and 3 of those 6 participants coupled TA with RF activity following Cue-Only$_{Control}$ trials approximately 15min post-conditioning (Figure 2.2). Compared to Cue-Only$_{Conditioning}$ trials, TA activity onsets and amplitudes in Cue-Only$_{Control}$ trials were not significantly different ($t(5)=2.75$, $p=0.040$ and $t(5)=3.11$, $p=0.027$, respectively. All participants that generated TA and/or RF EMG activity in Cue-Only$_{Control}$ trials also generated shank displacement and acceleration profiles that were similar to those observed in Cue-Only$_{Conditioning}$ trials (Figure 2.4). Three additional participants in Group #1 also had shank displacements in Cue-Only$_{Control}$ trials that were similar to those in Cue-Only$_{Conditioning}$ trials despite the absence of observable activity in TA or RF. Upon further investigation, it was revealed that these kinematic changes occurred in conjunction with tonic SOL inhibition rather than from excitatory contributions from TA or RF. There were no significant differences observed in the onset latencies ($t(8)=2.52$, $p=0.036$), times-to-peak

($t(8)=0.016$, $p=0.988$) or amplitudes ($t(8)=2.26$, $p=0.053$) of negative angular accelerations of the shank between Cue-Only$_{Control}$ Cue-Only$_{Conditioning}$ trials.

It was possible that the auditory stimulus used throughout the experiment could induce audio-spinal reflexes or acoustic startle responses. The potential for this confound was examined in Group #2 participants who experienced the Cue-Only$_{Control}$ trial before the Cue-Only$_{Conditioning}$ trial. As shown in Figure 2.2, participants in Group #2 did not generate visually discernible muscle activity in either TA or RF following Cue-Only$_{Control}$ trials. We can therefore be confident that the evoked responses that occurred in either Cue-Only$_{Control}$ or Cue-Only$_{Conditioning}$ trials were the result of the conditioning procedure.

2.5.4 Extinction of conditioned responses

As expected, the neutral auditory stimulus did not elicit observable muscular activity in any participants during the first series of Tone-Only trials in Experiment #2. Akin to the responses observed in Experiment #1, the conditioning trials in Experiment #2 evoked characteristic stretch reflexes in SOL and PRs in TA and RF whose amplitudes decreased with subsequent trials. In order to be admitted to further analysis in Experiment #2, participants needed to reach a criterion level of conditioning where they were capable of generating a conditioned PR to at least the first Tone-Only trial that immediately followed the conditioning procedure. Of the 6 tested, 5 subjects generated conditioned PRs in TA. Similar to Experiment #1, conditioned PRs in most instances also involved excitation of RF and inhibition of SOL (Trial #1 in Figure 2.5A) thus indicating that multi-muscle postural synergies were conditioned and triggered by the auditory cue. Subsequent Tone-Only trials, however, did not reliably evoke conditioned PRs as did the first few. As can been seen in representative subject data in Figure 2.5A, the excitation and inhibition that were triggered by the Tone-Only trials became less prevalent over the course of extinction. This trend eventually led to all muscles becoming totally unresponsive to the auditory stimulus by trial #4 of extinction. The very same trend existed across all subjects as the total number of subjects who triggered a detectable conditioned PR within TA, RF or SOL decreased as the extinction block progressed (Figure 2.5B). By the 4th extinction trial, all of the subjects that triggered conditioned PRs immediately post-conditioning, became entirely unresponsive to

the Tone-Only trials; generating neither excitation nor inhibition of any of the muscles examined.

2.6 Discussion

The purpose of the current study was to utilize a classical conditioning paradigm to determine whether PRs could be triggered in the absence of the sensory cues normally generated by a support surface tilt. The results demonstrate that PRs can be conditioned by pairing a perturbation with a neutral stimulus such as an auditory cue, and that following conditioning, postural synergies can be triggered in the absence of the sensory cues normally generated by the perturbation. Although reduced in amplitude compared to those produced in Conditioning trials, the responses triggered in Cue-Only trials had a biomechanical consequence as the shank was observed to rotate in a context-specific manner in the direction opposite to that caused by the balance perturbation. In addition to understanding the role of sensory feedback in triggering PRs, our results provide novel insights into PR modulation and adaptation. In particular, sensory feedback appears to play a key role in the modulation of PR amplitude. Furthermore, PRs have the potential to quickly adapt through associative learning and to retain such adaptations in memory for extended periods of time.

2.6.1 Conditioning of PRs

By coupling an auditory cue with a postural perturbation, we have demonstrated that a complex postural synergy involving multiple muscles of the lower-limb could be conditioned using a trace conditioning paradigm. There are a number of criteria used to determine whether or not behaviours have been conditioned; of them are the characteristic changes that occur to the evoked response during paired CS-US trials, including earlier onsets and reduced amplitudes compared to responses triggered by the US alone (Woodruff-Pak and Disterhoft, 2008). Further evidence for conditioning is the ability to elicit conditioned responses to the Cue-Only in the absence of the US (Bouton and Moody, 2004), as well as the ability to extinguish the conditioned response through repeated presentations of the Cue alone (Bouton and Moody, 2004). Based on the above criteria, the results of our study clearly indicate that the conditioning of PRs has occurred. We have shown that over the course of conditioning, PRs in TA and RF developed earlier onset latencies and reduced

amplitudes compared to trials in the Control blocks. These findings replicate the changes in PRs observed during conditioning reported by Kolb et al., (2002). We have provided further evidence of conditioning by demonstrating that multi-muscle PRs can be triggered by an auditory cue (i.e. CS-Only) in the absence of a support-surface tilt (i.e. an US) and by showing that the conditioned PRs could be extinguished over the course of 5 serially-presented Tone-Only trials.

We have also provided strong evidence to confirm that PR activity triggered by the Cue-Only trials are independent of audio-spinal reflexes, acoustic startle responses and anticipatory reactions that could be related to the auditory cue. The earliest mean onsets of conditioned responses occurring after Cue-Only$_{Conditioning}$ trials (Table 2.1) appeared approximately 200ms after the known time periods of audio-spinal reflex (Delwaide and Schepens, 1995) or startle response (Valls-Solé et al., 1999) facilitation. Muscular responses that would be consistent with audio-spinal reflexes or acoustic startle responses were absent in all subjects who received the Cue-Only$_{Control}$ trial prior to the conditioning protocols (refer to subjects in Group #2 of Experiment #1 (n=10)), and the subjects in Experiment #2 (n=6) who received 5 Tone-Only trials during extinction. Furthermore, although onset latencies of postural muscles showed a propensity to decrease over the course of conditioning, participants consistently generated muscular activity *after* the expected onsets of the postural perturbation and therefore did not satisfy a key criterion to consider the triggered responses as anticipatory in nature. The decrease in response onset latencies of during paired CS-US trials is a known consequence of the associative learning that subserves classical conditioning (Desmond and Moore, 1988). Therefore, we would argue that the observation of decreased onset latencies of PRs in Conditioning compared to those produced in Control blocks indicates that PRs were undergoing conditioning to the Cue and not that participants were preemptively generating PRs in anticipation of a balance disturbance.

Further evidence that the responses elicited by Cue-Only trials were, in fact, PRs can be drawn from the strong correlations observed between the Cue-Only and Conditioning trials for the timing of EMG onsets and shank accelerations. These results indicate that the between-subject variability in the timing of PRs elicited by the Conditioning trials was strongly related to the variability in responses observed during Cue-Only trials. In other words, individual differences in response timing are preserved independent of whether, or

not, the PRs were elicited by the perturbation or auditory stimulus alone. Such strong associations in timing of these EMG and kinematic events would not be expected if the responses were of different origins.

2.6.2 Sensory feedback and PR initiation

Our results indicate that an auditory cue can initiate PRs in multiple muscles of the lower-limb in the absence of the sensory feedback normally generated by a balance perturbation. This primary finding suggests that neither the vestibular, visual nor somatosensory systems can be considered the sole source of triggering information needed by the CNS to initiate a PR as previously supposed. Instead, it appears that the CNS can use other sensory inputs not directly linked to the physical perturbation to trigger appropriate PRs. These results may help to explain why previous studies have failed to demonstrate significant influences on the timing of PR initiation, when individual sensory inputs were systematically removed, or made unreliable through clinical lesions, or experimental manipulation. Specifically, PR onset latencies in muscle of the lower-limb were not different between normals and those with either lower-leg proprioceptive (Bloem et al., 2002) or bilateral vestibular loss (Allum and Pfaltz, 1984). As well, removing vision via eye closure (Allum and Pfaltz, 1984) or plantar cutaneous sense through foot sole anesthesia (Do et al., 1994) has also been proven incapable of altering the typical onset latencies of PRs seen under normal sensory conditions. By triggering the PRs using an auditory cue in the absence of all other normally relevant sensory inputs, we propose that the CNS does not necessarily rely on any one sensory system in particular to trigger the onset of PRs. Instead, it appears that the CNS can learn to elicit PRs using multiple sources of available sensory information, provided that 1) there is adequate prior experience to make connections between the sensory stimuli and postural disturbances and 2) that the sensory signal reliably codes for the onset of the perturbation itself. In this light, it could be argued that vestibular, visual and somatosensory information may all play an important role in triggering PRs, if they are the most relevant sources of information available to the CNS at a given time. This theory takes advantage of the multiple sources of redundant sensory information available to the CNS for providing possible triggering inputs for PR initiation. Under sensory deprived conditions, this theory would predict that as long as one sensory system could provide the CNS with the

relevant details regarding perturbation onset within a timeframe that would allow for the observed latencies of lower-leg PRs, the CNS could initiate PRs with minimal delay. Thus, the inability of previous work to significantly delay PR onsets when removing or altering sensory inputs may be more indicative of the capability of the CNS to adapt to altered sensory conditions than it is the influence that each sensory system has over PR initiation.

2.6.3 PR amplitude modulation and balance-relevant sensory feedback

Results indicated that amplitudes of PRs triggered by the Cue-Only$_{Conditioning}$ trials were significantly reduced compared to those that were evoked in the presence of postural perturbations. These observations corroborate with previous work that has demonstrated a robust relationship between decreases in the amount or the reliability of sensory inputs during balance perturbations and decreases in the amplitudes of the evoked PRs. For example, PRs amplitudes have been shown to decrease in situations of absent vestibular (Allum and Pfaltz, 1984), lower-leg proprioceptive (Bloem et al., 2002) and cutaneous sense (Do et al., 1990) compared to those PRs that were generated under normal sensory conditions. However, the decreased amplitudes observed in these previous studies were not to the same magnitudes as those observed in the current investigation. This discrepancy is likely explained by the fact that there was still relevant sensory information available from intact sensory systems in previous studies. Although all of the sensory systems remained intact in the current investigation, not one of them was influenced by a balance perturbation and therefore they were not able participate in amplifying the evoked PRs; thus producing PRs in Cue-Only trials that were markedly attenuated compared to those produced in Conditioning trials.

Because PRs, albeit reduced in amplitude, remain even in the absence of postural perturbations, it appears that a central mechanism may be capable of releasing a set of motor commands that is later shaped by afferent inputs depending on the parameters of the balance perturbation, the context in which they are delivered and the state of the motoneuron pool at the time of perturbation onset. These postulates were captured previously by Allum (1975) where it was proposed that a servo-like 'test pulse' mechanism was involved in the earliest phases of PR development (Allum, 1975). Under this proposed mechanism, PR amplitude modulation would be achieved through the combined efforts of supra-spinal outflow (the

'test pulse') and afferent feedback to CNS centres. This mechanism would then trigger a 'default' level of activation in postural muscles that would be minimal when not met with peripheral sensory feedback (Chan, 1983; Diener et al., 1988). Assuming this to be true, conditioned PRs elicited by Cue-Only trials in the current investigation would represent responses whose timing was determined by central command and whose amplitudes were unmodified by balance-relevant sensory inputs. Under normal circumstances then, the sensory feedback normally generated by balance perturbations would have a dual role in both triggering the onset of directionally appropriate PRs and in shaping the response according to the parameters of the perturbation and the state of the lower motoneuron (LMN) pools subserving control of postural muscles. By way of classical conditioning, the current investigation has demonstrated the ability of the postural system to trigger PRs using an auditory cue when all other sources of peripheral sensory feedback that could potentially modulate the amplitude of the 'default' motor program were absent. Triggering PRs under these unique circumstances has allowed for a relatively unmodified PR to be quantified in lower-limb postural muscles. Although the conditioned PRs would be unchanged by stimuli related to the balance perturbation, the amplitudes of the conditioned PRs would be largely dependent on the excitability of the LMN pools at the time of conditioned PR production; which has been known to change over the course of conditioning limb reflexes (Wolpaw, 1997).

An alternative explanation for the reduced amplitudes of PRs in Cue-Only trials is that the CNS may have the capability to trigger and to generate the complete postural synergy, but it is terminated as soon as the absence of a postural perturbation is detected. This theory is supported by work that has investigated the independent processes of response production and response termination (Logan et al., 1984; McGarry and Franks, 1997). By varying the stimulus onset asynchrony (SOA) between 'go' and 'stop' stimuli and analyzing the effects of the SOA duration on the evoked response, it was suggested that independent mechanisms subserve the triggering and the termination of movement (McGarry and Franks, 1997). It has been concluded that when excitatory impulses triggered by the 'go' stimulus are followed in short duration by the inhibitory impulses generated by the 'stop' stimulus, the EMG responses in prime movers decreased while still maintaining the profiles of the earliest components of the response triggered by the 'go' stimulus alone. It is reasonable to suggest

that in the current investigation, the lack of a balance perturbation in Cue-Only$_{Conditioning}$ trials acted as a 'stop' signal to terminate what could have been the full postural synergy triggered by the CS. Therefore, the reduced amplitudes of conditioned PRs may be a consequence of early response termination rather than servo-like 'test pulses'. In either case, both theories help to describe why conditioned PRs would be shortened and attenuated as observed in the current results.

2.6.4 Associative learning and memory involved in conditioning PRs

Retention of conditioned PRs and the sustained ability to generate conditioned PRs 15min after paired CS-US trials strongly implicates that higher-order associative learning and memory storage subserve classical conditioning of PRs. Previous work in classical conditioning has demonstrated that the memory trace containing the CS-US relationships is housed within cortical and subcortical regions. The animal model has been instrumental in allowing researchers to examine such influences of these neural centres on memory trace acquisition and retention. The cortical influence in memory trace acquisition and retention was revealed in a two part experiment by Galvez and colleagues (2007) using whisker deflection as the US to elicit eye-blink reflexes in rabbits. Firstly, they showed that a lesion to the relevant part of the primary somatosensory cortex that was most responsive to the CS *before* conditioning caused the eye-blink reflexes to not express the characteristic changes during paired CS-US trials that would otherwise indicate that memory traces were forming. In the second phase of their protocol, Galvez and colleagues (2007) performed the cortical lesion only *after* another set of rabbits were brought to criterion levels of conditioned response development; having showed robust abilities to generate conditioned responses in retention tests. Following these cortical lesions, the ability to generate conditioned eye-blinks was lost, therefore suggesting that areas in the primary somatosensory cortex have functions in both acquisition and retention of stimuli associations. Others have shown in similar fashion that subcortical and cerebellar centres are responsible for trace formation and retention (see Christian and Thompson, 2003 for review). Beyond lesioning studies, other work has also shown that subcortical centres participate heavily in the development of stimulus association and memory trace formation. Specifically, that of McVea and Pearson (2007) corroborate with much of what we have found regarding the carry-over effect to

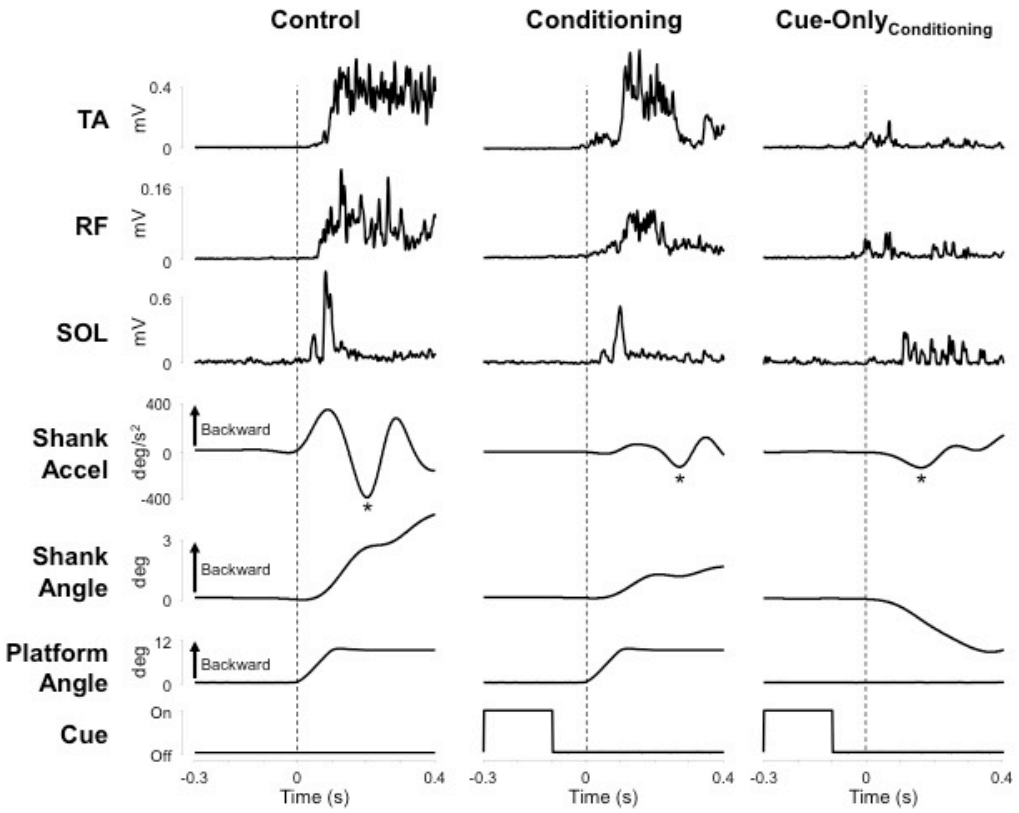
further support the possible influence of subcortical centres in potentiating stimulus-response associations and memory trace retention. Using a feline model, McVea and Pearson (2007) showed that after as few as 20 perturbations to the rear limb while walking on a treadmill, the modification to the cat's step height that was evoked by physical perturbation remained even when the impediment to normal walking was removed. They also observed that this stepping height modification carried-over to subsequent trials performed 20min and up to 24hr post-conditioning. Like McVea and Pearson (2007), we used a similar number of perturbations to upright stance and were able to elicit carry-over responses approximately 15min after the initial conditioning procedure. Although McVea and Pearson (2007) argue that their paradigm was only "superficially" similar to classical conditioning because it did not employ the explicit use of a CS (such as an auditory cue), they suggest that the contextual specificity observed in the carry-over responses imply the involvement of fore-brain centres in memory trace retention (McVea and Pearson, 2007). In the current investigation, the EMG responses evoked in Cue-Only trials caused context-specific angular displacements of the shank that were consistently in the opposite direction to the displacements caused by the balance perturbation. Therefore, the ability to generate context-specific carry-over responses strongly advocates for CNS engagement in retention of the memory trace required for triggering conditioned PRs long after the initial conditioning procedure. Collectively, these studies have shown that specific brain regions known to be involved in memory formation and retention are potentially responsible for producing the carry-over responses observed in the current study. Although there is still much to understand regarding the mechanisms underlying the learning and the retention of conditioned PRs, having access to such neural processes through classical conditioning highlights the potential to apply similar paradigms towards balance training or development of therapeutic prostheses. In situations where sensory loss is significant, such techniques and devices could exercise the adaptability of the CNS to circumvent weaknesses in balance perturbation onset detection and encourage persistent changes to the neural mechanics underlying postural control.

2.7 Conclusion

Classical conditioning provides a unique paradigm through which the triggering dynamics and amplitude modulation of PRs can be examined. Our initial results have

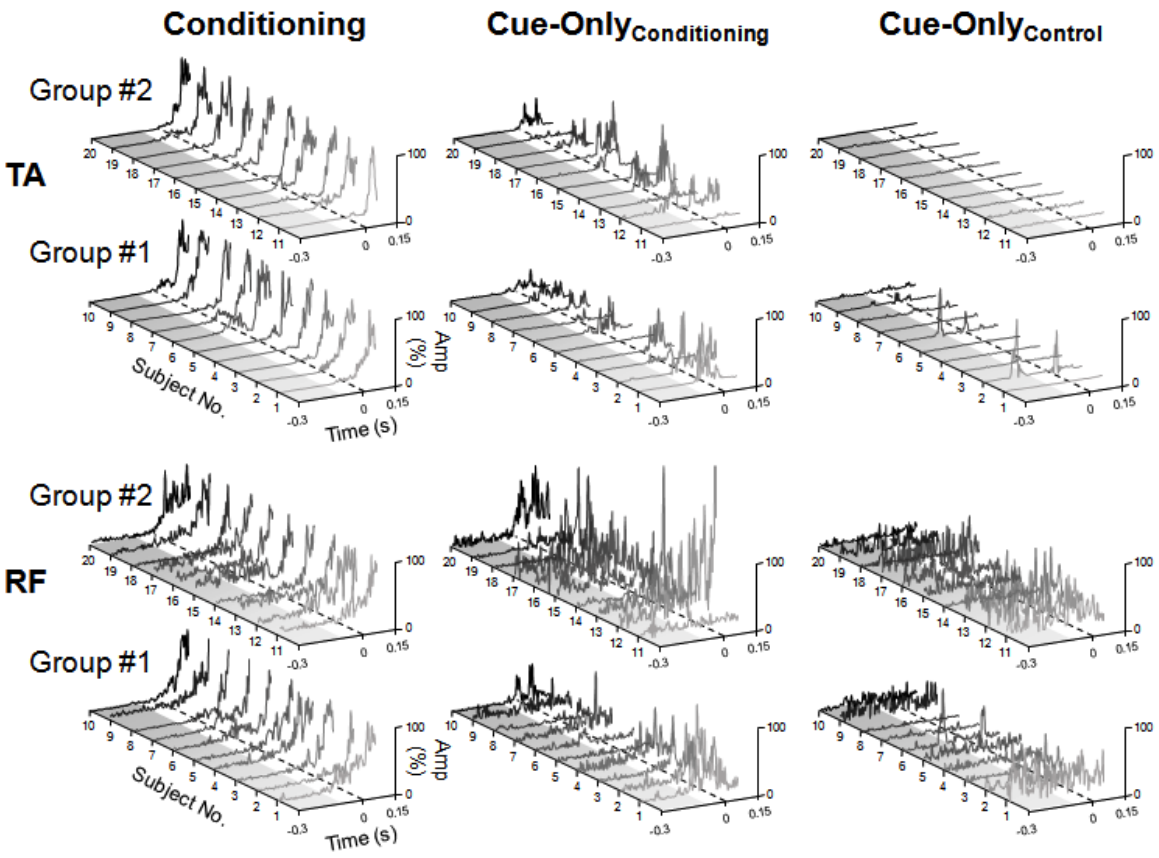
demonstrated for the first time that through classical conditioning, somatosensory, visual and vestibular stimuli related to balance perturbations appear not to be necessary requisites in triggering the earliest components of PRs in multiple lower-limb muscles. The results also provided a unique glimpse into the role of sensory feedback in PR amplitude modulation that could not be achieved through conventional methodological practices. Finally, because the processes of classical conditioning allow access to neural centres responsible for stimuli association and memory trace retention, we feel that classical conditioning of PRs could provide the groundwork from which assistive devices and balance training protocols could be developed.

Figure 2.1: Representative subject EMG and kinematic data



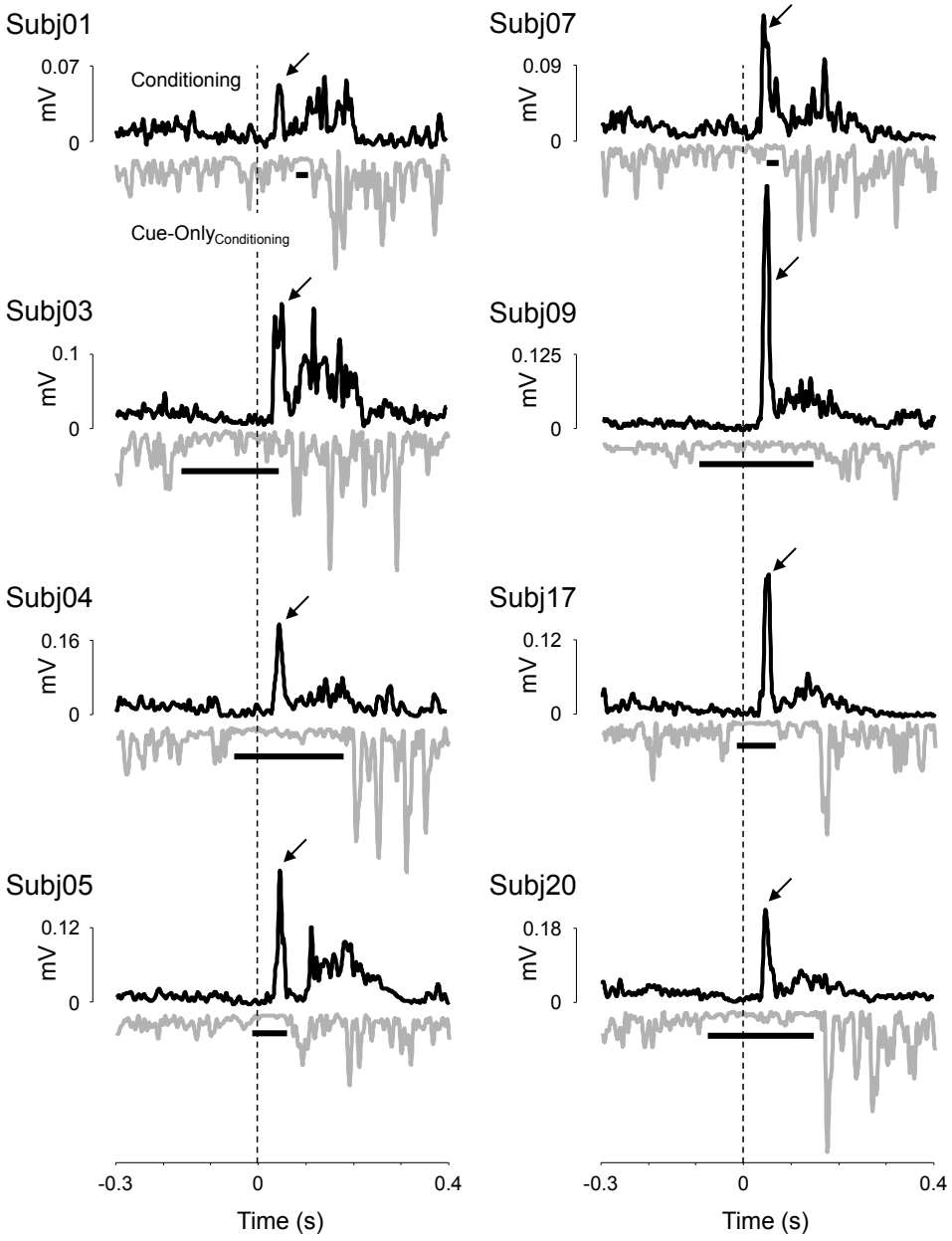
Tibialis anterior (TA), rectus femoris (RF) and soleus (SOL) muscle activities, shank acceleration and shank angle changes during Control, Conditioning and Cue-Only$_{Conditioning}$ trials for a representative subject. Traces in Control and Conditioning columns are averages from the last 5 trials in each respective block. Positive acceleration and angular changes denote movement in the same direction as the toes-up (backward) support-surface tilt. The vertical dashed lines represent the onsets of the actual support-surface tilts in Control and Conditioning trials, and the expected support-surface tilt in the Cue-Only$_{Conditioning}$ trial. The negative peaks of shank angular accelerations that were of interest in statistical analysis are denoted by (*). Note the different Cue and Platform combinations that are specific to each type of trial.

Figure 2.2: Waterfall plots of TA and RF EMG data



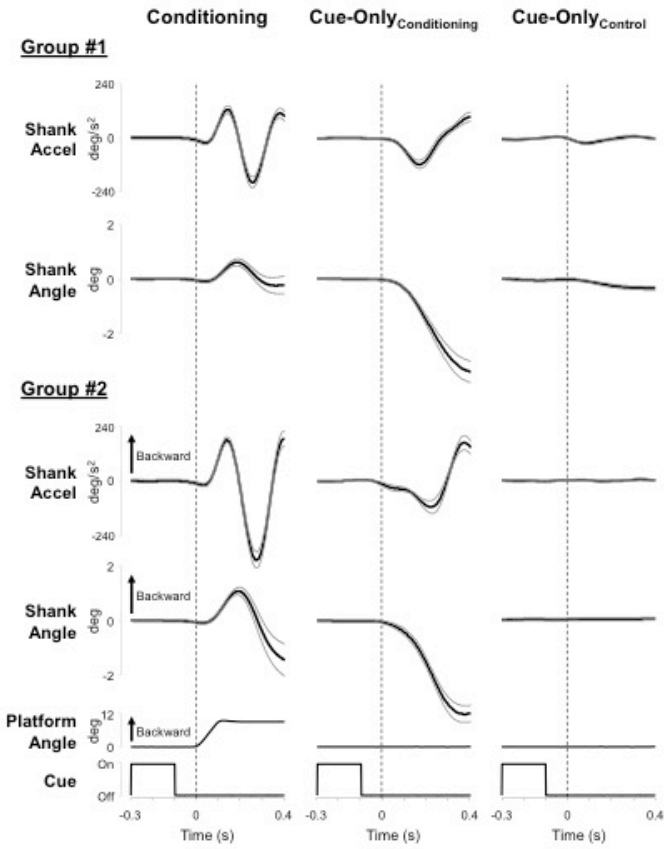
Waterfall plots of tibialis anterior (TA) and rectus femoris (RF) muscle activity in Conditioning, Cue-Only$_{Conditioning}$ and Cue-Only$_{Control}$ trials for all participants (n=20). Subjects were split into 2 groups based on the order in which they experienced the blocks of Conditioning and Control trials; Group #1 received the Conditioning trials first whereas Group #2 received the Control trials first. The grey area represents the time period of the 200msec auditory cue. The dashed line denotes (1) the true onsets of support-surface tilts in Conditioning trials and (2) the expected but absent onsets of support-surface tilts in Cue-Only$_{Conditioning}$ and Cue-Only$_{Control}$ trials. Conditioning traces were based on the average of the last 5 Conditioning trials for each subject. For each subject and trial type, TA and RF traces were normalized to their peak amplitudes in Conditioning trials.

Figure 2.3: Inhibition of SOL EMG during Cue-Only$_{Conditioning}$ trials



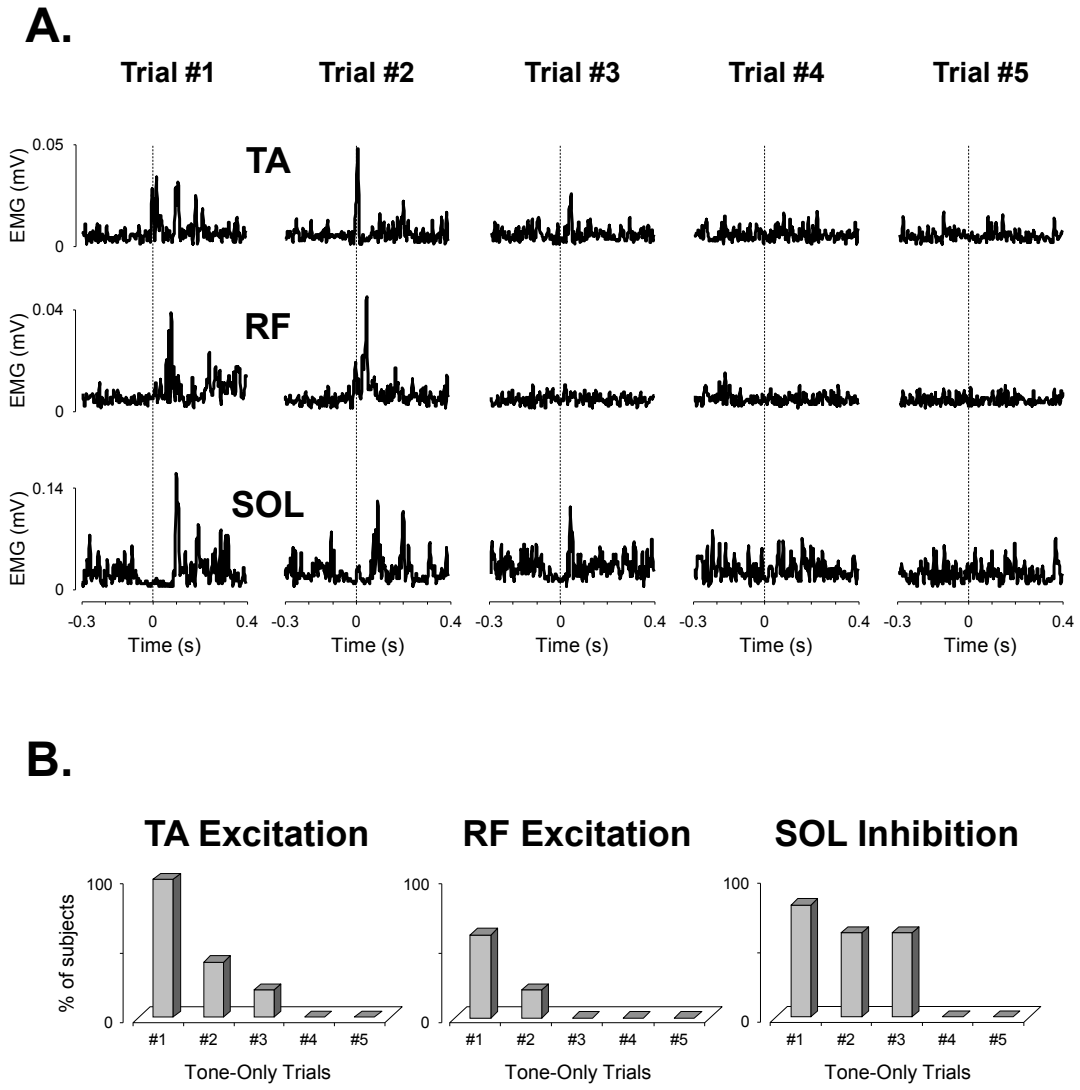
Each pair of trials represents soleus (SOL) muscle activity in Conditioning (black; positive y-axis values) and Cue-Only$_{Conditioning}$ (grey; negative y-axis values) trials. The presence of stretch reflexes in Conditioning trials are denoted by arrows, whereas horizontal black bars represent the locations of tonic SOL inhibition during Cue-Only$_{Conditioning}$ trials. The vertical dashed lines indicate (1) the true onsets of support-surface tilts in Conditioning trials and (2) the expected but absent onsets of support-surface tilts in Cue-Only$_{Conditioning}$ trials.

Figure 2.4: Group differences in shank kinematics between trial types



Traces represent the mean (thick black) ± 1 standard error (thin grey) of shank accelerations and shank angles for all participants in Group #1 and Group #2 during Conditioning, Cue-Only$_{Conditioning}$ and Cue-Only$_{Control}$ trials. Group differences were based on the order of Conditioning and Control trial presentation; Group #1 received the Conditioning trials first whereas Group #2 received the Control trials first. The vertical dashed lines represent (1) the true onsets of support-surface tilts in Conditioning trials and (2) the expected but absent onsets of support-surface tilts in Cue-Only$_{Conditioning}$ and Cue-Only$_{Control}$ trials. Positive angles and accelerations represent movements in the same direction as the toes-up (backward) support-surface tilt. Note the different Cue and Platform combinations that are specific to each type of trial.

Figure 2.5: Extinction of TA, RF and SOL conditioned responses



(A) Representative subject EMG responses for tibialis anterior (TA), rectus femoris (RF) and soleus (SOL) during the 5 Tone-Only trials that immediately followed conditioning in Experiment #2. Note that as trials progress, the conditioned postural responses become less evident and were fully extinguished by Trial #4. Vertical lines denote the expected onset of the support-surface tilt in conditioning trials.

(B) Bar charts indicate the percent of subjects (n=6) who expressed either TA or RF excitation, or SOL inhibition in the 5 Tone-Only trials that immediately followed the conditioning block of Experiment #2. Note that by Trial #4, the conditioned postural responses were completely extinguished in all subjects.

Table 2.1: Summary measures of EMG data

	Control		Conditioning		Cue-Only$_{Conditioning}$	
	Latency (ms)	Amplitude (μVs)	Latency (ms)	Amplitude (μVs)	Latency (ms)	Amplitude (μVs)
SOL	32.8 ± 1.1	11.9 ± 1.2	34.2 ± 2.1†	6.1 ± 0.9*†	132.0 ± 8.3	3.3 ± 0.5
TA	97.4 ± 3.1	36.8 ± 2.8	36.4 ± 8.8*†	21.3 ± 2.4*†	-10.5 ± 10.2	5.7 ± 1.0
RF	104.3 ± 4.9	8.6 ± 1.5	66.1 ± 8.6*	2.5 ± 0.3*†	29.4 ± 20.2	1.1 ± 0.2

** represents significant differences within measures between Control and Conditioning trials.*
† represents significant differences within measures between Conditioning and Cue-Only$_{Conditioning}$ trials.
Significance levels were set a p≤0.017 for all statistical tests.

Soleus (SOL), tibialis anterior (TA) and rectus femoris (RF) muscular responses during Control, Conditioning and Cue-Only$_{Conditioning}$ trials. Values represent mean ± 1SE. See Methods section for calculations.

Chapter 3: Startle induces early initiation of conditioned postural responses

3.1 Introduction

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.

3.2 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.

3.2.1 Experimental setup

Kinetics and kinematics

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.

3.2.2 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 3.1) 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 3.1). 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/s^2 acceleration) presented 300ms after the onset of the auditory CS (Figure 3.1). 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 3.1) 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 3.1). 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.

3.2.3 Measures

Kinetics and kinematics

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 (Carlsen et al., 2011b).

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.

3.2.4 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). Consequently, kinetic and kinematic dataset reduction based on the prevalence of generalized startle responses was not conducted.

3.2.5 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 ≤ 0.05 and significant trends were considered at *p*-values between >0.05 and ≤ 0.10.

3.3 Results

3.3.1 Kinetics and kinematics

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 3.2). This initial response was quickly followed by ankle inversion and hip abduction and leftward COP displacements (Figure 3.2). Over the course of the 15 Conditioning trials, onsets of COP displacements progressively decreased and plateaued by the end of the block (Figure 3.3), 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 3.2). 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 3.3), 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 3.2) that was highly consistent across subjects (Figure 3.4). 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 3.2 & 3.5). 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 3.5 & Table 3.1). 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 3.5 & 3.6). 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 3.5 & Table 3.1). 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 3.1).

3.3.2 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 3.7). As shown in Figure 3.3, 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 3.3). 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 3.3).

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 3.8 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 3.8) 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 $_R$GM 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 $_R$GM were significantly larger in post-Conditioning Startle compared to CS-Only trials (t(9)=2.49, p=0.034).

3.4 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.

3.4.1 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 $_{R}$TA and $_{R}$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 (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.

3.4.2 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.

3.4.3 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 (Carlsen et al., 2011a; Alibiglou and MacKinnon, 2012). 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.

3.4.4 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 $_{R}$GM, and to a limited extent $_{R}$TA, 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

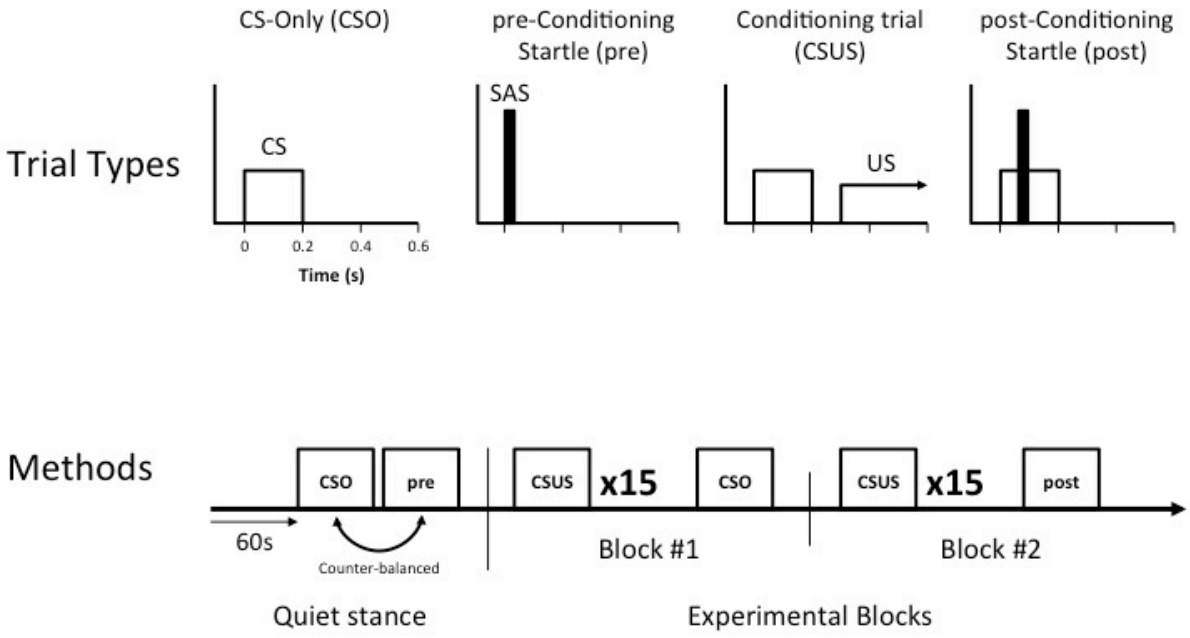
conditioning where the effects of the US and the associated responses are limited specifically to the eye.

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.

3.4.5 Conclusions

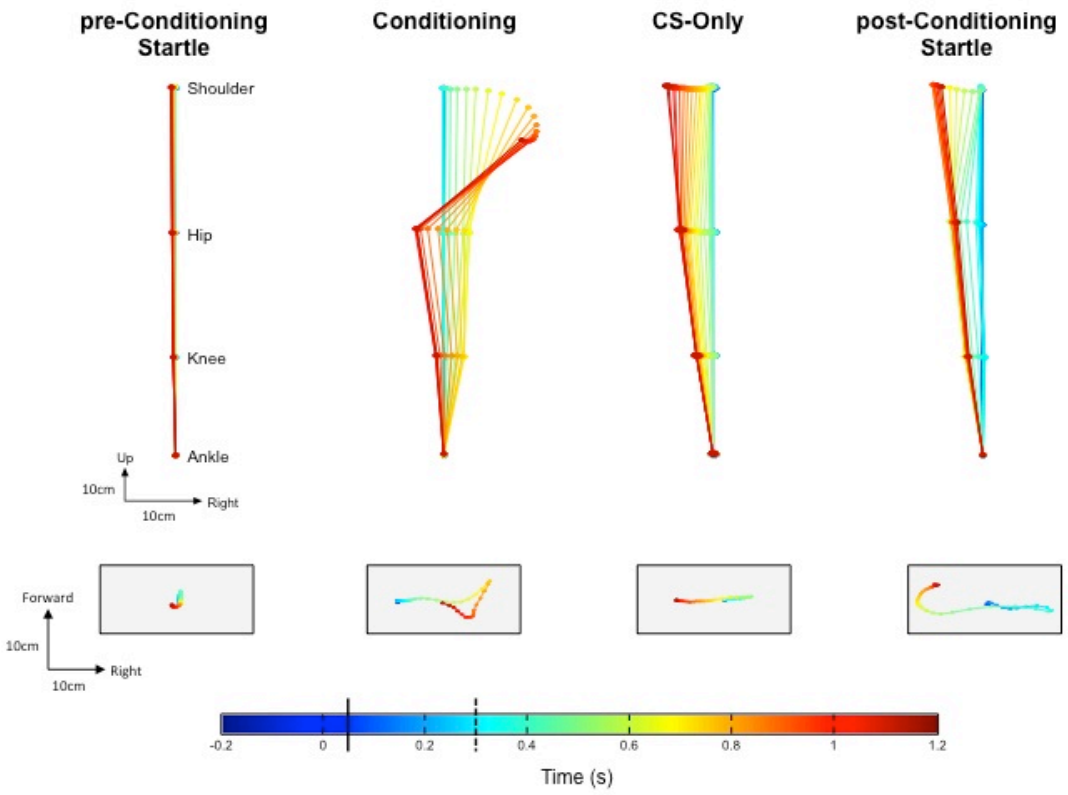
We have demonstrated that a SAS can induce the rapid initiation of a PR conditioned to a cue and a lateral support-surface translation. In doing so, we have discovered a potential neural link between dynamic postural control and processes responsible for classical conditioning and motor preparation. 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.

Figure 3.1: Schematic of trial-types and methodology



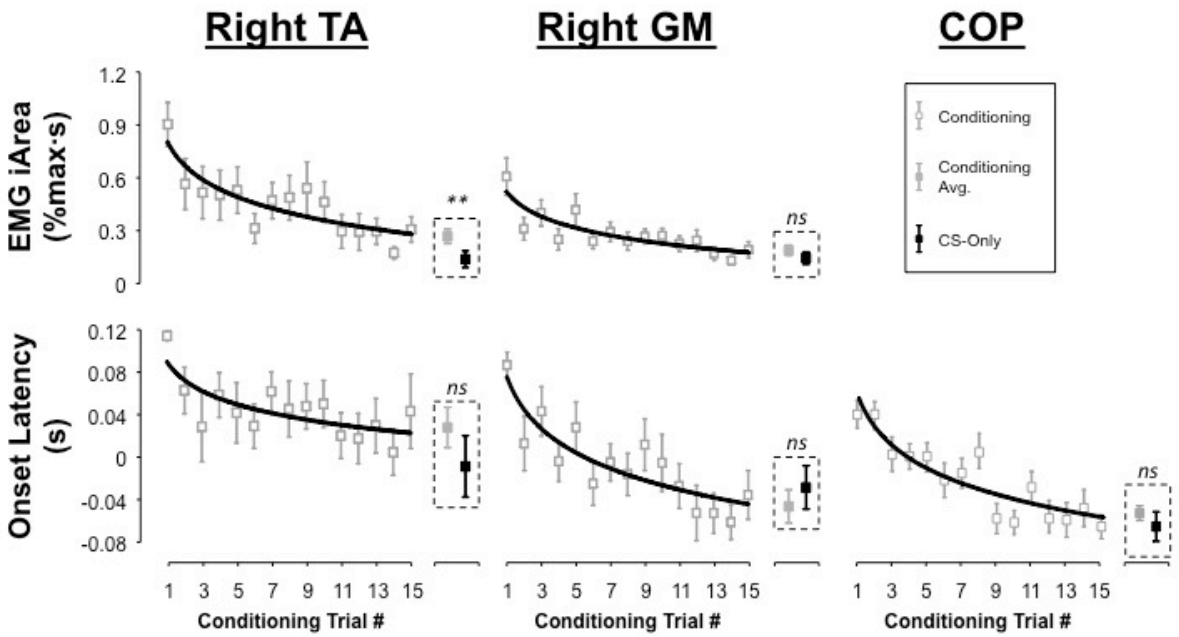
Four distinct 'trial types' (CS-Only, pre-Conditioning Startle, Conditioning, post-Conditioning Startle) were used during this experiment; each with a particular set of stimuli present within them (i.e. CS: conditioned stimulus; SAS: startling acoustic stimulus; US: unconditioned stimulus). The experimental 'methods' depicts a schematic of the order in which trials were presented to subjects. Trial types were identified within the methods schematic using abbreviated terms located in the bracketed titles.

Figure 3.2: Color-coded, time-based kinematic and COP displacements



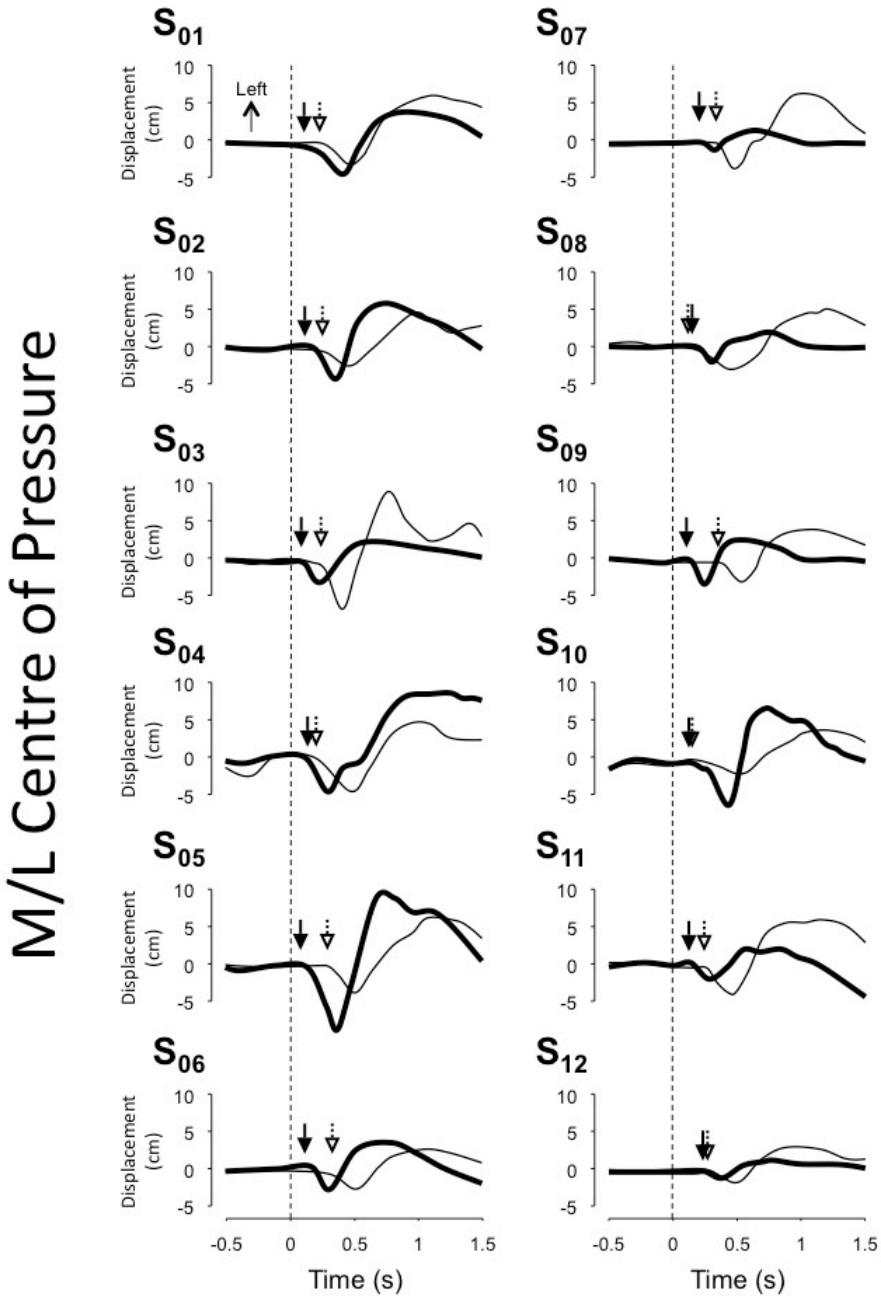
For a single representative subject, posterior frontal-plane view of stick figures illustrating kinematics of right ankle, knee, hip and shoulder displacements during pre-Conditioning Startle, Conditioning, CS-Only and post-Conditioning Startle trials. Insets located below each stick figure represent 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 3.1 for details.

Figure 3.3: Progression of conditioned response acquisition in $_{R}$TA and $_{R}$GM



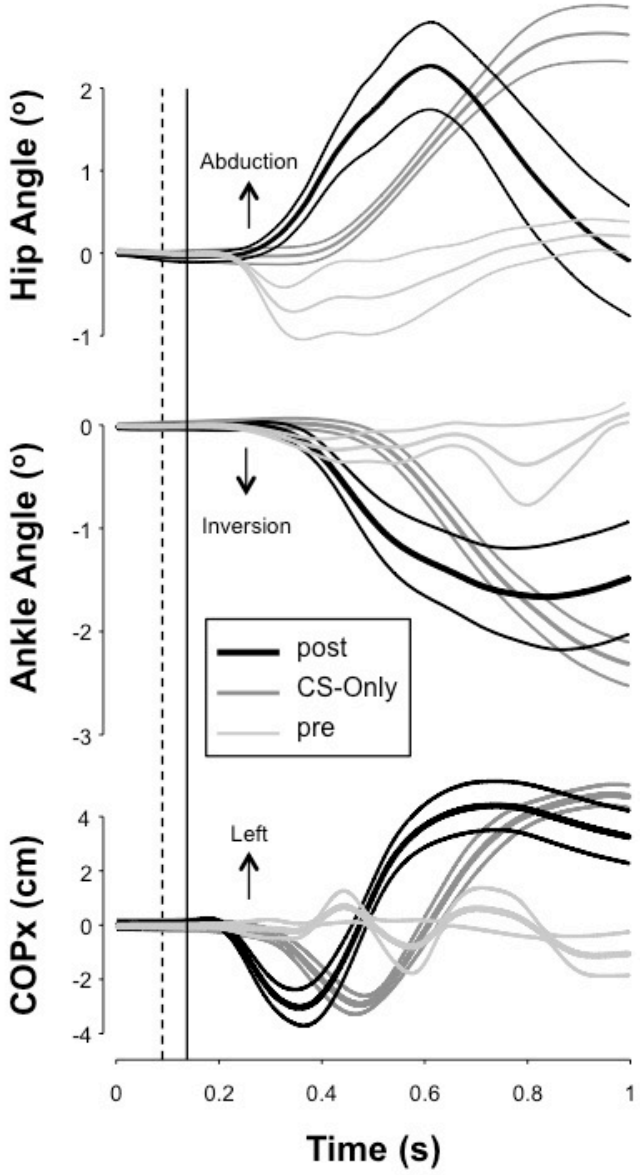
Group mean (±1SE) onsets and amplitudes of right tibialis anterior (TA) and right gluteus medius (GM) EMG activity and onsets of COP for each of the 15 trials during the first block of Conditioning. For onsets, time 'zero' represents the onset of the balance perturbation. Amplitudes of EMG are scaled 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 3.4: M/L COP displacments during CS-Only and post-Conditioning Startle trials



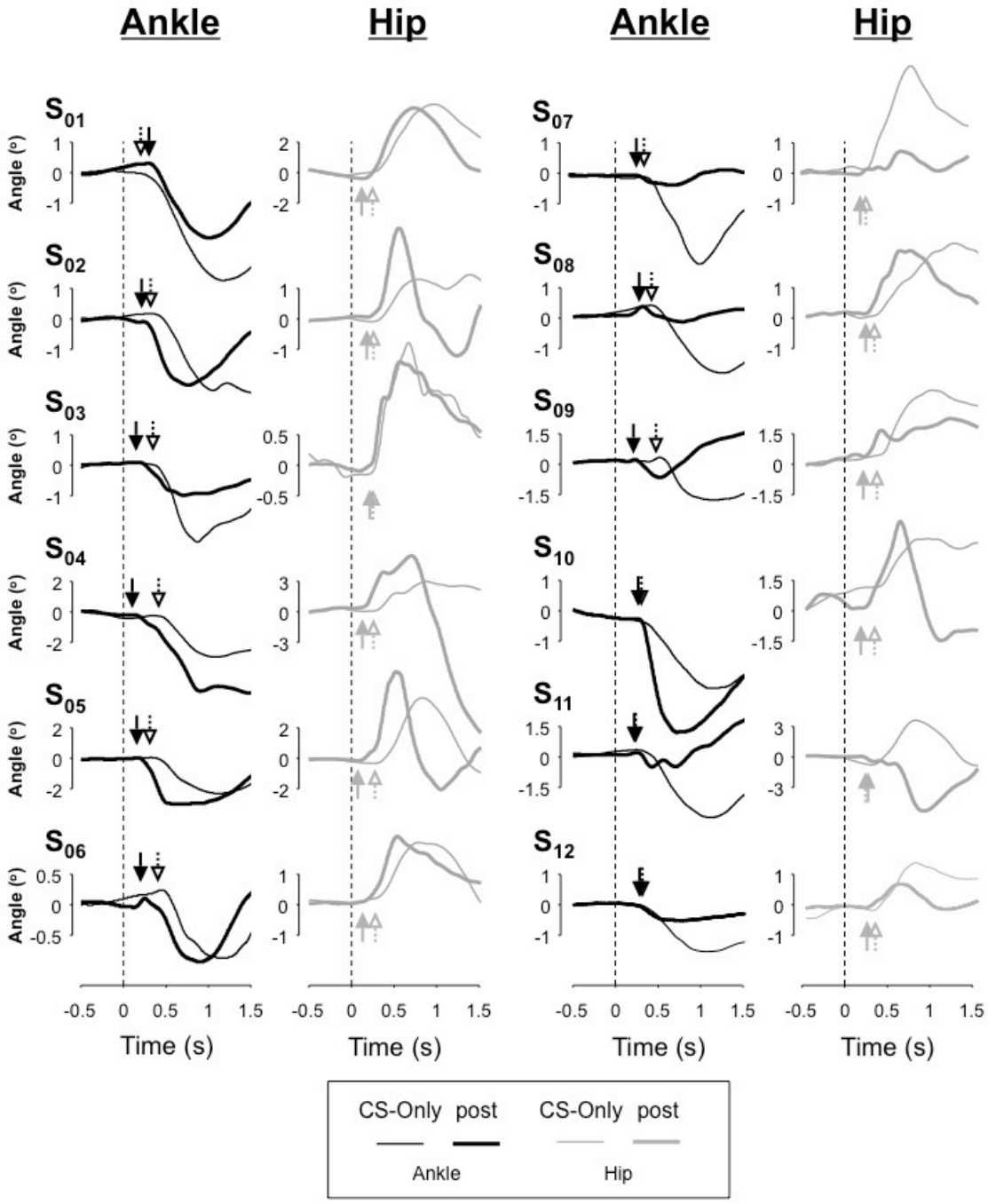
M/L COP displacements produced during CS-Only (thin black) and post-Conditioning Startle (thick black) trials for all 12 subjects (i.e. S_{01}-S_{12}). Arrows indicate onsets for each trial; hollow arrowheads for CS-Only trials and solid arrowheads for post-Conditioning Startle trials. Vertical hashed lines denote onsets of the CS in each trial.

Figure 3.5: Average traces of M/L COPx, and frontal-plane ankle and hip displacements



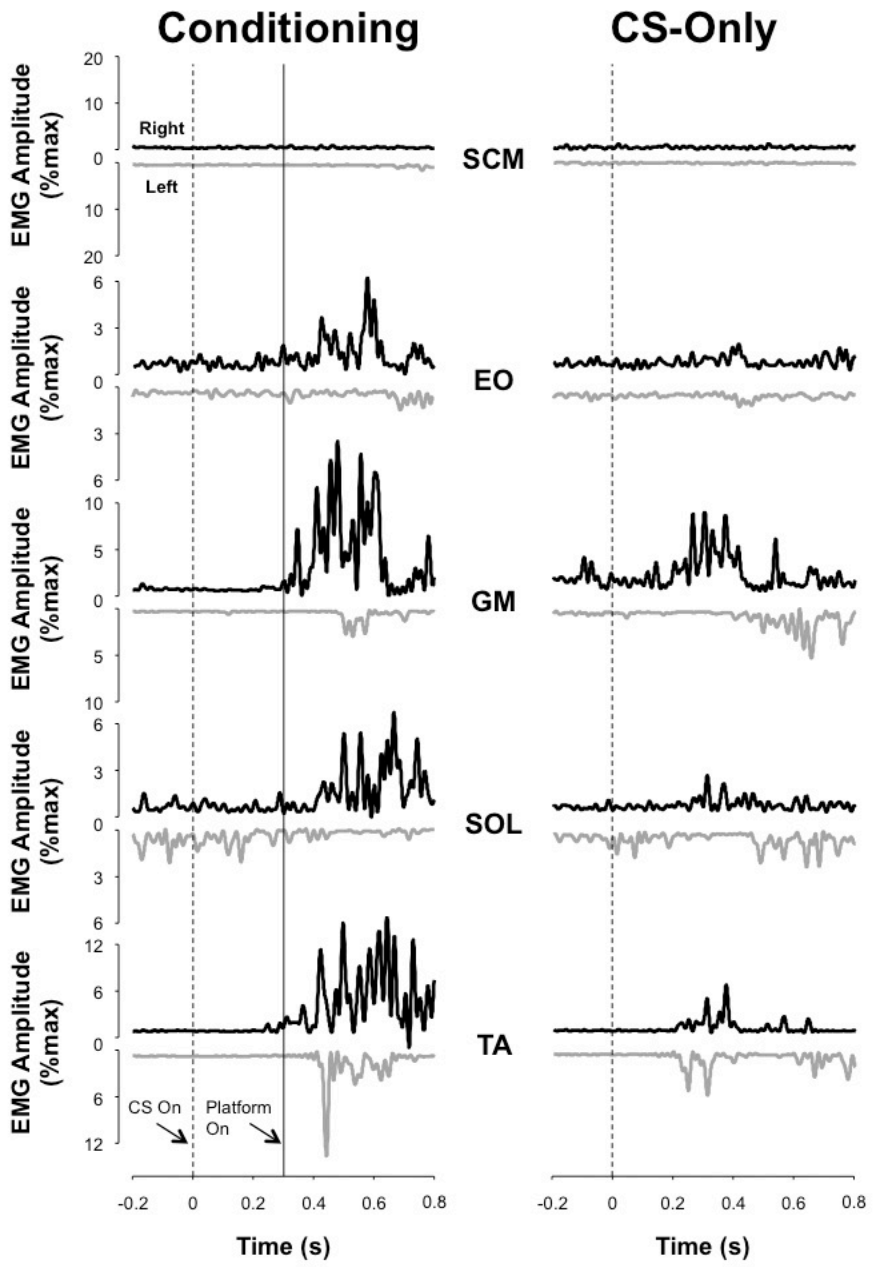
Group mean (±1SE) displacements of the right hip, right ankle and M/L COP in post-Conditioning Startle (post; black), CS-Only (dark grey) and generalized startle responses observed during pre-Conditioning Startle trials (pre; light grey). The vertical dashed line represents the onset of the CS and the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

Figure 3.6: Ankle and hip displacements in CS-Only and post-Conditioning Startle trials



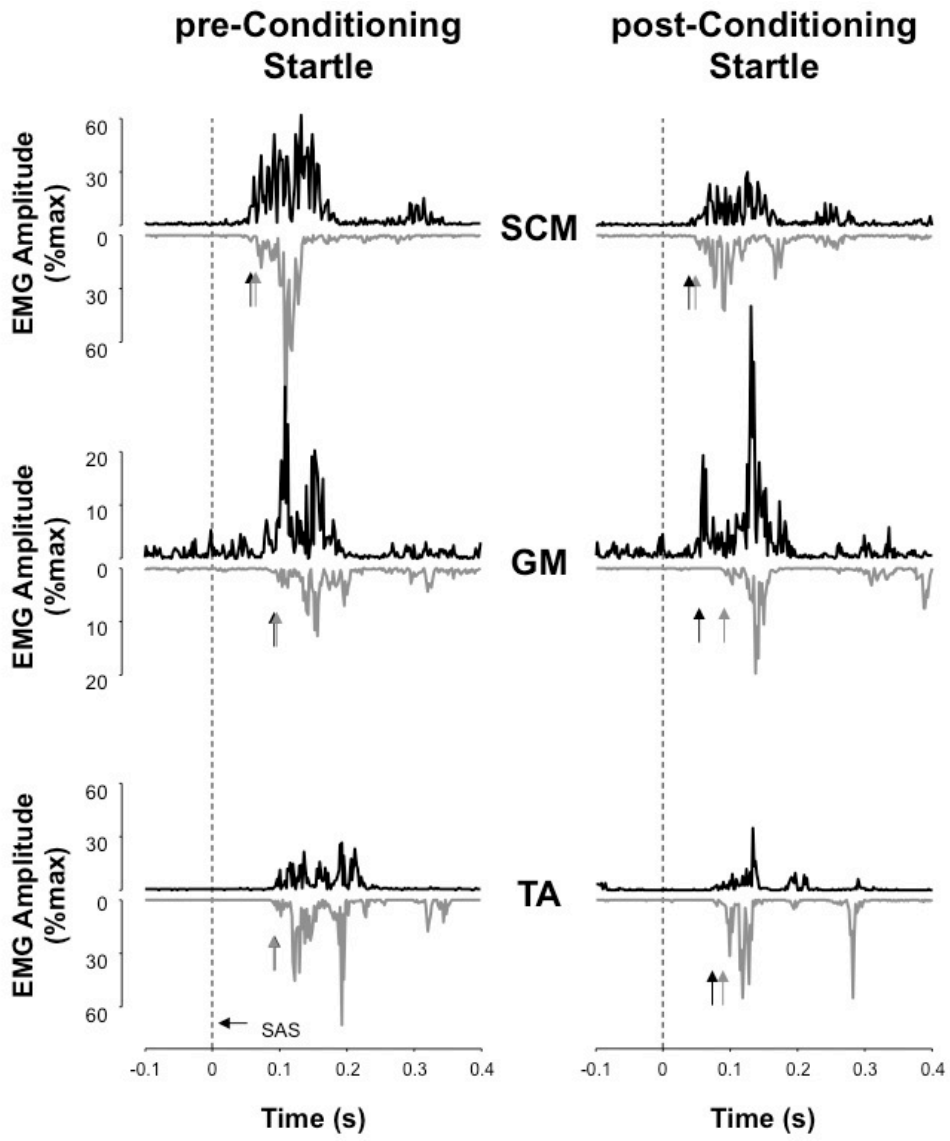
Angular displacements of the right ankle (black) and right hip (grey) during both CS-Only (thin lines) trials and post-Conditioning Startle (post; thick lines) trials for all 12 subjects (i.e. S_{01}-S_{12}). Vertical dashed lines denote onset of the conditioned stimulus (CS) in each trial. Onsets of angular accelerations are indicated by solid arrows during PCS trials and by hollow arrows during CS-Only trials.

Figure 3.7: Representative subject EMG responses in Conditioning and CS-Only trials



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 3.8: Representative subject EMG data in pre- and post-Conditioning Startle trials



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.

Table 3.1: Summary COPx, ankle and hip displacement measures

	COPx		Ankle		Hip	
	CS-Only	post	CS-Only	post	CS-Only	post
Onset	215 ±18ms	104 ±13ms**	318 ±25ms	191 ±16ms**	237 ±15ms	150 ±16ms**
Peak Displacement	-2.75 ±0.40cm	-3.06 ±0.57cm	-2.18 ±0.19deg	-1.71 ±0.45deg	2.55 ±0.30deg	1.90 ±0.79deg
Time-to-Peak	198 ±14ms	153 ±19ms‡	608 ±43ms	433 ±53ms**	518 ±42ms	393 ±37ms‡

data in cells 'CS-Only' and 'post' represents the mean ±1SE

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

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

Onset, peak displacement and time-to-peak measures of COPx, Ankle and Hip during CS-Only and post-Conditioning Startle (post) trials.

Chapter 4: Unexpected balance perturbations facilitate motor preparation of postural responses

4.1 Introduction

Voluntary motor behaviours require multiple stages of processing (Donders, 1869) that eventually lead to preparation of the motor response. This preparation acts to simplify movement control by concentrating individual response elements (such as timing, amplitude and coordination parameters) into a reduced set of units before movement onset (Keele and Summers, 1976; Klapp, 1995). The nature of motor preparation has been extensively studied during voluntary movements (Valls-Solé et al., 1999; Siegmund et al., 2001; Carlsen et al., 2004; Queralt et al., 2008ab). Recent evidence suggests that motor preparation may also influence movements that aim to regain stability following balance perturbations.

A postural response (PR), evoked by an external balance perturbation, is a type of reactive behaviour (Nashner and Cordo, 1981; Horak and MacPherson, 1986; Carpenter et al., 1999) that may be influenced by motor preparation. Using a classical conditioning paradigm, results from Chapter 3 recently demonstrated that aspects of directionally-specific PRs could be prepared when participants were highly experienced with balance perturbations that were each preceded with time-locked auditory cues; in which case, the onset of balance perturbations were considered predictable. These results have led to two major questions. First, can PRs be prepared in advance of balance perturbations that are not preceded by cues and therefore are unpredictable in time (hereafter, termed 'unexpected' to be in accordance with the literature)? Second, does experience influence PR motor preparation? These questions are particularly relevant towards understanding the mechanisms involved in dynamic postural control and the experimental techniques commonly used to study PRs.

The characteristics of prepared movements and whether they change with experience can both be examined using loud, startling acoustic stimuli (SAS) (Carlsen et al., 2011; Maslovat et al., 2008; 2009; 2011). When a person is at rest and not engaged in specific motor preparation, unexpected SAS evoke only generalized startle responses characterized as bilaterally symmetric, whole body flexion reactions (Landis and Hunt, 1939; Rossignol, 1975; Brown et al., 1991). In contrast, SAS have also been shown to evoke a variety of contextually-specific movements when these movements are specifically prepared in

advance of the stimulus (Valls-Solé et al., 1999; Siegmund et al., 2001; Carlsen et al., 2004). Not only do the movements triggered by SAS depend on the type of movement prepared, the features (e.g., amplitude, timing) of the movement also undergo experience-related changes that reflect the extent of preparation. Sufficient experience can facilitate near complete preparation; consequently, movements evoked by SAS occur more rapidly (Carlsen and MacKinnon, 2010) and retain many relative timing and spatial characteristics observed during control trials (Carlsen et al., 2011; Maslovat et al., 2011). In contrast, less than fully prepared movements have delayed onsets (Carlsen and MacKinnon, 2010), are notably reduced in amplitude and are less likely to emerge following SAS presentation (MacKinnon et al., 2007; Carlsen and MacKinnon, 2010; Maslovat, PhD thesis). Thus, by characterizing movements evoked by SAS that differ from generalized startle responses, it is possible to determine both 'if' motor preparation has been undertaken and whether the extent of preparation was affected by factors such as experience.

The objectives of this experiment were to determine 1) if unexpected balance perturbations could induce advanced motor preparation of PRs and 2) if the extent of PR motor preparation would be influenced by repeated experience with balance perturbations. We hypothesized that SAS would induce prepared PRs after repeated experiences with balance perturbations that would differ from generalized startle responses. We also hypothesized with experience, SAS would induce prepared PRs of greater magnitude and with greater probability.

4.2 Methods

Twelve completely naïve subjects (6 males, mean ±1SD age, height and body mass; 21.1±1.9 years, 1.74±0.11m and 70.25±12.74kg, respectively) volunteered to participate in this experiment. Upon entering the laboratory, each subject was individually briefed on all methodological procedures and data collection techniques prior to providing their informed consent to participate. The University of British Columbia Research Ethics Board approved all experimental procedures.

4.2.1 Data collection and processing

Kinetics

Two forceplates were used in this experiment. One of the forceplates (BP400600, Advanced Mechanical Technologies, Incorporated, USA) was mounted flush with the top surface of a stage (1.23m wide; 0.61m long) affixed to a moveable platform (DR Stage, H2W Technologies Incorporated, USA). The other forceplate (#K00407, Bertec Corporation, USA) was placed on the ground located directly behind the stage.

For both forceplates, ground reaction forces and moments were sampled at 1000Hz (Power1401, Cambridge Electronic Design, UK) and digitally lowpass filtered offline at 5Hz (Spike2, Cambridge Electronic Design, UK) prior to calculating centre of pressure (COP) in frontal- and sagittal-planes.

Kinematics

Clusters of 3 non-collinear infra-red light emitting diodes (iREDS) were placed on the feet, shanks and thighs as well as on the trunk of each subject. 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 which required defining the location of the acromion processes, greater trochanters and lateral malleoli on both sides of the body using virtual markers (Visual 3D, C-Motion, USA). Offline, raw marker positions were lowpass Butterworth filtered at 5Hz (Visual3D, C-Motion, USA).

Using the locations of the virtual markers, a 2-segment model was developed to characterize whole-body sway in the frontal plane. The upper-body segment was defined between the average locations of bilateral acromion processes and the greater trochanters. The lower-body segment was defined between the average locations of bilateral greater trochanter markers and the average of the lateral malleoli markers. For each segment, 2-dimensional absolute angular displacements were calculated in the frontal-plane (Matlab 7.1, Mathworks, USA).

Surface electromyography (EMG)

EMG activity was recorded from bilateral sternocleidomastoid (SCM) muscles using pre-gelled Ag/AgCl surface electrodes. An experimenter first identified the locations of each muscle belly, then shaved and cleaned the skin with alcohol swabs. EMG data were grounded off a single electrode placed on the acromion process of the right scapula. Raw EMG data were pre-amplified at 500x, sampled at 3000Hz and band-pass filtered between 10-500Hz (Telemyo 2400R, Noraxon, USA) before being A/D converted at 1000Hz (Power1401, Cambridge Electronic Design, UK). Offline, these data were digitally high-pass filtered at 30Hz (Spike2, Cambridge Electronic Design, UK) in order to remove heart rate artifacts then baseline corrected and full-wave rectified.

4.2.2 Experimental procedures

Quiet stance and generalized startle trials

Prior to entering the laboratory, subjects were randomly placed into one of 2 possible groups (Group 1 (n=6); Group 2 (n=6)), which differed in the direction of support-surface translations they would experience in later trials. Each subject, regardless of group, performed two 60s quiet standing trials (the first took place on the forceplate mounted onto the moveable platform and the second took place on the forceplate located on the ground). In each trial, subjects stood relaxed and looked straight ahead at an eye-level target located ~2m away with stance equal to 100% of their measured foot length. An experimenter informed all subjects that at no time would the location of either forceplate be mechanically displaced during quiet stance. At the end of each 60s trial, 2 auditory stimuli, which originated from the same speaker located ~1m over the subject's head, were randomly presented at least 20s apart to assess their effectiveness in evoking reactions prior to experiences with balance perturbations. One stimulus was a non-startling auditory cue (<80dB, 1000Hz, 40ms duration), which at this point was not expected to induce any detectable movements. The other stimulus was a SAS (~120dB, 1000Hz, 40ms duration, ~1ms rise-time) (CR:251B Impulse sound level meter, Cirrus Research plc, UK) which was only expected to induce generalized startle responses after quiet stance. If generalized startle responses evoked while standing on the moveable platform were found to be directionally

biased in the frontal-plane (see Results), generalized startle responses evoked on the second forceplate would be used to characterize SAS-induced sway in an environment with no threat of the support-surface being displaced.

Visual and physical experiences with balance perturbations

In the next phase of testing, subjects were given 3 separate opportunities to observe platform translations (1m displacement, 0.25m/s velocity, 1.3m/s^2 acceleration) (Figure 4.1) while standing on the ground and therefore in an environment with relatively low threat of balance being compromised. The direction of the observed platform displacements was dependent upon group. For subjects in Group 1, the platform only translated leftwards. For subjects in Group 2, the platform only translated rightwards. Prior to the first observation, subjects were told that they would eventually receive numerous physical balance perturbations identical those they were to be shown. At no time had any participant experienced similar balance perturbations in the current or prior experiments in our laboratory. Therefore, these observation periods were subjects' first opportunities to experience the mechanical translations of the support-surface.

After the 3rd visual experience, subjects stepped onto the forceplate mounted onto the moveable platform. In this phase of the experiment, subjects were made aware that they would receive a block of balance perturbations as well as intermittent SAS and non-startling cues. Subjects were also told that each stimulus would only occur in isolation of the others (i.e. SAS and perturbations would never occur within the same trial) and they were totally unaware of exactly how many of each stimulus they would receive and when each stimulus would be presented.

After subjects were again standing comfortably on the moveable platform, the first experimental trial they experienced was an unexpected pre-Perturbation Startle (SAS_{PRE}) trial (Figure 4.1). Since subjects had yet to physically experience the platform movement, SAS_{PRE} trials were used to determine if only visual experiences with balance perturbations were sufficient to induce motor preparation of directionally-specific PRs. Following SAS_{PRE}, subjects received a single unexpected balance perturbation (left or right depending on Group). This was followed by a second, unexpected SAS trial (SAS_1) that was used to determine if only one physical experience with balance perturbations could induce motor

preparation of PRs (Figure 4.1). Subjects then received 11 identical perturbations (leftward or rightward depending on Group) spaced 10-20s apart. Two trials involving only non-startling cues (Tone-Only) presented during quiet stance were interspersed among these perturbations (after perturbation 4 and 8) (Figure 4.1) to assess whether subjects utilized auditory cues to initiate volitional motor behaviours. After the 12th physical experience with the balance perturbation, a 3rd and final unexpected SAS trial (i.e. SAS_{12}) was presented during quiet stance (Figure 4.1).

4.2.3 Data analysis

Onset latencies

For COP, lower- and upper-body angular displacements, background accelerations were determined as the mean ±2SD calculated within a 500ms epoch immediately before each SAS. Onsets were calculated as the first time in which accelerations surpassed and remained beyond threshold for a minimum of 150ms.

For EMG, threshold values were quantified from the mean plus 2SD of background activity calculated for 500ms of data immediately preceding the onset of each trial. Using a semi-automated algorithm, onsets of processed EMG activity were determined to be the time at which EMG activity first surpassed and remained above threshold for a minimum of 30ms while at no time dipping below for >3ms (Carpenter et al., 2008).

Amplitudes

For trials with detectable onsets, COP, lower- and upper-body kinematic angular displacement magnitudes were determined as the first displacement peak or trough (i.e. zero velocity) minus the mean background position calculated 500ms prior to SAS onset. Polarities of peak response amplitudes were used to classify movements as ipsilateral/contralateral to perturbation direction. Time-to-peaks were determined as the delay between response onset and zero velocity.

In muscles with detectable onsets, response amplitudes were determined from integrals of EMG calculated for 100ms following response onset minus background activity calculated 100ms prior to the beginning of each trial.

4.2.4 Data reduction

SAS trials (i.e. SAS_{PRE}, SAS_1 and SAS_{12}) were first examined to ensure that they evoked a startle response in SCM. For measures (either kinetic or kinematic) to be included for analysis of motor preparation during any SAS trial, onsets of at least 1 SCM muscle must have occurred within 90ms of SAS onset (Carlsen et al., 2011). If a startle response was not detected, all data within the respective trial was removed from further analyses.

It was expected that generalized startle responses evoked by SAS presentation would elicit at least some frontal-plane sway independent of the potential influences of motor preparation (Chapter 3). Therefore, data from SAS trials were included only if frontal-plane sway response magnitudes surpassed thresholds established from generalized startle trials. In establishing thresholds, we assumed that 1) the initial components of sway induced by generalized startle responses would not be directionally biased in the frontal-plane and 2) that generalized startle response amplitudes would be greatest during the first unexpected exposure to SAS (Oude Nijhuis et al., 2010). Thresholds were developed for each subject by determining the absolute value of maximal frontal-plane sway amplitudes induced by generalized startle responses (see Amplitudes section). Based on our first assumption, the absolute value of maximal sway amplitudes achieved in generalized startle response were used to set an equivalent range above and below resting values for subsequent SAS trials in each subject. Based on our second assumption, responses evoked by SAS trials (i.e. SAS_{PRE}, SAS_1 and SAS_{12}) were removed from analysis if their magnitudes did not surpass thresholds and thus were not discernable from generalized startle responses.

4.2.5 Statistical analyses

Our experiment centred on determining the ability for SAS to induce prepared frontal-plane PRs discernable from generalized startle responses and the effects of repeated balance perturbations on PR motor preparation. The null hypothesis was that no amount of experience with balance perturbations would induce prepared sway responses and thus SAS would evoke only generalized startle responses. To test the validity of the null hypothesis, we tabulated the total number of subjects who produced a sway response that surpassed generalized startle response thresholds (see Data reduction section) for each SAS trial and represented them as percentages of the total possible (out of 12). For the responses that

surpassed threshold, binomial statistics were performed in each SAS trial condition to determine if the probability of the observed sway directions (ipsilateral or contralateral to direction of support-surface translation) differed from 50%. To ensure suitable power of the binomial statistic, we focused analyses only on those variables that had a least n=6 (Schlich, 1993). Significant results from binomial tests would suggest that support-surface displacements significantly influenced the direction sway induced by SAS and would further suggest that SAS responses were distinct from generalized startle responses. Non-significant binomial statistics would suggest SAS induced randomly directed sway.

The effects of experience on response magnitude parameters (i.e. onsets, amplitude and time-to-peak) were compared across SAS trials using 3-way (SAS_{PRE}, SAS_1, SAS_{12}) repeated measures ANOVAs. P-values for statistical tests of main effects were set at ≤ 0.05. Post-hoc analyses were conducted using paired t-tests with p-values corrected for multiple comparisons by the Bonferroni method (i.e. $p \leq 0.017$).

4.3 Results

4.3.1 Generalized startle responses

Generalized startle responses induced rapid responses that were primarily in the sagittal-plane. In contrast, relatively small COP, hip and ankle displacements were observed in the frontal-plane with mean (±1SE) amplitudes of 0.72±0.22cm, 0.63±0.15deg and 0.19±0.04deg, respectively. Binomial statistics confirmed our earlier assumption that generalized startle responses would induce COP, upper- and lower-body displacements that were not directionally biased in the frontal plane (p=0.774, p=1.000 and p=0.777, respectively).

4.3.2 Perturbation-induced sway responses

Lateral support-surface translations evoked stereotypical multi-phasic kinematic and COP displacement profiles (Figure 4.2). Perturbations induced initial passive sway characterized by both upper- and lower-body segment rotations and COP displacements contralateral to the direction of the support-surface. The latter phases of the movement involved active compensatory movements that arrested passive displacements and stabilized

posture. Compared to the first trial, lower- and upper-body segment rotation amplitudes observed 12th perturbation were markedly attenuated, whereas amplitudes of COP displacements increased slightly with experience (Figure 4.2).

4.3.3 Probability and directionality of sway induced by SAS

COP

For SAS_1 and SAS_{12}, 50% (n=6) and 67% (n=8) of subjects produced COP responses distinct from generalized startle responses, respectively. Interestingly, after only observing platform movements, SAS_{PRE} trials also induced COP displacements that were of greater magnitude than generalized startle responses in 75% (n=9) of the subject pool.

For SAS trials, every subject that produced a response greater than generalized startle also produced COP displacements in the contralateral direction to the movement of the support-surface. For subjects in Group 1, expectations of leftward support-surface translations consistently led SAS to evoke rightward COP displacements and vise versa for subjects in Group 2 (Figure 4.3). Binomial statistics confirmed that COP displacements were directionally-specific for all SAS trials (Table 4.1). Repeated measures ANOVA across subjects with detectable responses in all SAS trials suggested that measures of COP onset, peak and time-to-peak were not significantly influenced by experience (p=0.719, p=0.609 and p=0.159, respectively) (Figure 4.4AB).

Lower- and upper-body kinematics

SAS_{PRE}, SAS_1 and SAS_{12} trials, induced frontal-plane lower-body rotations greater than generalized startle responses in 92% (n=11), 75% (n=9) and 83% (n=10) of subjects, respectively. Binomial statistics (Table 4.1) confirmed that displacements of the proximal aspect of the segment consistently occurred in the ipsilateral direction to the support-surface translation (Figure 4.4A). Results from repeated measures ANOVA suggested that onsets, peaks and time-to-peaks of lower-body rotations were not significantly influenced by experience (p=0.501, p=0.610 and p=0.219, respectively) (Figure 4.4B).

For the upper-body segment, SAS_{PRE}, SAS_1 and SAS_{12} trials only induced frontal-plane angular rotations greater than generalized startle responses in 33% (n=4), 25% (n=3)

and 25% (*n*=3) of subjects, respectively (Figure 4.4A). Binomial and ANOVA statistics were not performed on remaining data because <50% of subjects produced upper-body rotations distinct from generalized startle responses during SAS trials.

4.3.4 Tone-Only trials

Tone-Only trials presented during the repeated series of balance perturbations induced detectable displacements of COP and lower-body segments displacements. For TO_1, 50% (*n*=6) and 25% (*n*=3) of subjects produced detectable COP and lower-body displacements, respectively. For TO_2, 67% (*n*=8) and 42% (*n*=5) of subjects produced detectable COP and lower-leg displacements, respectively. Binomial statistics determined that these displacements were not directionally specific and thus could not be differentiated from random sway (Table 4.1).

4.4 Discussion

The two aims of this experiment were to determine if unexpected balance perturbations could induce motor preparation of PRs and to determine if such preparation could be influenced by repeated experiences with balance perturbations. Using SAS, we have confirmed that unexpected support-surface translations lead to multiple aspects of directionally-specific PRs being prepared. Our results have also demonstrated that parameters of prepared PRs were unaltered throughout the combined visual and physical experiences with balance perturbations.

4.4.1 Overt cues are not critical for motor preparation of reactive PRs

Previous evidence of PR motor preparation was developed from classical conditioning protocols that required an overt cue to precede balance perturbations (Chapter 3). As such, cues provided specific information pertaining to the exact timing of balance perturbation onsets. Although critical to the formation of conditioned PRs, it was unclear if cues, and therefore temporal certainty, were necessary to prepare PRs. The current experiment has shown that PRs could be prepared in the absence of cues and thus in the absence of temporal certainty surrounding perturbation onsets. Thus, we have demonstrated that the ability to induce motor preparation of PRs is not limited to situations involving

classical conditioning. Rather, our results suggest that aspects of reactive PR can be prepared even when balance perturbation onsets are unexpected.

Although perturbation timing was unexpected, their spatial parameters were held constant throughout testing. Knowledge of required response characteristics, even with uncertainty in the relationship between cues and movement onset timing (Cressman et al., 2006), is known in simple reaction time literature as a critical prerequisite to advanced motor preparation (see Carlsen et al., 2011 for review). This is in contrast to choice reaction time paradigms where motor preparation cannot reliably precede even predictable IS onsets because of the existence of multiple response alternatives and the possibility of negative consequence associated with pre-selecting an inappropriate response (Carlsen et al., 2007). When balance perturbation parameters (i.e. direction) were held constant, so too were certain aspects of the PRs required to prevent falls (i.e. direction of sway). In the absence of time-based cues, PR motor preparation observed in the current experiment was therefore most likely facilitated by the consistent spatial characteristics of balance perturbations.

Having evoked aspects of reactive PRs with SAS supports a growing body of work suggesting that PRs themselves are not entirely a sequence of stretch reflexes (Nashner and Cordo, 1982); rather, subcortical centres may be involved in dynamic postural control (Stapley and Drew, 2009; Honeycutt et al., 2009; Jacobs and Horak, 2007). It is assumed that information processing, a component of which is motor preparation (Donders, 1869), contributes specifically to the higher-order control of movement and therefore is not involved in the regulation of simple reflexive behaviours. Furthermore, even for movements that are governed by higher-order control, such as voluntary motor behaviours, SAS is thought to only induce prepared movements if the muscles involved are highly innervated by subcortical (i.e. reticular) pathways (Carlsen et al., 2009). Using a novel technique, our results further support a body of work that suggests PRs may be partially governed by non-reflexive processes and are perhaps mediated by supra-spinal centres.

4.4.2 Experience and PR motor preparation

Although previous work identified aspects of prepared PRs (Chapter 3), the time course of prepared PR formation has never been established. The current experiment, however, presented SAS before and after many repeated perturbations and thus offers

important and more detailed information pertaining to the timeline of prepared PR formation and the nature of prepared PRs at each stage.

Our results have collectively demonstrated that experience with balance perturbations had a constant effect on motor preparation of PRs. SAS trials consistently evoked prepared PRs after as many as 12 physical experiences with unexpected physical perturbations and after as few as 3 visual experiences. At every stage of experience, SAS evoked prepared PRs with high probability, and with unchanged temporal and amplitude parameters. Thus, our data are the first to suggest that aspects of prepared movement could be formed from only minimal experience with unexpected balance perturbations, which persisted during repeated exposures and remained unchanged throughout.

The absence of changes to prepared response parameters evoked during SAS trials suggests that they may represent elements of highly skilled movements. Early in skill acquisition of more novel motor behaviours, experience is known to foster greater 'chunking' of individual response parameters (Klapp, 1995), which can lead to greater response amplitudes, earlier response onsets and a greater likelihood of a response being evoked by SAS (MacKinnon et al., 2007; Carlsen and MacKinnon, 2011). The absence of observable changes to these motor preparation variables that are sensitive to the effects of experience, suggests that prepared PRs were not aspects of a novel behaviour; rather, they may represent a type of learned and highly skilled movement that underwent experience-related changes before entering the laboratory.

4.4.3 Possible causes of PR motor preparation influenced by visual observation

Visual exposure to perturbing stimuli perceived as threatening can lead to motor imagery of physical behaviours (Fusi et al., 2005; Green et al., 2010). Motor imagery involves mental rehearsal of movement and has been shown induce partial motor preparation of movement that can be evoked by SAS (Maslovat, PhD thesis). Although we did not specifically measure perceived threat associated with our particular balance perturbation, previous evidence suggests that postural perturbations are considered threatening to the extent that they increase arousal, and may even alter underlying reflexes (Horslen et al., submitted). Thus, our results may support previous reports of threat induced by balance perturbations and further indicate that these situations may lead to motor imagery and thus

motor preparation of PRs. Alternatively, it is also plausible that motor imagery led to unintended movements and thus activation of peripheral aspects of motor and sensory systems (Gandevia et al., 1997). If true, PR preparation induced by visual observation could have been the result of unintended and undetected physical practice.

Although we expected that visual experience might influence motor preparation, we did not expect prepared PRs to remain mostly unchanged after 12 physical balance perturbations. In the only study that has to date used SAS to examine motor preparation during imagined and physically practiced movements, imagined movements led to preparation of low amplitude responses that were evoked by SAS only 20% of the time (Maslovat, PhD thesis). For physically practiced movements, however, SAS induced prepared responses with near total certainty and whose amplitudes closely approximated those observed in control trials. Assuming that visual experience facilitated motor imagery of PRs, our results would both align with previous work (Maslovat, PhD thesis) and be the first to demonstrate that the extent of preparation can be similar between imagined and physically practiced movement.

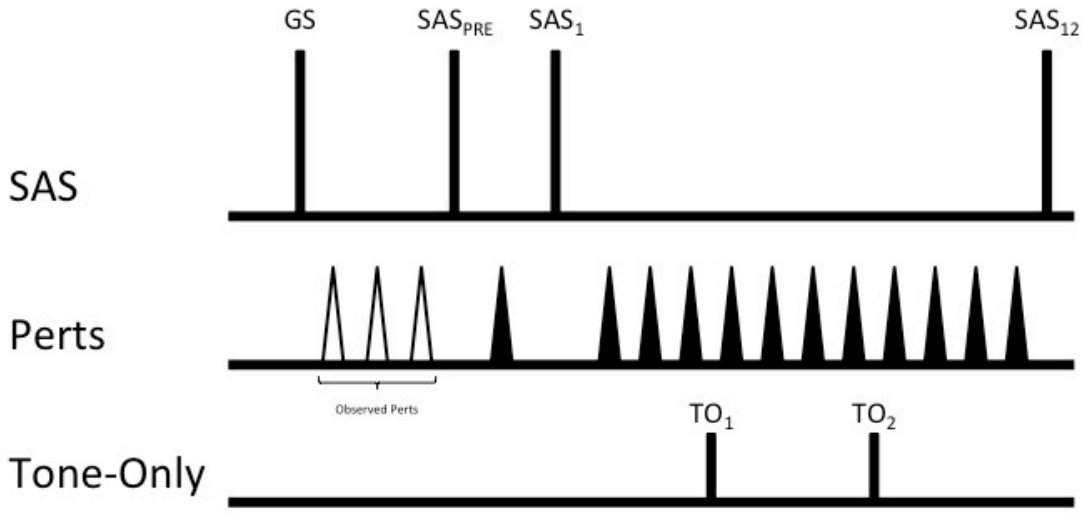
4.4.4 Limitations

The fact that prepared PRs were observed after only visual experiences with balance perturbations suggests the possibility that physical experience was not alone responsible for the formation of prepared PRs. Due to the fixed temporal sequence between visual observation and physical experience, it is possible that either the effects of visual observation persisted throughout the experiment, or that physical experience acted only to maintain the effect that originated with visual observation. Regardless, the impacts of these findings are substantial given the highly common practice of both allowing subjects to observe the devices on which they will ultimately stand and repeatedly exposing subjects to identical balance perturbations. Coupled with the highly uncommon practice of addressing the potential for PR motor preparation to influence dynamic postural control measures, this experiment has provided unique insight into how methodological procedures influence neural mechanisms involved in dynamic postural control.

4.5 Conclusions

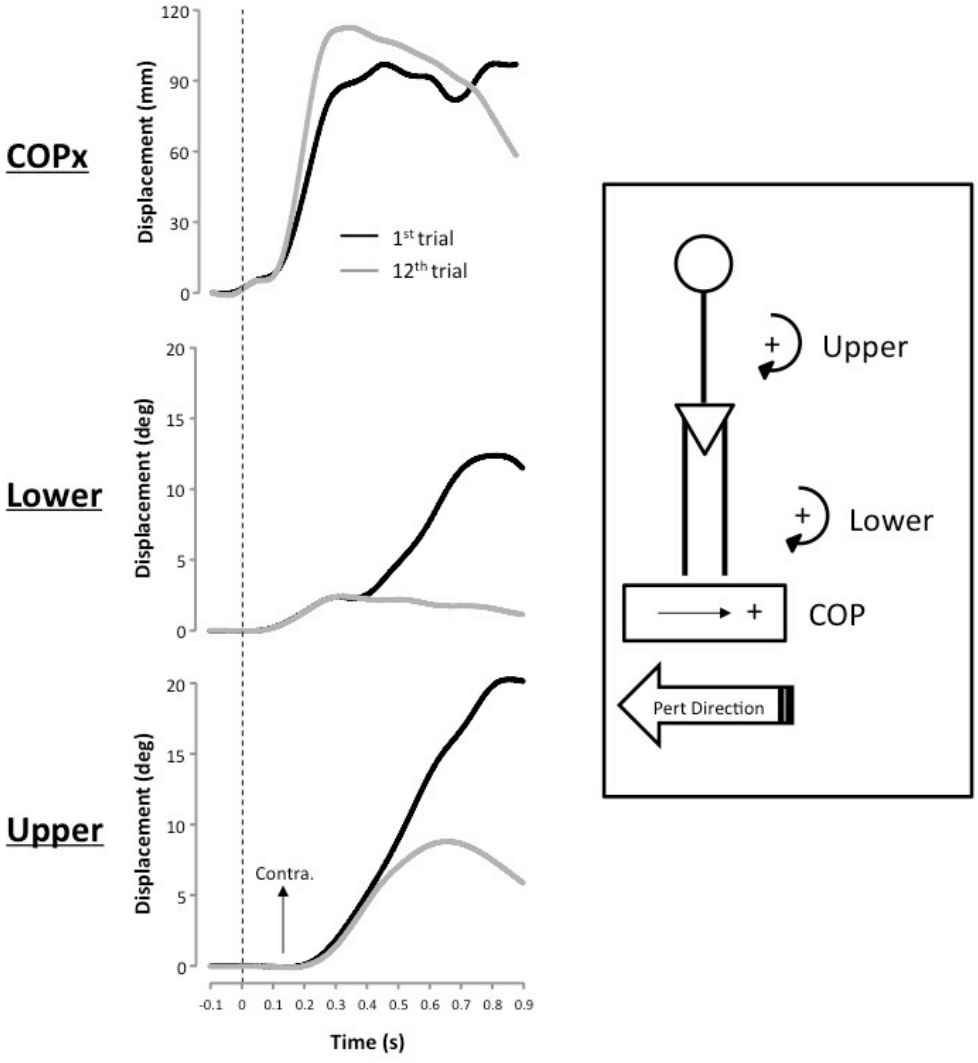
We have demonstrated for the first time that experience with unexpected balance perturbations can facilitate motor preparation of PRs. Future research is indeed necessary to better understand the possible influences that PR motor preparation may have had on interpretations of previous work where repeated sequences of unexpected balance perturbations were used to examine the dynamic postural control system. Furthermore, it would be important to understand the extent to which motor preparation may be involved in healthy balance and perhaps if it may become compromised in individuals with known postural deficits.

Figure 4.1: Schematic of methodology



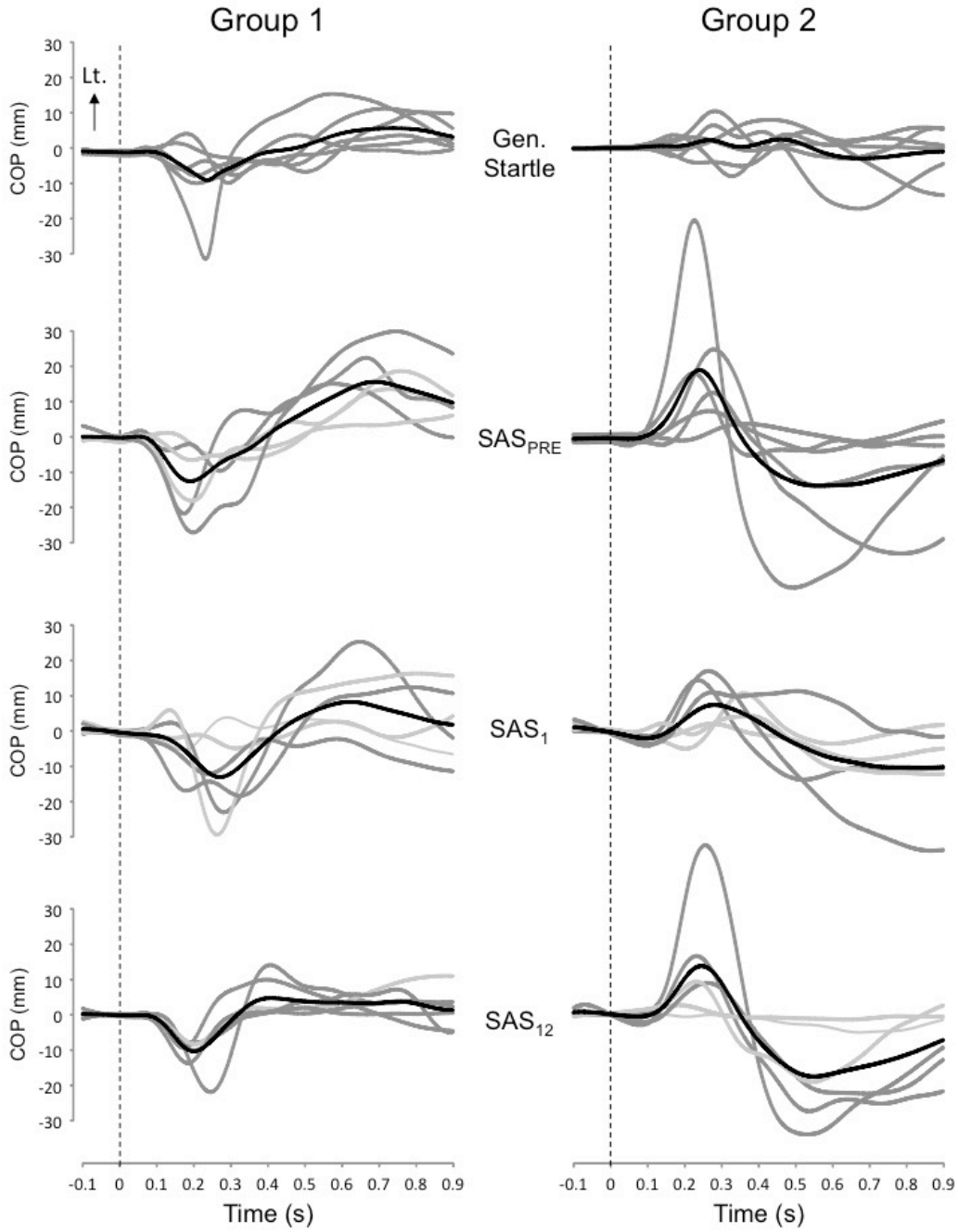
Schematic of methodology. The progression of time passes from left to right. The first trial experienced by subjects were SAS to induce generalized startle responses (GS), followed by 3 visual experiences with perturbations (unfilled triangles), a SAS_{PRE} trial, a subsequent physical experience with a balance perturbation (filled triangle), a SAS_1 trial and a repeated sequence of physical perturbations with 2 interspersed Tone-Only trials (TO_1 and TO_2). After the 12th physical experience, a final SAS trial (SAS_{12}) was presented.

Figure 4.2: COPx, lower- and upper-body displacements



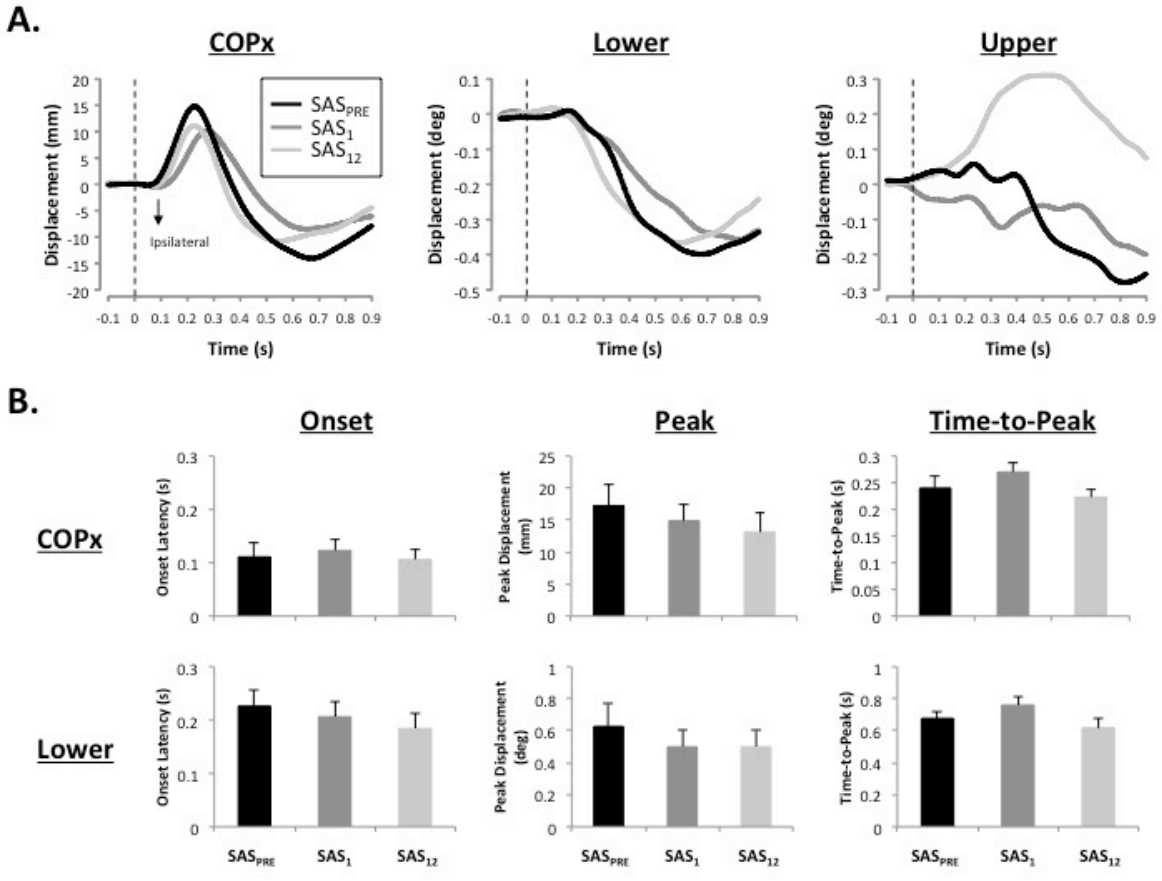
Group averages of COPx, lower- and upper-body displacements evoked by 1st (black line) and 12th (grey line) physical exposures to balance perturbations. Vertical dashed line denotes onset of balance perturbations.

Figure 4.3: COPx displacments in Group 1 and Group 2



Individual subject (split by Group) COPx displacements evoked in generalized startle trials (Gen. Startle), SAS_{PRE}, SAS_1 and SAS_{12} trials. Light grey traces represent subject data that were not greater in amplitude than generalized startle responses. Black line denotes group-wise average of COP displacements. Vertical dashed line represents startle stimulus onset.

Figure 4.4: Summary traces and plots for COPx, lower- and upper-body displacements



(A) Average traces of COPx, lower- and upper-body displacements evoked in SAS_{PRE} (black), SAS_1 (dark grey) and SAS_{12} (light grey) trials. Vertical dashed lines denote onset of startle stimulus.

(B) Bar charts of mean onsets, peak and time-to-peak displacements of COPx and lower-body angular displacements in SAS_{PRE} (black), SAS_1 (dark grey) and SAS_{12} (light grey) trials

Table 4.1: Summary of binomial statistics

	COPx	Ankle	Hip
GS	12 (7/5)	11 (5/6)	12 (5/7)
SAS_{PRE}	9 (9/0)**	11 (0/11)**	4 (1/3)
SAS_1	6 (6/0)**	9 (4/5)	3 (1/2)
SAS_{12}	8 (8/0)**	10 (1/9)**	3 (3/0)
TO_1	6 (5/1)	3 (0/3)	
TO_2	8 (4/4)	5 (3/2)	

Total number and directionality of detectable COPx, Ankle and Hip responses in Generalized Startle (GS), SAS_{PRE}, SAS_1, SAS_{12}, TO_1 and TO_2 trials. The number located outside of the bracket denotes total *n* with a detectable response. Within the bracket denotes the directionality of each response. For GS trials, 12 (7/5) denotes 12 subject with a detectable response where 7 displaced COP to the left and 5 displaced COP to the right. For remaining trials, total *n* denotes responses with displacement amplitudes greater than GS trials and the fraction denotes contralateral versus ipsilateral to the direction of the support-surface translation. For example, 9 (9/0) denotes 9 total subjects with a COP response greater than GS where all 9 subjects produced a COP displacement in the contralateral direction to the support-surface translation. ** denotes significant directional specificity.

Chapter 5: First trial and StartReact effects induced by balance perturbations to upright stance

5.1 Introduction

The first in a sequence of balance perturbations is known to induce responses that differ markedly from those that follow (Keshner et al., 1987). Responses observed in the first exposure to balance perturbations (i.e. first-trial) are known to be over-amplified and to rapidly habituate with repeated trials (Nashner, 1976; Keshner et al., 1987; Oude Nijhuis et al., 2009, 2010), induce directionally non-specific sway (Oude Nijhuis et al., 2009) and involve non-postural muscles (Oude Nijhuis et al., 2010). However, the nature of the first-trial effect (FTE) is currently unknown (Oude Nijhuis et al., 2009).

The characteristics of responses evoked in first-trials and the observed changes in subsequent trials suggest that FTEs may be mediated by startle responses induced by balance perturbations that are super-imposed onto triggered postural responses (PRs) (Hansen et al., 1988; Bloem et al., 1998; Blouin et al., 2007; Oude Nijhuis et al., 2009). However, whether in fact a startle response is even evoked by balance perturbation remains admittedly inconclusive (Siegmund et al., 2008). FTEs have been compared to startle responses evoked by known startle stimuli (i.e. SAS) and while there are similarities in some aspects of the response pattern, marked delays exist between SCM onsets evoked by perturbations and known startle responses evoked by SAS (Oude Nijhuis et al., 2010). Furthermore, previous conclusions were partially based on correlational analyses, which limit inferences of causation (Siegmund et al., 2008). Thus, questions remain whether startle responses are evoked by balance perturbations and whether they should be considered as factors mediating FTEs.

If startle is involved in mediating FTEs, there are at least two ways in which startle can affect the PRs evoked by balance perturbations. One way is that during a sequence of balance of perturbations, startle responses are only evoked on the first trial and not on remaining trials. This position is supported by significant coherence of startle-like frequencies in bilateral sternocleidomastoid (SCM) responses only in first trial perturbations to seated posture (Blouin et al., 2006) and by the dramatic habituation of kinematic displacement amplitudes observed between the first and second in a repeated sequence of

perturbations to upright stance (Oude Nijhuis et al., 2009). The other possibility is that startle responses evoked by perturbations habituates progressively with repeated exposures and therefore may persist beyond the first trial (Blouin et al., 2007). Thus, perturbations may act as startling stimuli beyond the first trial while the evoked startle response habituates over time.

The StartReact effect offers a unique way to probe whether a stimulus is startling. The StartReact effect involves the involuntary release of a prepared motor response as a result of being startled (see Carlsen et al., 2011 for review). Theoretically, any type of stimulus that can induce a startle response 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 complex step initiation (Nieuwenhuijzen et al., 2000) and PRs (Campbell et al., in review). StartReact effects are focal, task-specific and highly distinguishable from startle responses. Thus, determining the presence of the StartReact effect provide a relatively independent method for determining if a particular stimulus is capable of inducing a startle response.

The main purpose of this experiment was to determine if and how FTEs are mediated by startle induced by balance perturbations. After a sequence of reaction time trials, balance perturbations were introduced simultaneously with the instruction to initiate a wrist extension task to determine if onsets could be facilitated to the extent induced by known startling stimuli (i.e. SAS). If the first trial response is distinguishable from subsequent trials because of an isolated startling effect, as suggested by Oude Nijhuis et al., 2010), we hypothesized that StartReact effects and startle responses would be observed only on the first trial and not in subsequent trials of balance perturbations.

5.2 Methodology

Twelve (5 males; 1 left-handed) healthy individuals (mean ±1SD age: 24.8 ±3.4years, height: 1.71 ±0.07m, weight: 65.90 ±9.40kg) volunteered to participate in the study. All subjects were entirely naïve of the experimental protocol prior to entering the laboratory and each of them was 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.

5.2.1 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) and flexor carpi radialis (FCR) 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 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.

5.2.2 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 imperative stimulus (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 imperative stimulus (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 5.1). 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 an IS presented simultaneously with a SAS (i.e. $TEST_{SAS}$) (Figure 5.1). For the PERT block, TEST trials involved the WARN cue followed 1.5-3.5s by the IS presented simultaneously with a toes-up support-surface rotation (12°, 120°/s, 100ms duration) (i.e. $TEST_{PERT}$) (Figure 5.1). Subjects were entirely unaware of when TEST trials were to be presented and how many would be in each block. Subjects were also informed that there would be instances interspersed throughout each experimental block where the WARN cue would not be followed by an IS (CATCH trials) (Figure 5.1) 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. Subjects

were told that the IS would never be illuminated and thus they were no longer to complete the reaction time task.

5.2.3 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 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). Peak velocity of wrist extension was calculated as the maximal rate of wrist displacements achieved in the time period between wrist extension onset and peak displacement.

ECR and FCR EMG

Onsets and amplitudes of EMG responses of ECR and FCR 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 then determined as the first time 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). 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 both for consistency with which PRs are typically analyzed (Carpenter et al., 2008; Campbell et al., 2009) but also because it is a period where sensory feedback has limited influence over triggered reactions (Wadman et al., 1979). Absolute onsets were determined for each muscle with IS onset as a reference. Relative onsets between muscles were calculated by subtracting ECR from FCR onsets for each trial.

Postural responses

Both EMG and kinematic data quantified PRs evoked by support-surface rotations. For EMG, absolute onsets and amplitudes were determined using the same algorithm applied to records of ECR and FCR muscle activity. For the kinematic dataset, onsets of absolute shank, trunk and upper-arm angular displacements were determined as the latency between perturbation onset and the time where angular displacements surpassed a mean ±2SD threshold of resting positions calculated from 500ms of data that immediately preceded the onset of the IS and remained beyond threshold for 200ms. Peak displacement and peak velocity measures were also calculated as the greatest change achieved within 800ms of perturbation onset.

5.2.4 Data analysis

Reaction time

To be included in the dataset, subjects were required to have deviated their wrist position into extension for at least 200ms and to have had produced onsets of SCM activity within 90ms of SAS onset during $TEST_{SAS}$ trials to ensure that they were sufficiently startled (Carlsen et al., 2011). All dependent measures for wrist kinematics as well as EMG for ECR and FCR were averaged across CONTROL trials from both blocks and were compared to the first response to $TEST_{SAS}$ and $TEST_{PERT}$ trials in a 1-way ANOVA with 3 levels (CONTROL, $TEST_{SAS}$, $TEST_{PERT}$). Because SCM activity was neither expected nor observed during CONTROL trials, SCM response dependent measures were compared only between $TEST_{SAS}$ and $TEST_{PERT}$ trials using paired *t*-tests.

To analyze the effects of repeated exposures to auditory startle and balance perturbations on reaction time, a 2 X 2 (trial number X TEST trial-type) repeated measures ANOVA was used to compare the changes from the 1st to the 10th experience with both $TEST_{SAS}$ and $TEST_{PERT}$ trials for all subjects independent of the presence of SCM. To determine whether the presence of SCM influenced these results, a similar analysis was also conduced involving only a subset of subjects that produced SCM activity throughout all experimental levels. Interaction effects would be used to identify whether experience-related changes to response parameters differed between $TEST_{SAS}$ and $TEST_{PERT}$ trials.

To determine the presence of FTEs on PRs, *t*-tests were conducted to compare EMG and kinematic responses observed in the 1st and 10th experiences with $TEST_{PERT}$ trials. P-values were set at 0.05 for all statistical tests, which were corrected for multiple comparisons using the Bonferonni method.

5.3 Results

5.3.1 Reaction time facilitation by first-trial exposures to SAS and balance perturbations

Examples of a typical series of responses evoked during reaction time trials across CONTROL and first-trial exposures to $TEST_{SAS}$ and $TEST_{PERT}$ trials are depicted in Figure 5.2 for a representative subject. For CONTROL trials, onsets of wrist extension onsets occurred, on average, within 247 ±27ms of the IS and achieved average peak displacements of 57.5 ±12.3°. Prior to wrist extension onset, sequential onsets of ECR and then FCR (195 ±28ms and 211 ±32ms, respectively) burst were consistently observed whose amplitudes quickly rose above background during the initial phases of movement and persisted as subjects held the wrist in full extension. In no instances during CONTROL trials were SCM bursts detected. $TEST_{SAS}$ and $TEST_{PERT}$ trials evoked a similar pattern of wrist extension kinematics and EMG of ECR and FCR. However, compared to CONTROL trials, onsets occurred earlier and large SCM bursts were observed. Significant main effects were observed across trial types for onsets ($F_{(2,20)}$=8.51, p=0.002) and peak velocity ($F_{(2,20)}$=10.95, p=0.000) measures of wrist extension. Post-hoc analyses indicated that both $TEST_{SAS}$ and $TEST_{PERT}$ trials facilitated significantly earlier onsets compared to CONTROL trials ($p \leq 0.017$) while no differences were observed between TEST trial conditions ($p > 0.017$) (Figure 5.3A). Post-hoc analyses also revealed that peak wrist velocities observed in CONTROL and $TEST_{SAS}$ trials were significantly greater than in $TEST_{PERT}$ trials ($p \leq 0.017$) (Figure 5.3A). Wrist extension amplitudes did not differ between trial conditions (p=0.620) (Figure 5.3A).

Significant main effects were observed across trial types for ECR and FCR onsets ($F_{(2,20)}$=55.16, p<0.001 and $F_{(2,20)}$=52.88, p<0.001, respectively). Post-hoc analyses confirmed that both $TEST_{SAS}$ and $TEST_{PERT}$ trials elicited significantly earlier onsets of ECR and FCR

compared to CONTROL trials ($p \leq 0.017$) (Figure 5.3B) while no differences were observed between TEST trial conditions ($p > 0.017$). EMG amplitudes were unaffected by trial type (ECR ($p=0.481$); FCR ($p=0.158$)) (Figure 5.3B).

In CONTROL trials, a consistent pattern of muscle onsets was observed between ECR and FCR such that onsets of ECR preceded those of FCR by an average (±1SE) of 18±4ms. This relative pattern was not significantly affected by trial type ($p=0.938$) (Figure 5.3B).

T-test revealed that SCM onsets observed in $TEST_{SAS}$ trials (59 ±5ms) were significantly earlier than those observed during $TEST_{PERT}$ trials (94 ±7ms) ($t(11)=6.55$, $p<0.001$) (Figure 5.3B). SCM response amplitudes were not significantly different between TEST trials ($p=0.179$) (Figure 5.3B).

5.3.2 Repeated TEST trials and reaction time

SCM EMG

The probability of evoking SCM activity decreased from the first trial exposure of each TEST trial to the 10th (Figure 5.4). Of the 12 subjects tested, 11 subjects produced detectable SCM activity in the first trial exposure to $TEST_{SAS}$ and all 12 subjects had detectable SCM onsets following first trial exposure to $TEST_{PERT}$ trials. By the 10th exposure, only 5 subjects produced SCM onsets to $TEST_{SAS}$ trials whereas 8 had detectable onsets in $TEST_{PERT}$ trials. All 5 subjects who had detectable SCM onsets in the 10th $TEST_{SAS}$ trial also did so for the 10th $TEST_{PERT}$ trial. For those subjects who had detectable SCM activity in the first and last exposure to both TEST trials ($n=5$), significant main effects of TEST trial were observed for SCM onset latency ($F_{(1,4)}=24.26$, $p=0.008$) where post-hoc analyses revealed that SCM onsets were significantly earlier in $TEST_{SAS}$ trials compared to $TEST_{PERT}$ trials. Significant main effects of experience were also observed for SCM amplitude ($F_{(1,4)}=53.44$, $p=0.002$) where significantly smaller amplitudes were observed in the 10th TEST trials compared to the 1st (Figure 5.5A) independent of stimulus type. No significant interactions were observed (see Table 5.1A for statistical summary).

Analyses of repeated TEST trial effects on reaction time parameters, PRs, wrist kinematics and EMG were conducted across all subjects independent of whether or not SCM

activity was observed. A secondary analyses was conducted on a subset of 5 subjects that produced detectable SCM responses across all experimental levels.

ECR and FCR EMG

Despite marked changes to SCM indicators of startle, repeated exposure to TEST trials had only marginal effects on reaction time parameters and movement profiles (Figure 5.2). Neither stimulus type nor experience significantly influenced onsets of ECR (stimulus: p=0.555; experience: p=0.346) or FCR (stimulus: p=0.325; experience: p=0.909) (Figure 5.4A). Significant main effects of ECR amplitudes were observed between the TEST condition ($F_{(1,11)}$=4.73, p=0.050) driven by greater amplitudes of ECR observed in $TEST_{SAS}$ compared to $TEST_{PERT}$ trials. Experience significantly affected ECR amplitudes ($F_{(1,11)}$=8.18, p=0.016) that was due to greater amplitudes observed in the last trial of TEST blocks compared to the first ($p \leq 0.017$). No significant interactions were observed for onset (p=0.934) or amplitude (p=0.641) measures of ECR activity. FCR amplitudes were not significantly affected by either stimulus (p=0.632) or experience (p=0.943). No significant interaction effects were observed for FCR onsets (p=0.991) or amplitudes (p=0.904).

Supplementary analyses on subjects with SCM responses throughout all experimental conditions validated the results from the larger dataset (Table 5.1B). Main effects of TEST or experience conditions were not significant for ECR onsets (p=0.615 and p=0.643, respectively) or amplitudes (p=0.103 and p=0.369, respectively). Similarly, main effects were not significant for FCR onsets (p=0.848 and p=0.593) or amplitudes (p=0.301 and p=0.783) in TEST or experience conditions, respectively. No significant interactions were observed (p>0.05).

Wrist kinematics

Compared to the first trial exposures to $TEST_{SAS}$ and $TEST_{PERT}$ trials, the 10^{th} exposure induced similar wrist kinematic profiles (Figure 5.2). ANOVA determined that onset latencies (p=0.496), peak (p=0.862) and peak velocity (p=0.065) of wrist extension were independent of stimulus type (Figure 5.6A). Main effects of experience were observed only for peak velocity ($F_{(1,11)}$=8.03, p=0.016) where greater mean velocities were observed in

the last trial of TEST blocks compared to the first. No significant interactions were observed for onset (p=0.195), peak (p=0.680) or peak velocity (p=0.237) measures of wrist kinematics.

Supplementary analyses also validated that measures of wrist kinematics were independent of the presence of SCM. Main effects of TEST and experience conditions were non-significant for onset (p=0.742 and p=0.157, respectively) and amplitude (p=0.176 and p=0.998, respectively). Interactions were non-significant for onset (p=0.961) and amplitude (p=0.617).

5.3.3 FTEs evoked by $TEST_{PERT}$ trials

EMG

Support-surface rotations evoked characteristic EMG activity in various postural muscles. Following the onset of balance perturbation, stretch reflexes observed in SOL occurred earlier than PRs in TA, RF and EO (Figure 5.7). Repeated experience with balance perturbations during $TEST_{PERT}$ did not significantly influence the onset latencies of any of the postural muscle activity examined (Figure 5.5B). However, EMG amplitudes significantly decreased from the 1st to the 10th experience with support-surface rotations in all muscles other than TA (Figure 5.5B).

Kinematics

Rotations of the support-surface induced angular displacements of body segments in the sagittal-plane. Toes-up support-surface tilts caused the shank and upper-arm segments to initially rotate backwards whereas the trunk rotated forwards (Figure 5.7). Onsets of shank, trunk or upper-arm angular displacements were not significantly influenced by experience (Figure 5.6B). However, peak angular displacements and peak angular velocities of both trunk and upper-arm segments were significantly attenuated in the 10th trial compared to the 1st experience with $TEST_{PERT}$ trials. Significant influences of experience were not observed in shank angular displacements or peak angular velocities.

5.4 Discussion

The main purpose of this experiment was to determine if and how FTEs are mediated by startle induced by balance perturbations. We hypothesized that, if the first-trial is distinguishable from subsequent trials because of an isolated startling effect (Oude Nijhuis et al., 2010) StartReact effects and startle responses would be observed only in the first-trial and not in subsequent trials of balance perturbations.

We have demonstrated balance perturbations are a type of startling stimulus that can induce StartReact effects of prepared movement. First trial exposures to both balance perturbations and SAS independently facilitated early onsets of wrist extension compared to CONTROL trials with no apparent disparities in reaction time or relative movement characteristics between $TEST_{PERT}$ and $TEST_{SAS}$ trials. Not only did balance perturbations induce similar StartReact effects as other known startle stimuli in the first-trial, they persisted over repeated $TEST_{PERT}$ trials. These data contradict the notion that balance perturbations induce startle responses only in the first trials. During repeated $TEST_{PERT}$ trials, indicators of startle responses (i.e. SCM amplitude) decreased (Figure 5.5A), however, StartReact effects were conserved over 10 trials and were similar to those evoked by known startle stimuli (i.e. SAS). Thus, we believe our data support the possibility that FTEs are likely mediated by startle response evoked by balance perturbations, yet we also believe that our data directly challenge the hypothesis that the influences of startle responses are reserved only for the first-trial exposure to balance perturbations.

5.4.1 What are FTEs?

The common assumptions amongst research into the nature of FTEs are that startle responses and PRs evoked by balance perturbations are superimposed onto one another (Blouin et al., 2006) and that changes observed with repeated perturbations are the result of response habituation (Keshner et al., 1987). PRs evoked in first-trials have been eliminated from further analyses for the very fact that they were assumed to contain a component that existed only in first trials and not in remaining ones (Allum et al., 2002). Our results confirm that balance perturbations induced a startling event during first-trials, evidenced by high probability of startle indicators and the similarities in StartReact effects induced by both balance perturbations and SAS. However, our data further suggests that although the

strength of startle response indicators (i.e. SCM amplitude and probability) decreased with repeated exposure to perturbations, the capacity for balance perturbations to induce StartReact effects remained unchanged and similar to the effects induced by SAS. Thus, the possibility exists that FTEs are not mediated by an all-or-none mechanism where startle responses and their influence on postural control, only exist during first trials. As suggested by evidence of StartReact effects induced by repeated $TEST_{PERT}$ trials, the potential influences of startle responses may persist beyond the first trial and thus have the capacity to influence PRs during entire blocks of repeated perturbations.

5.4.2 Implications

The obvious implication of our findings is that startle response and related mechanisms should be considered as potential mediating factors in trials beyond the first in a sequence of perturbations. Additionally, our results offer a unique perspective on previous work that utilizes voluntary movement to assess dynamic postural control as well as the underlying mechanisms that govern StartReact effects.

Incorporating voluntary movement into dynamic postural control studies has been a relatively common approach to understanding cognitive load and adaptation processes involved in balance control. One major aspect of balance research that has utilized voluntary tasks are dual-task paradigms where the onset latencies of voluntary movements are used as a metric to deduce the cognitive load applied to dynamic postural control at various times before and during balance perturbations (Redfern et al., 2002; Woollacott and Shumway-Cook, 2002; Müller et al., 2004, 2007). Out of 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., 2004a, 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.

For over 70 years, the motor responses evoked by startle stimuli have been examined scientifically (Landis et al., 1939). In the last 13 years, additional influences of startle stimuli on prepared motor responses been studied (Valls-Solé et al., 1999) and only recently has evidence emerged suggesting that startle responses and StartReact effects are perhaps mediated by partially independent circuits (Valls-Solé et al., 2005; Alibiglou and MacKinnon, 2012). Early descriptions of startle responses include, among many factors, neck muscle responses that have been repeatedly observed in SCM in more contemporary research. For their robustness, early SCM responses have been used as a measure to indicate the existence of startle responses (see Carlsen et al., 2011) and whether to include trials in experiments examining the StartReact effect. SCM latencies have even been used as relative markers for onsets of other responses induced by startle stimuli (Brown et al., 1991a). Although SCM responses have previously been linked and tightly integrated into the discussion of startle mechanisms, our work along with recent evidence suggests that separate circuits may mediate startle responses and StartReact effects. 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 startle 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 $TEST_{PERT}$ and $TEST_{SAS}$ trials suggested that the timing of the startle response 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 startle responses and StartReact effects were mediated by the same mechanism, that the ~50ms delay in SCM onsets between

$TEST_{PERT}$ and $TEST_{SAS}$ trials would have carried over into a similar delay in StartReact effects. That not being the case aligns with recent work suggesting that startle responses and StartReact effects are the end results of 2 partially disassociated neural mechanisms.

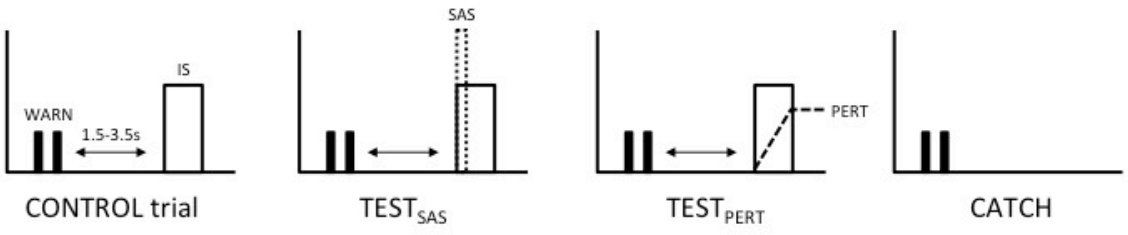
5.4.3 Limitations

A limitation of the current investigation is the result of the voluntary task used to assess the StartReact effect. StartReact effects were assessed using a wrist extension task because SAS has previously shown to influence its onset (Valls-Solé et al., 1999). However, wrist extension was also partially affected by whole-body perturbation itself, independent of the reaction time task (Figure 8). Thus, although the absolute magnitude of responses observed during $TEST_{PERT}$ trials may have been a composite of StartReact effects and other unrelated wrist movements, the early onsets and the comparably large amplitude of wrist extension observed during $TEST_{PERT}$ trials (Figure 8) suggest that indeed a StartReact effect was evoked by balance perturbations. Perhaps future investigations may benefit from utilizing an alternative task that is better insulated from the influences of whole-body balance perturbations. Vocalizations have recently been described as behaviours that are susceptible to StartReact effects (Chiu et al., 2011) and may be relatively unaffected by physical displacements of the body caused by balance perturbations compared to wrist extension.

5.5 Conclusions and future directions

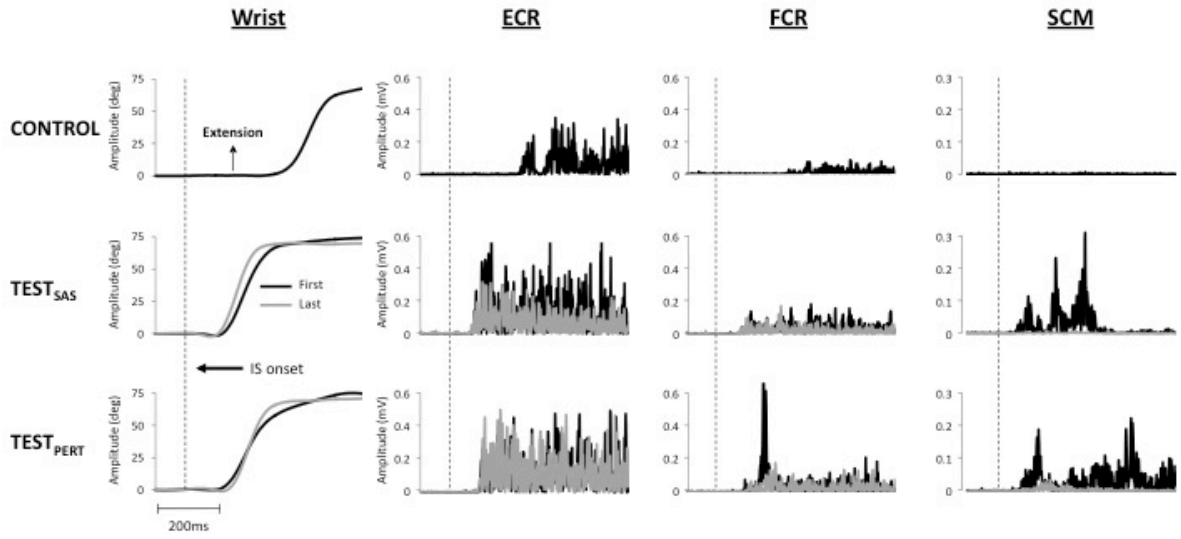
Our results have supported the notion that FTEs are mediated by a startle response induced by balance perturbations and have added further details regarding the persistent effects of startle induced by repeated balance perturbations. Startle responses appear not only to be a mediating factor in a single trial, but possibly in as many 10 repeated trials. Thus, the role and importance of startle responses in trials beyond the first must be considered. Future experiments in dynamic postural control must not ignore the possibility for startle to influence their measures, especially if they are temporally based. Furthermore, considering startle responses 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, where both over-sensitivity to startle stimuli and postural instability are known to co-exist (Brown et al., 1991a).

Figure 5.1: Schematic of individual trials



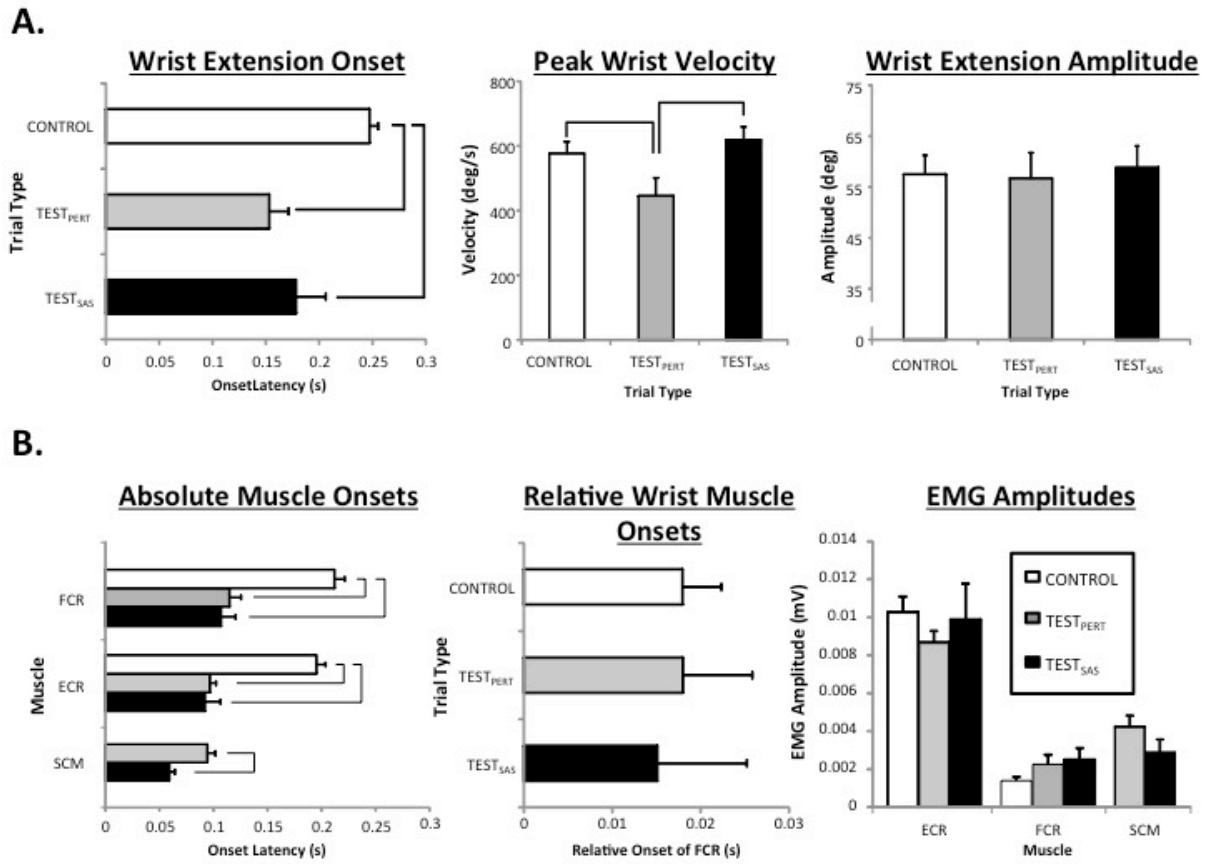
Graphic illustration of the stimuli involved in each trial type (CONTROL, $TEST_{SAS}$, $TEST_{PERT}$ and CATCH). In CONTROL trials, a warning cue (WARN; 2 black rectangles) involving 2 50ms auditory stimuli, preceded the onset of an imperative stimulus (IS; white-filled rectangle) by a random 1.5-3.5s fore-period. $TEST_{SAS}$ trials involved a WARN cue that preceded the simultaneous onset of the IS and a startling acoustic stimulus (SAS; tall rectangle). $TEST_{PERT}$ 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 5.2: Representative subject wrist displacements, ECR, FCR and SCM EMG data



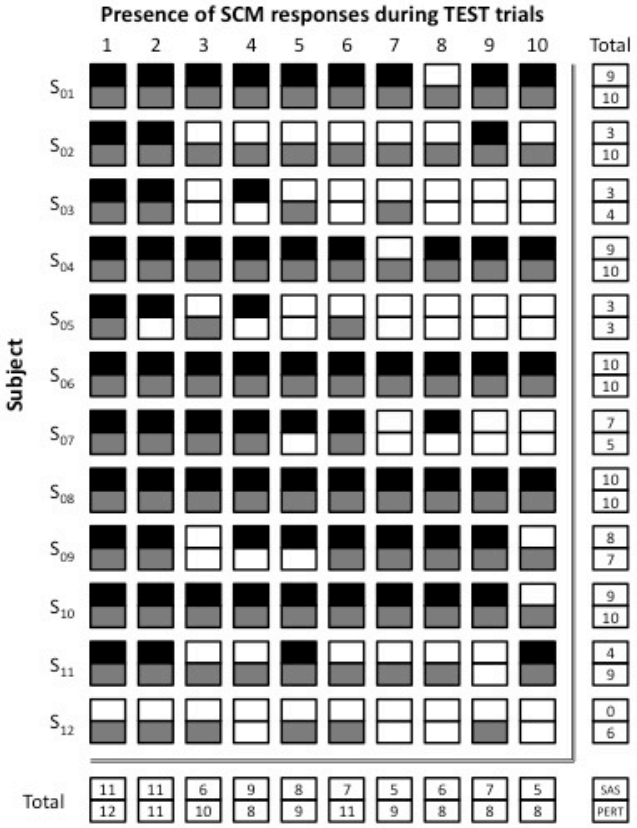
Representative subject wrist kinematic displacements as well as extensor carpi radialis (ECR), flexor carpi radialis (FCR) and sternocleidomastoid (SCM) during single CONTROL, $TEST_{SAS}$ and $TEST_{PERT}$ trials. For $TEST_{SAS}$ and $TEST_{PERT}$ trials, black traces denote the first trial exposure whereas grey traces represented the 10^{th} trial exposure. Vertical dashed line represents the onset of the imperative stimulus (IS) in each trial.

Figure 5.3: Summary plots of wrist kinematics and EMG



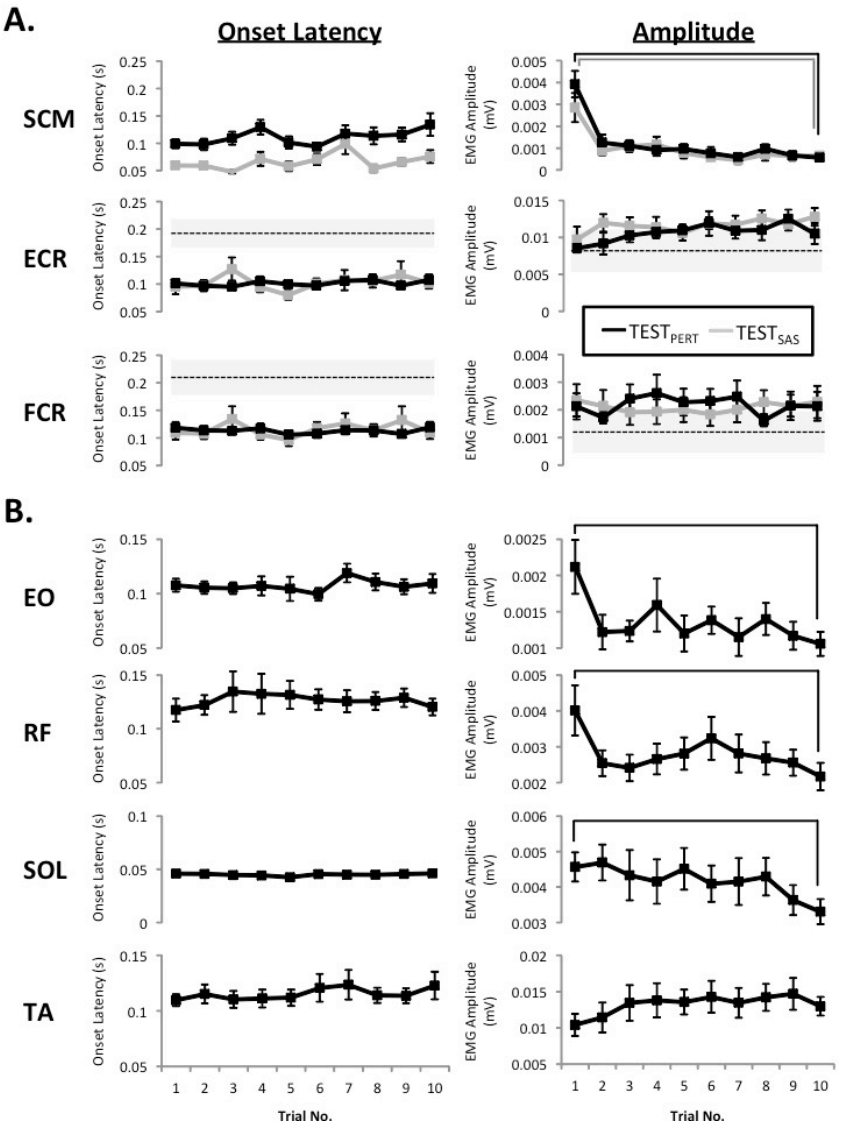
(A) Bar charts illustrating (left to right) mean wrist extension onset, peak wrist extension velocity, and peak wrist displacement for CONTROL (white bars), $TEST_{PERT}$ (grey bars) and $TEST_{SAS}$ (black bars). Connector lines highlight significant effects between trials.
(B) The left-most bar chart illustrates mean absolute onsets of extensor carpi radialis (ECR) and flexor carpi radialis (FCR) EMG evoked during CONTROL, $TEST_{PERT}$ and $TEST_{SAS}$ trials. SCM onsets are also depicted but only for $TEST_{PERT}$ and $TEST_{SAS}$ trials. The middle plot denotes the relative timing between ECR and FCR in each trial type where positive values indicate the timing of FCR after ECR. The right-most plot denotes EMG amplitudes of ECR and FCR between all 3 trial types, and of SCM between only $TEST_{PERT}$ and $TEST_{SAS}$ trials. Connector lines highlight significant effects between trials.

Figure 5.4: Presence of SCM responses during repeated $TEST_{SAS}$ and $TEST_{PERT}$ trials



The presence of SCM during TEST trials depicted as a 2D matrix with subjects (S_{01}-S_{12}) on the y-axis and TEST trial number on the x-axis. Each cell contains a rectangle with a filled (black) top half indicates a detectable SCM response within a given $TEST_{SAS}$ trial and a filled (grey) bottom half indicates a detectable SCM response within a given $TEST_{PERT}$ trial. The 'total' row summates the number of SCM responses observed across subjects for each TEST trial. The 'total' column summates the total number of SCM responses observed across TEST trials within each subject.

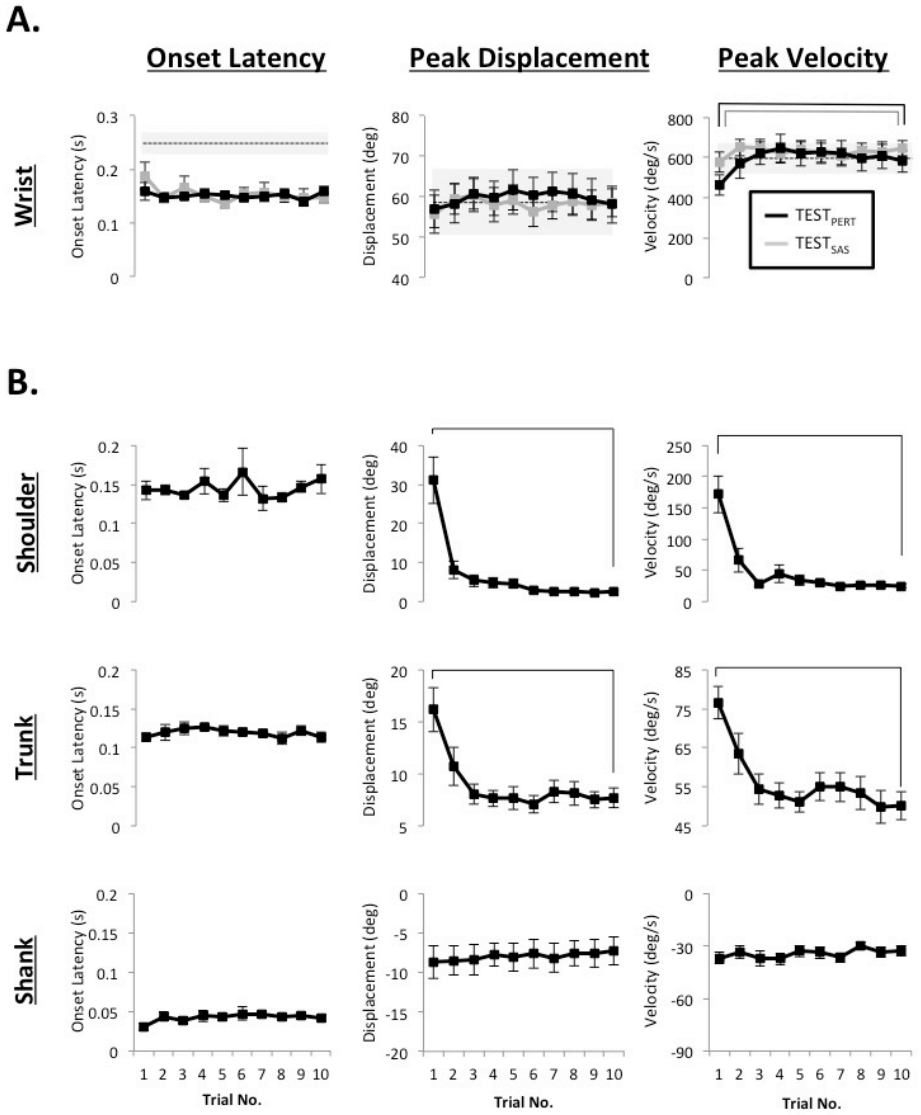
Figure 5.5: EMG onsets and amplitude during repeated $TEST_{PERT}$ and $TEST_{SAS}$ trials



(A) Average (±1SE) onsets and amplitudes of sternocleidomastoid (SCM), extensor carpi radialis (ECR) ad flexor carpi radialis (FCR) during $TEST_{SAS}$ (grey) and $TEST_{PERT}$ (black) trials. For ECR and FCR plot, horizontal dashed line and grey areas denote mean (± 1SD) of respective responses observed in CONTROL trials. Grey and black connector lines denote significant differences between 1st and 10th trials in $TEST_{SAS}$ and $TEST_{PERT}$ trials, respectively.

(B) Average (±1SE) onsets and amplitudes of external oblique (EO), rectus femoris (RF), soleus (SOL) and tibialis anterior (TA) during $TEST_{PERT}$ trials. Black connector lines denote significant differences between 1st and 10th $TEST_{PERT}$ trials.

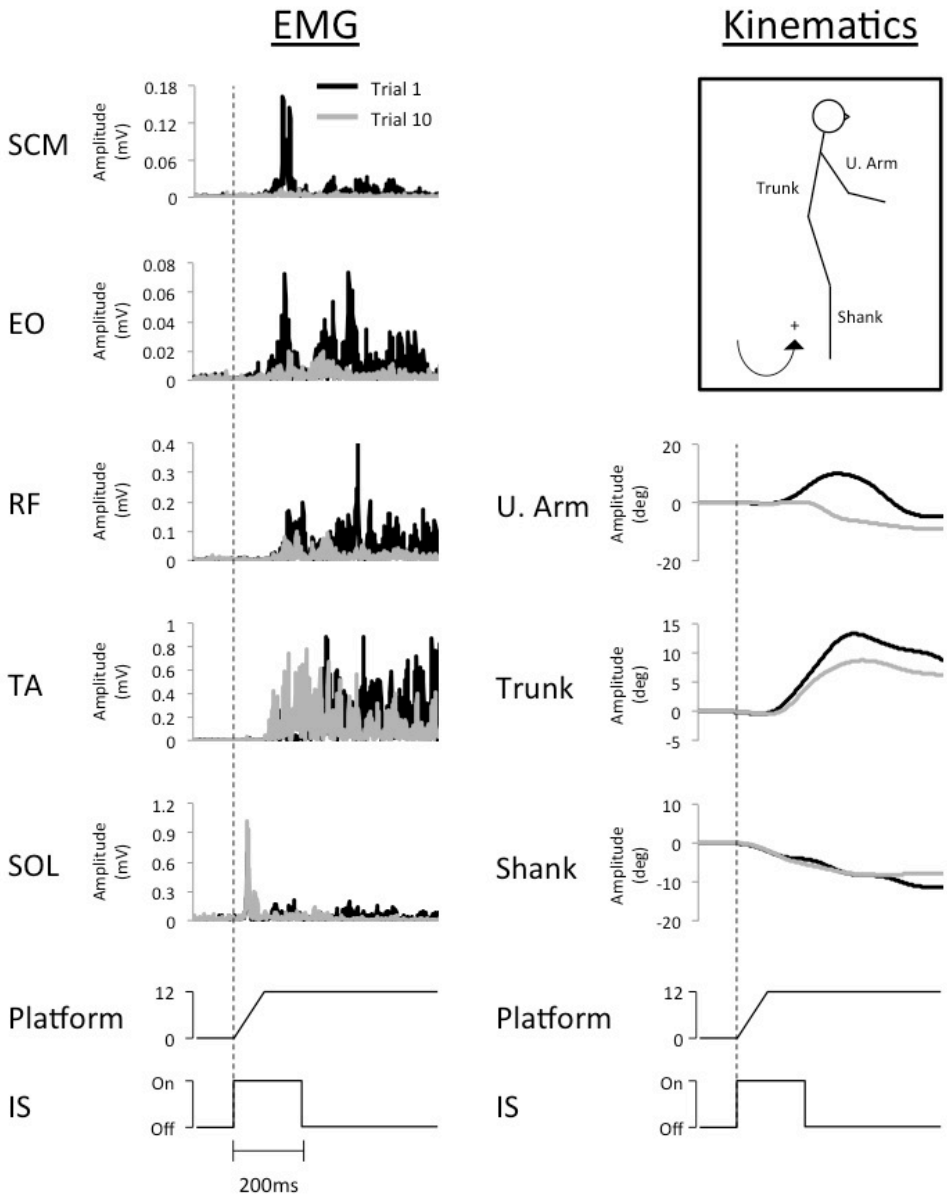
Figure 5.6: Mean kinematic measures during $TEST_{PERT}$ and $TEST_{SAS}$ trials



(A) Average (±1SE) onsets, peak angular displacement and peak velocity of wrist extension during repeated $TEST_{SAS}$ (grey) and $TEST_{PERT}$ (black) trials. Horizontal dashed line and grey areas denote mean (± 1SD) of respective responses observed in CONTROL trials. Grey and black connector lines denote significant differences between 1st and 10th trials in $TEST_{SAS}$ and $TEST_{PERT}$ trials, respectively.

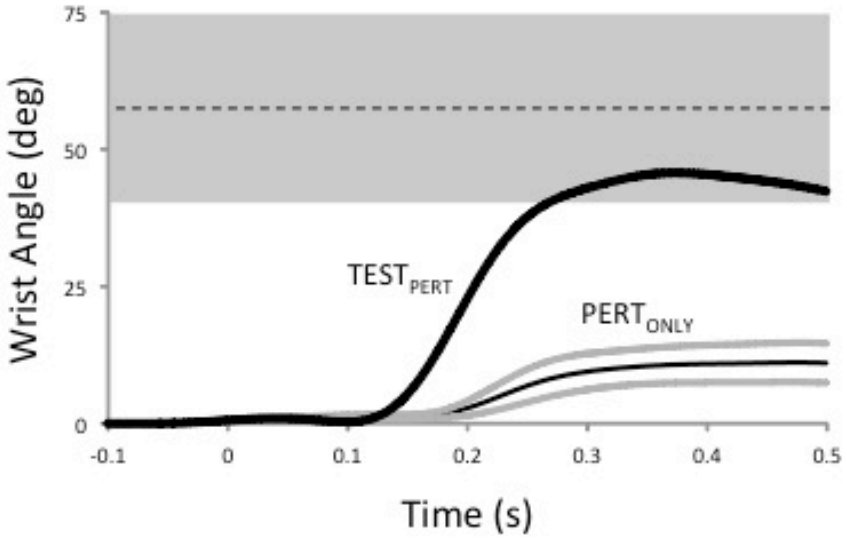
(B) Average (±1SE) onsets, peak displacement and peak velocity of shoulder, trunk and shank kinematic segments during $TEST_{PERT}$ trials. Black connector lines denote significant differences between 1st and 10th $TEST_{PERT}$ trials.

Figure 5.7: Representative EMG and kinematic plots in 1st and 10th $TEST_{PERT}$ trials



Individual subject EMG (left column: sternocleidomastoid (SCM); external oblique (EO); rectus femoris (RF); tibialis anterior (TA); soleus (SOL)) and kinematic (right column: upper-arm, trunk and shank) plots from the 1st (black) and 10th (grey) $TEST_{PERT}$ trials. Vertical dashed line denotes the simultaneous onset of the support-surface rotation (Platform) and the imperative stimulus (IS).

Figure 5.8: Wrist kinematics in $TEST_{PERT}$ and Pert-Only trials



Wrist extension response for a single $TEST_{PERT}$ trial and the average (±1SE) wrist displacements observed across 5 perturbation-only trials for all subjects. Horizontal dashed line and grey areas denote mean (black) (±1SD; grey) of average peak wrist extension observed across all subjects during $TEST_{PERT}$ trials. Time 'zero' denotes simultaneous onset of imperative stimulus and perturbation in $TEST_{PERT}$ trials and the onset of the support-surface displacement during perturbation-only trials.

Table 5.1: Summary measures and statistics of wrist kinematics and EMG responses

A) Independent of SCM (*n*=12)

		Control Trial	TEST Trial						
			$TEST_{SAS}$		$TEST_{PERT}$		Stat (2 X 2) ANOVA		
			Experience		Experience				
			First	Last	First	Last	TEST	Experience	Interaction
Wrist Kinematics	Onset (ms)	247 ±8	188 ±25	147 ±10	159 ±17	158 ±11	*ns*	*ns*	*ns*
	Peak Displacement (°)	57.5 ±3.7	55.6 ±4.8	58.3 ±3.5	57.0 ±4.6	58.0 ±4.6	*ns*	*ns*	*ns*
	Peak Velocity (°/s)	576.5 ±36.4	578.6 ±50.4	645.4 ±37.8	463.8 ±52.5	582.7 ±54.8	*ns*	·	*ns*
ECR	Onset (ms)	195 ±8	95 ±14	102 ±10	101 ±7	107 ±10	*ns*	*ns*	*ns*
	Area (mV·s)	0.010 ±0.001	0.010 ±0.002	0.013 ±0.001	0.009 ±0.001	0.011 ±0.001	*ns*	·	*ns*
FCR	Onset (ms)	211 ±10	109 ±12	110 ±12	118 ±11	119 ±10	*ns*	*ns*	*ns*
	Area (mV·s)	0.001 ±0.000	0.002 ±0.000	0.002 ±0.000	0.002 ±0.000	0.002 ±0.000	*ns*	*ns*	*ns*
SCM	Onset (ms)	N/A	58 ±11	60 ±7	85 ±10	99 ±11	·	*ns*	*ns*
	Area (uV·s)	N/A	3.4 ±0.7	0.5 ±0.1	4.3 ±1	0.5 ±0.14	*ns*	·	*ns*

B) Only SCM+ (*n*=5)

		Control Trial	TEST Trial						
			$TEST_{SAS}$		$TEST_{PERT}$		Stat (2 X 2) ANOVA		
			Experience		Experience				
			First	Last	First	Last	TEST	Experience	Interaction
Wrist Kinematics	Onset (ms)	237 ±7	179 ±21	139 ±13	172 ±35	133 ±9	*ns*	*ns*	*ns*
	Peak Displacement (°)	60.6 ±6.9	61.5 ±8.3	59.6 ±6.4	57.3 ±9.1	59.2 ±6.2	*ns*	*ns*	*ns*
	Peak Velocity (°/s)	627.0 ±65.3	676.6 ±71.7	689.7 ±63.4	558.8 ±47.9	601.7 ±52.6	*ns*	*ns*	*ns*
ECR	Onset (ms)	186 ±7	99 ±25	95 ±11	91 ±3	85 ±6	*ns*	*ns*	*ns*
	Area (mV·s)	0.011 ±0.001	0.012 ±0.003	0.014 ±0.002	0.009 ±0.001	0.011 ±0.003	*ns*	*ns*	*ns*
FCR	Onset (ms)	203 ±14	98 ±16	98 ±16	108 ±16	95 ±10	*ns*	*ns*	*ns*
	Area (mV·s)	0.002 ±0.000	0.004 ±0.001	0.003 ±0.001	0.002 ±0.000	0.003 ±0.001	*ns*	*ns*	*ns*
SCM	Onset (ms)	N/A	58 ±11	60 ±7	85 ±10	99 ±11	·	*ns*	*ns*
	Area (uV·s)	N/A	3.4 ±0.7	0.5 ±0.1	4.3 ±1	0.5 ±0.14	*ns*	·	*ns*

"·" denotes significance for corresponding measure and level of 2 X 2 ANOVA
"ns" denotes non-significant result for corresponding measure and level of 2 X 2 ANOVA

each numerical cell value represents mean ±1SE

Summary measures of wrist kinematics, ECR, FCR and SCM in all subjects (*n*=12) (A) and for a subset of subjects (*n*=5) where SCM activity was observed in all 4 levels of the 2 X 2 ANOVA.

Chapter 6: Conclusion

Many experiences in everyday life challenge the state of postural stability and if left unchecked, would lead to falls. The main focus of this thesis was to better understand the neurophysiology of corrective PRs that are elicited to counter balance perturbations to upright stance. In a sequence of 4 studies, this thesis utilized novel applications of established techniques, such as classical conditioning and startle paradigms, to address questions regarding the role of sensory feedback in the initiation of PRs and the nature of PRs that are evoked by balance perturbations.

6.1 Thesis contributions to scientific understanding of dynamic postural control

The results of this thesis have provided a number of novel findings that may help to better understand PRs. Firstly, this thesis has demonstrated that experience with either expected or unexpected (i.e. cued or uncued) balance perturbations facilitated development of motor responses that could be evoked in the complete absence of postural instability. Contextually-specific sequences of muscle responses and appropriate biomechanical displacement profiles were consistently evoked with non-perturbing sensory stimuli (i.e. auditory cues). These responses were elicited during classical conditioning and startle paradigms, both in isolation (Studies 1 and 3) and in combination (Study 2). In particular, auditory cues in the form of CS during conditioning paradigms and SAS during unexpected balance perturbations induced detectable responses in numerous postural muscles throughout the body which were of sufficient amplitude to induce kinematic and kinetic changes that ultimately led to posture-stabilizing body sway. In each experiment, the responses evoked by cues were shaped by the particular experience of instability provided by balance perturbations. In Study 1, where posteriorly-directed instability was induced by toes-up support-surface rotations, isolated presentations of the CS induced activity in postural agonists, inhibition in muscles that further destabilize posture, and kinematic changes that would lead to forward sway about the ankle. In Studies 2 and 3, an entirely different array of muscle responses, kinematic and kinetic changes emerged which where tuned to the repeated experiences with lateral support-surface translations and were evoked by SAS in the absence of balance perturbations. Auditory stimuli (either CS or SAS) presented at various stages

throughout Studies 2 and 3 induced frontal-plane sway in the direction of stability via a combination of contextually-specific sequences of lower- and upper-body displacements, centre of pressure displacements, and muscle activity.

The second major contribution of this thesis confirms the presence of perturbation-induced responses that may potentially mask the true nature of evoked PRs. The results from Study 4 suggest that startle responses may be a type of motor behaviour evoked by balance perturbations. Their presence during first-trial exposure to balance perturbations, as well as up to the 10th repeated exposure, strongly suggests that PRs are not the only type of motor response evoked by balance perturbations. These results highlight the potential confounding presence of startle responses that may exist when evoking PRs using balance perturbations, and further underscores the potential weaknesses of earlier attempts to understand the nature of PRs in situations where other confounding factors may have been present.

6.2 Specific contributions of each study

In the first experiment, a classical conditioning paradigm was used to examine the link between sensory feedback derived from balance perturbations and PR initiation. Auditory cues that are normally unrelated to balance perturbations were capable of inducing motor responses after being repeatedly coupled to balance perturbations. In the absence of perturbations, motor responses included contextually-specific excitation of postural agonists in balance correction to a toes-up support-surface rotation (TA and RF) and inhibition of plantar-flexor activity. This muscle activity was accompanied by shank rotations in the direction appropriate for countering the expected toes-up support-surface rotation. Through acquisition of the conditioned PRs and with their subsequent extinction, this study also provided evidence of an adaptive mechanism whereby PR-related responses can be learned, and retained in memory for an extended period of time.

The second experiment replicated the ability to condition PRs to a cue and to evoke them in the absence of balance perturbations, while extending these observations to lateral directions. These conditioned PRs included an extensive array of muscle activity: most notably TA, GM and others that control lateral movement of the legs and trunk. Muscle responses induced angular displacement of the ankle and hip joints and coupled with COP displacements induced contextually-specific whole body sway that would be corrective for

the expected direction of perturbation-induced instability. Additionally, data from Study 2 provided novel evidence that conditioned PRs were prepared and stored in the nervous system and could be evoked by SAS. Compared to conditioned PRs evoked by only non-startling cues, SAS-induced responses were observed significantly earlier, yet contained preserved aspects of relative timing between COP and kinematic changes in the ankle and hip joint as well as absolute amplitudes of individual responses.

The third study extended the findings of Study 1 and 2 by demonstrating that the ability to prepare a PR was not limited to situations where perturbations were accompanied by a cue. Prior to any experiences with unexpected and uncued balance perturbations, SAS presented during quiet stance evoked generalized startle responses characterized by mostly sagittal-plane sway induced by bilateral flexion reactions and bilateral activation of SCM. However, experience garnered by simply watching an uncued lateral-support surface translation, or physically experiencing the perturbation once or 12 times, was sufficient to allow SAS to induce directionally- and contextually-specific movements characterized by kinematic changes in the lower-body and COP profiles that differed markedly from the generalized startle response.

The fourth and final study incorporated support-surface toes-up rotations into a simple reaction time paradigm to examine whether FTEs are related to a startling effect induced by balance perturbations. Balance perturbations showed a highly consistent and robust ability to induce the StartReact effect, similar to other known startle stimuli (i.e. SAS), not only in the first trial exposures, but also in as many as 10 repeated trials. In contrast, the generalized startle response, as indicated by bilateral SCM activity, habituated after the first trial in both frequency and amplitude. These data provided evidence to both support the role of startle responses in mediating FTEs and additionally to demonstrate a persistent startle effect beyond the first trial.

6.3 Novel insights into responses triggered by balance perturbations

Evidence provided by temporal and spatial characteristics of responses evoked across studies suggest that, in the absence of balance perturbations, aspects of PRs could be evoked by stimuli that do not physically destabilize posture. For example, in Study 1, toes-up support-surface rotations induced a relative pattern of TA and RF onsets, as observed in

previous investigations using similar perturbations (Nashner and Cordo, 1981; Carpenter et al., 1999). This activation pattern was conserved in responses evoked only by CS (Table 2.1). Additionally, the absolute timing and direction of sway responses evoked by cues further suggest that they could serve a stabilizing role following balance perturbations. The absolute onsets of muscle responses evoked by CS straddled the onsets of balance perturbations (Table 2.1 and Figure 3.3). In Study 2 and 3, movements evoked by SAS were within the established latency range of PRs evoked by balance perturbations. For example, in Study 2, mean COP, ankle and hip displacement onsets all occurred within 200ms (Table 2.1). Likewise, in Study 3, mean onsets of COP and lower-body angular displacements were observed under 150ms and at approximately 200ms, respectively.

In Studies 1-3, the movements induced by cues or SAS contained biomechanical characteristics that could serve a specific role in regaining stability following particular experiences with either support-surface rotations or translations. In Study 1 where posteriorly-directed instability was induced by toes-up support-surface rotations, cued movements evoked muscle contractions and shank movements that induced re-stabilizing forward body sway. In Studies 2 and 3 where frontal-plane instability was induced by support-surface translations, CS- and SAS-induced responses consistent with those that would displace the body in the ipsilateral direction of the support-surface movements, and therefore towards stability (Figures 3.2 and 4.4).

Assuming that the responses evoked in the absence of balance perturbations contain elements of perturbation-induced PRs, they provide a unique opportunity to address questions regarding 1) the sensory contributions to PR initiation, and 2) the nature of PRs evoked by balance perturbations.

6.4 Sensory contributions to PR initiation

Multiple theories have been described which aim to explain how sensory feedback arising from balance perturbations leads to PR initiation. The notion that a single sensory system may alone be responsible for initiating PRs has been the motivation for many studies (Allum and Pfaltz, 1985; Keshner et al., 1987; Horak et al., 1990; Allum et al., 1994; Do et al., 1994; Allum and Honegger, 1997, 1998; Runge et al., 1998; Perry et al., 2000; Nataka and Yabe, 2001; Bloem et al., 2002). Assuming that a single source of feedback was

critically responsible for initiating PRs, these experiments predicted that removing a sensory source would substantially delay PR onsets or prevent them from being initiated altogether. In the absence of a consistent effect between studies and within tests of the same sensory system, researchers postulated that a single source of feedback may not be able to induce the array of muscle responses that emerge following balance perturbations. Thus, a multi-sensory trigger theory was proposed which ultimately stated that when perturbed, feedback from any sensory system could contribute to PR initiation (Nashner et al., 1982; Allum et al., 1995). Regardless of the conceptual differences between theories, they collectively assert that PRs are directly the result of the sensory feedback derived from the destabilizing forces applied to the body when balance is perturbed.

In stark contrast to previous work that used balance-perturbing forces to evoke PRs, the use of classical conditioning paradigms and startle techniques in this thesis offer unique perspectives on sensory contributions to PR initiation. In the presence of balance perturbations, sensory feedback is critical to trigger PRs; however, this thesis has provided many examples of situations where sensory feedback generated specifically by balance perturbations may not be critical towards initiating all aspects of PRs. The first example is the ability to induce aspects of PRs in the complete absence of balance perturbations and related sensory feedback. The second example is the ability for alternative sensory cues (i.e. auditory cues) formerly unrelated to balance perturbations and with no known capacity to trigger PRs, to evoke aspects of PRs. Given previous theories, these results support the argument that sensory feedback from a single source is not tasked with initiating all aspects of PRs evoked by balance perturbations. Furthermore, it suggests that the mechanisms involved in PR initiation may be less 'reflexive' and more adaptable than previously thought.

Along with contributing to the previous theories of PR initiation, this thesis also provides new insight into an alternative sensory contribution to PR initiation. The results from Study 4 suggest that balance perturbations to upright stance, like SAS, are a type of stimulus that can induce not only startle responses, but also prepared motor behaviours via StartReact effects. This mechanism has been shown to induce intended movements of various limbs (Valls-Solé et al., 1999; Carlsen et al., 2004a) and whole-body behaviours (such as stepping and rising-to-toes) (Valls-Solé et al., 1999; MacKinnon et al., 2007; Queralt et al., 2008a,b). An interesting finding that emerged from this thesis was that certain

balance perturbations contained a startling element and thus carries with them, the potential to initiate prepared movement if they exist. Previous work by Ravichandran and colleagues (2009) has demonstrated that startle responses (indicated by SCM activity) and StartReact effects of prepared upper-limb movements could be induced by mechanical perturbations to elbow angle. These results suggest the possibility that whole body perturbations could induce startle responses as well as StartReact effects. Consolidating the results of Study 3 and those from Study 4 support this possibility. In Study 3, motor preparation was induced by experience with unexpected balance perturbations and in Study 4, the perturbations themselves induced StartReact effects of prepared movements. Future studies could be done to further explore the potential for startle to influence PRs. The use of techniques such as pre-pulse or transcranial magnetic stimulation, which are capable of selectively influencing generalized startle responses (Maslovat et al., 2012) and StartReact effects (Alibiglou and MacKinnon, 2010), respectively, could provide an opportunity to determine the extent to which PRs are influenced by generalized startle responses and prepared PRs can be evoked by balance perturbations.

6.5 The nature of PRs evoked by balance perturbations

Balance perturbations induce a broad array of muscle responses throughout the body. One theory of PRs suggests that they are a sequence of functional stretch reflexes (Nashner, 1977) or long-latency reflexes (Diener et al., 1984, 1985) that originate from perturbation-induced stretch of lower-limb muscles. Another theory of PRs describes the collective pattern of muscle responses as postural strategies (Horak and Nashner, 1989; Runge et al., 1999) or synergies (Ting and McKay, 2007) that are shaped and tuned to the specific characteristics of balance perturbation parameters. It has been argued that neither description of PRs in isolation can explain all aspects of responses evoked by balance perturbations. Functional stretch reflex or long-latency reflex theories of PRs are not supported by previous evidence of upper-body muscle responses at latencies similar to stretch reflexes in the lower-limb (Keshner et al., 1988) and evidence of PRs in muscles that cannot produce a stretch reflex (Allum and Honegger, 1997; Bloem et al., 2002). The results from Studies 1-3 are also inconsistent with PRs being a sequence of stretch-related reflexes. Evidence of PRs evoked in the absence of balance perturbations and associated muscle lengthening, which

may in fact be components of PRs, are in direct contradiction to a pure stretch-reflex driven response governing the entirety of induced PRs. In addition to refuting stretch-reflex contributions, data provided by this thesis support the existence of pre-assembled PRs.

The multi-muscle patterns observed in response to balance perturbations have been described based on net biomechanical outcomes (Runge et al., 1999) and by an assembly of elementary response patterns (Ting and McKay, 2007). However, they have not yet been validated as an entity that exists within the nervous system (Ting and McKay, 2007). The results of this thesis have provided evidence for the existence of pre-structured PRs, which align with aspects of postural synergies. Responses evoked by cues induced contextually-specific multi-muscle and multi-joint responses that lead to corrective sway.

Assuming that cue- and SAS-induced PRs are postural synergies, their consistent attenuation in the absence of balance perturbations may provide new information about how postural synergies are influenced by sensory feedback. One explanation for the discrepancies in amplitude is that the observed responses evoked by cues may have been an attenuated version of a much larger response. A 'horse-race' between initiation and termination mechanisms has been proposed as a potential mediator of intended response amplitudes (Logan et al., 1984; McGarry and Franks, 1997). If the absence of a balance perturbation signaled a mechanism to terminate a PR, then it would be plausible that the observed response amplitudes could have been reduced before reaching their peak. However, the fact that SAS induced PRs with the same magnitude as those evoked by non-startling cues (Table 4.1) suggest that PRs were likely not terminated early. Prepared motor responses triggered by SAS occur at such short latencies that they are typically executed to completion (Carlsen et al., 2011a,b). Therefore, the possibility exists for prepared movement characteristics to reflect an unaltered postural synergy. If true, questions then remain as to how low-amplitude postural synergies become amplified to the levels observed following balance perturbations. One possibility is that they are modulated in an online feedback manner when combined with local sensory inputs induced by balance perturbations (Chan, 1983; Diener et al., 1988). Another possibility is that they are modulated in a feedforward manner by the specific sensory inputs induced during prior experiences with balance perturbations. The directionally-specific movements induced by SAS after only visual experience with balance perturbations argue indirectly against the feedforward model of

control. Consistent with previous examples of sensory contributions to PR amplitude modulation (Diener et al., 1983; Do et al., 1994; Allum and Honegger, 1997), the results of this thesis suggest that the role of sensory feedback may be to act in real time to influence the amplitude of evoked postural synergies.

6.6 Supra-spinal contributions to dynamic postural control

Various lines of evidence have emerged in the literature to support a supra-spinal role in dynamic postural control. One line of evidence comes from animal preparations and the negative consequences to dynamic postural stability observed in spinalized cats (MacPherson and Fung, 1999). Using a feline animal model, Stapley and colleagues (2009) have also observed patterns of supra-spinal neural activity located in the reticular formation of the brainstem that related to specific instances of quadrupedal instability and postural corrections. In humans, evidence of supra-spinal involvement in dynamic postural control has emerged from various experimental procedures. Firstly, robust and highly consistent motor- (Jacobs et al., 2008; Mochizuki et al., 2008) and sensory-related (Adkin et al., 2008) cortical activity has been observed prior to cued perturbations. Secondly, PRs are influenced by higher-order mechanisms such as in attention, cognition, experience and emotion (Horak et al., 1989; Redfern et al., 2001; Carpenter et al., 2004; Maki and McIlroy, 2007) that presumably involve supra-spinal processing.

The work in this thesis further supports a supra-spinal contribution to dynamic postural control. Classical conditioning is an associative learning process whose underlying neural circuitry has been extensively studied. In the eye-blink model, the neural substrates that mediate conditioned response acquisition, retention and initiation are widely dispersed throughout the nervous system, but critical aspects (such as the hippocampus, cerebellum, motor and sensory cortices) reside supra-spinally (see Christian and Thompson, 2003 for review). Although the circuits involved in classical conditioning vary greatly depending on the type of movement being conditioned, the modality of the CS (Holland, 1977), the temporal relationship between CS and US (Reynolds, 1945; Christian and Thompson, 2003), and the ability to classically condition a response requires intimate interactions amongst supra-spinal centres. It follows that the ability to induce a conditioned PR requires the potential involvement of supra-spinal centres. Interestingly, like PRs (Carpenter et al.,

2004), conditioned eye-blink response amplitudes are augmented in the presence of fear (Maschke et al., 2000), which suggests a potential similarity between mechanisms involved in conditioning PRs and supra-spinal processing that is known to influence postural control.

Motor preparation is another process that is known to involve supra-spinal processing. The theoretical model of motor control proposed by Donders (1869) implies that higher-order supra-spinal processing was required for motor preparation. This has since been supported by repeated observations of a relationship between movement preparation and lateralized motor-related cortical potentials (Kutas and Donchin, 1980; Leuthold et al., 1996). Further evidence for supra-spinal processing in motor preparation emerges from the ability for SAS to induce the StartReact effect of prepared movements. While early work associated StartReact effects with the reticular formation and efferent reticulo-spinal tracts (Valls-Solé et al, 1999) more recent evidence suggests higher cortical regions may also be involved (Carlsen et al., 2011a; Alibiglou and MacKinnon, 2012). The support for supra-spinal processing in motor preparation provides grounds to suggest that perhaps similar mechanisms may contribute to PRs when triggered by SAS or other stimuli (like balance perturbations) that can induce prepared movements.

6.7 Implications and future directions

6.7.1 Startle-mediated emotional influences on PRs

Postural control is influenced by a number of psychological factors. Emotional factors such as fear and anxiety have been of interest to researchers, mostly for their supposed causal relationship to postural instability and falls (Yardley, 2004). In static balance, the physiological changes due to increases in fear that affect postural control have been relatively well documented (Carpenter et al., 1999; Davis et al., 2009, 2010, 2011). In dynamic balance situations, the influence of emotion on PRs has been relatively well documented, (Carpenter et al., 2004; Adkin et al., 2008) however, the physiological mechanisms underlying emotion-related changes to PRs are not well understood.

Perturbing balance while in a state of heightened anxiety is known to specifically increase the amplitudes of PRs, independent of stretch reflexes or voluntary stabilizing reactions (Carpenter et al., 2004). It has been proposed that mechanisms specifically

involved in governing PRs are sensitive to the influences of anxiety (Carpenter et al., 2004), however, the mediating factors underpinning these changes have yet to be described. The evidence provided by this thesis perhaps offers an explanation for the observed influences of anxiety on PR amplitudes. Results from Study 4 have suggested that balance perturbations, like SAS, can induce generalized startle responses that may be super-imposed onto PRs and may occur at similar latencies (Blouin et al., 2006, 2007). Generalized startle responses, evoked by SAS in states of heightened fear and anxiety, are known to be increased in amplitude compared to control (Grillon et al., 1991) by a phenomenon known as fear-potentiated startle. Given the sensitivity of generalized startle response amplitudes to states of fear and anxiety, it is plausible that increases in the perturbation-induced response amplitude observed at PR latencies (Carpenter et al., 2004) could have been the result of an augmented generalized startle response potentiated by fear.

6.7.2 Influences of StartReact effects induced by balance perturbations

Previous experiments have introduced voluntary movements as probes to assess the attentional demands (Müller et al., 2004, 2007) and the adaptability (Küng et al., 2009, 2010) of PRs. In both situations, latency measures of voluntary responses evoked by balance perturbations are critical to data interpretations. For example, in dual task paradigms, changes to reaction time are interpreted as evidence to support an attentional influence of PRs (Müller et al., 2004, 2007). In other paradigms, onset latencies of voluntary movements are used to assess the adaptability of reactive PRs (Küng et al., 2009, 2010). Given the potential for perturbations to induce StartReact effects of prepared movement (Study 4), it would be important for future studies to understand the extent to which previous measures of reaction time and voluntary movement onsets were influenced by startle induced by balance perturbations. It is possible that instances where voluntary movement onsets were shortened in the presence of balance perturbations, that StartReact effects may have facilitated them. Future studies could incorporate the use of SAS to determine whether voluntary movements produced along with PRs were influenced by motor preparation.

6.7.3 Future directions

One of the major contributions of this thesis was evidence in support of the ability for balance perturbations to evoke startle responses and prepared movement. Given these observations, questions are raised whether presence of these responses could have confounded previous attempts to understand sensory contributions to PR initiation. For studies that aimed to understand the role of specific sensory systems in PR initiation, change in onset latency was used as the primary measure (Allum and Pfaltz, 1985; Keshner et al., 1987; Horak et al., 1990; Allum et al., 1994; Do et al., 1994; Allum and Honegger, 1997, 1998; Runge et al., 1998; Perry et al., 2000; Nataka and Yabe, 2001; Bloem et al., 2002). Specifically, given the known timing of generalized startle responses observed in postural muscles (Brown et al., 1991a,b) and the observed rapid onset latencies of prepared PRs in Studies 2 and 3 evoked by SAS, the possibility exists that initial bursts of EMG activity induced by balance perturbation may be early components of generalized startle responses or prepared movement. Thus, failing to account for the effects of startle may have confounded the ability for prior work to assess the sensory contributions to PR initiation. Future work should first be done to understand the full extent to which startle can influence the early aspects of responses evoked by balance perturbations. Instead of using onset latencies of EMG activity as a primary measure, perhaps future experiments would be better served to use the onset latency of particular response frequencies that are related to startle responses. Generalized startle response frequencies have been characterized within the 10-20Hz range (Blouin et al., 2006), which is markedly distinct from frequency signatures of non-startle related EMG activity (Siegmund et al., 2008). Thus, even when responses are superimposed onto one another, temporal distinctions between responses could still be made.

Another question raised by this thesis is whether similar observations of PR motor preparation would occur in different balance paradigms. Prepared PRs that were observed in Studies 2 and 3 have provided the first lines of evidence to suggest a potential contribution of motor preparation in dynamic postural control. However, these data emerged from situations where either cues provided highly reliable information regarding perturbation onsets or balance perturbation parameters were highly predicable (i.e. direction and magnitude). Thus, in other situations where balance perturbation parameters and required PR characteristics are not known in advance, it is unclear whether prepared PRs would develop and what their

characteristics may be. Recent evidence from motor control literature suggests that even when the specific parameters of required movements are not known in advance (i.e. such as in choice-reaction time paradigms) that a 'default intermediate amplitude' response could be prepared that benefits completion of multiple possible tasks (Forgaard et al., 2011). Thus, although introducing multiple response alternatives can dampen the intensity of motor preparation effects, does not preclude the potential for motor preparation to be undertaken. It would therefore be of importance for further research to understand the extent to which PR motor preparation could occur in response to unpredictable and randomized perturbations. Furthermore, comparing the extent of PR preparation between serial and random, or expected and unexpected, perturbations may help further describe the factors other than central set (Horak et al., 1989) that mediate differences in PRs evoked by balance perturbations in these situations.

References

1. Adkin AL, Campbell AD, Chua R, Carpenter MG. The influence of postural threat on the cortical response to unpredictable and predictable postural perturbations. Neurosci Let 435:120-125, 2008.
2. Alibiglou L, MacKinnon CD. The early release of planned movement by acoustic startle can be delayed by transcranial magnetic stimulation over the motor cortex. J Physiol 4:919-935, 2012.
3. Allum JHJ. Responses to load disturbances in human shoulder muscles: The hypothesis that one component is a pulse test information signal. Exp Brain Res 22:307-326, 1975.
4. Allum JHJ, Bloem BR, Carpenter MG, Hulliger M, Hadders-Algra M. Proprioceptive control of posture: a review of new concepts. Gait Posture 8:214-242, 1998.
5. Allum JHJ, Büdingen HJ. Coupled stretch reflexes in ankle muscles: an evaluation of the contributions of active muscle mechanisms to human posture stability. Prog Brain Res 50:185-195, 1979.
6. Allum JHJ, Carpenter MG, Honegger F, Adkin AL, Bloem BR. Age-dependent variations in the directional sensitivity of balance corrections and compensatory arm movements in man. J Physiol 15:643-663, 2002.
7. Allum JHJ, Honegger F. Interactions between vestibular and proprioceptive inputs triggering and modulating human balance-correcting responses differ across muscles. Exp Brain Res 121:478-494, 1997.

8. Allum JHJ, Honegger F, Acuña H. Differential control of leg and trunk muscle activity by vestibulo-spinal and proprioceptive signals during human balance corrections. Acta Otolaryng 115:124-129, 1995.
9. Allum JHJ, Pfaltz CR. Visual and vestibular contributions to pitch sway stabilization in the ankle muscles of normals and patients with bilateral peripheral vestibular deficits. Exp Brain Res 58:82-94, 1985.
10. Anderson JR. Learning and memory: an integrated approach, 2nd edition. New York: John Wiley & Sons Inc, 2000, p. 56-57.
11. Bernstein NA. The co-ordination and regulation of movements. Oxford UK, Pergamon Press, 1967.
12. Bisdorff AR, Bronstein AM, Gresty MA. Responses in neck and facial muscles to sudden free fall and a startling auditory stimulus. Electroencephalogr Clin Neurophysiol 93:409-416, 1994.
13. Bloem BR, Allum JHJ, Carpenter MG, Verschuuren JJ, Honegger F. Triggering of balance corrections and compensatory strategies in a patient with total leg proprioceptive loss. Exp Brain Res 142:91-107, 2002.
14. Blouin JS, Inglis JT, Siegmund GP. Startle responses evoked by whiplash perturbations. J Physiol 573:857-867, 2006.
15. Blouin JS, Siegmund GP, Inglis JT. Interaction between acoustic startle and habituated neck postural responses in seated subjects. J App Physiol 102:1574-1586, 2007.

16. Bouisset S, Zattara M. Biomechanical study of the programming of anticipatory postural adjustments associated with voluntary movement. J Biomech 20: 735-742, 1987.
17. Bouton ME, Moody EW. Memory processes in classical conditioning. Neurosci Biobehav Rev 28:663-674, 2004.
18. Braun HW, Geiselhart R. Age differences in the acquisition and extinction of the conditioned eyelid response. J Exp Psychol 57:386-388, 1959.
19. Britton TC, Day BL, Brown P, Rothwell JC, Thompson PD, Marsden CD. Postural electromyographic responses in the arm and leg following galvanic vestibular stimulation in man. Exp Brain Res 94:143–151, 1993.
20. Brown P, Day BL, Rothwell JC, Thompson PD, Marsden CD. The effect of posture on the normal and pathological auditory startle reflex. J Neurol Neurosurg Psych 54:892-897, 1991a.
21. Brown P, Rothwell JC, Thompson PD, Britton TC, Day BL, Marsden CD. New observations of the normal auditory startle reflex in man. Brain 114:1891-1902, 1991b.
22. Campbell AD, Dakin CJ, Carpenter MG. Postural responses explored through classical conditioning. Neurosci 164:986-997, 2009.
23. Carlsen AN, Chua R, Inglis JT, Sanderson DJ, Franks IM. Can prepared response be stored subcortically? Exp Brain Res 159:301-309, 2004a.
24. Carlsen AN, Chua R, Inglis JT, Sanderson DJ, Franks IM. Prepared movements are elicited early by startle. J Motor Behav 36:253-264, 2004b.

25. Carlsen AN, Chua R, Inglis JT, Sanderson DJ, Franks IM. Differential effects of startle on reaction time for finger and arm movements. J Neurophysiol 110:306-314, 2009.
26. Carlsen AN, Dakin CJ, Chua R, Franks IM. Startle produces early response latencies that are distinct from stimulus intensity effects. Exp Brain Res 176:199-205, 2007.
27. Carlsen AN, MacKinnon CD. Motor preparation is modulated by the resolution of the response timing information. Brain Res 1322:38-49, 2010.
28. Carlsen AN, Maslovat D, Franks IM. Preparation for voluntary movement in healthy and clinical populations: Evidence from startle. Clin Neurophysiol doi:10.1016/j.clinph.2011.04.028, 2011a.
29. Carlsen AN, Maslovat D, Lam MY, Chua R, Franks IM. Considerations for the use of a startling acoustic stimulus in studies of motor preparation in humans. Neurosci Behav Rev 35:366-376, 2011b.
30. Carpenter MG, Allum JHJ, Honegger F. Vestibular influences on human postural control in combinations of pitch and roll planes reveal differences in spatiotemporal processing. Exp Brain Res 140:95-111, 2001.
31. Carpenter MG, Allum JHJ, Honegger F. Directional sensitivity of stretch reflexes and balance corrections for normal subjects in the roll and pitch planes. Exp Brain Res 129:93-113, 1999.
32. Carpenter MG, Frank JS. Slicher CP. Surface height effects on postural control: a hypothesis of a stiffness strategy for stance. J Ves Res 9:277-286, 1999.

33. Carpenter MG, Tokuno CD, Thorstensson A, Cresswell AG. Differential control of abdominal muscles during multi-directional support-surface translations in man. Exp Brain Res 188:445-455, 2008.
34. Castellote JM, Kumru H, Queralt A, Valls-Solé J. A startle speeds up the execution of externally guided saccades. Exp Brain Res 177:129-136, 2007.
35. Carpenter MG, Franks JS, Adkin AL, Paton A, Allum JHJ. Influence of postural anxiety on postural reactions to multi-directional surface rotations. J Neurophysiol 92:3255-3265, 2004.
36. Chan CW. Segmental versus suprasegmental contributions to long-latency stretch responses in man. Adv Neurol 39:467-487, 1983.
37. Chiu C, Stevenson A, Maslovat D, Chua R, Gick B, Franks IM. Feedforward control of phonetic gestures in consonant-vowel syllables: evidence from responses to auditory startle. J Acoust Soc Am 129:2455-2455, 2011.
38. Christian KM, Thompson RF. Neural substrates of eyeblink conditioning: Acquisition and retention. Learn Mem 10:427-455, 2003.
39. Clark RE, Manns JR, Squire LR. Classical conditioning, awareness, and brain systems. Trends Cogn Sci 6:524-531, 2002.
40. Clark RE, Squire LR. Classical conditioning and brain systems: the role of awareness. 280:77-81, 1998.
41. Cole JD, Sedgwick EM. The perceptions of force and of movement in a man without large myelinated sensory afferents below the neck. J Physiol 449:503–515, 1992.

42. Dakin CD, Lee Son GM, Inglis JT, Blouin JS. Frequency response of human vestibular reflexes characterized by stochastic stimuli. J Physiol 583:1117-1127, 2007.

43. Davis JR. Fear of falling, proprioception and spinal reflex modulation. circle.ubc.ca, 2010.

44. Davis JR, Campbell AD, Adkin AL, Carpenter MG. The relationship between fear of falling and human postural control. Gait Posture 29:275-279, 2009.

45. Davis JR, Horslen BC, Nishikawa K, Fukushima K, Chua R, Inglis JT, Carpenter MG. Human proprioceptive adaptations during states of height-induced fear and anxiety. J Neurophysiol 106:3082-3090, 2011.

46. Davis M. The mammalian startle response. In: Eaton, R.C. (Ed), Neural mechanisms of startle behaviour. Planum Press, New York, pp.287-351, 1984.

47. De Graaf B, Van Weperen W. The retention of balance: An exploratory study into the limits of acceleration the human body can withstand without losing equilibrium. Hum Factor 39:111-118, 1997.

48. Delwaide PJ, Schepens B. Auditory startle (audio-spinal) reaction in normal man: EMG responses and H reflex changes in antagonistic lower limb muscles. Electroencephalogr Clin Neurophysiol 97:416-423, 1995.

49. Desmond JE, Moore JW. Adaptive timing in neural networks: The conditioned response. Biol Cybern 58:405-415, 1988.

50. Deuschl G, Ludolph A, Schenck E, Lücking CH. The relations between long-latency reflexes in hand muscles, somatosensory evoked potentials and transcranial stimulation of motor tracts. Electroenceph Clin Neurophyiol 74:425-430, 1989.

51. Diener HC, Ackermann H, Dichgans J, Guschlbauer B. Medium- and long-latency responses to the displacements of the ankle joint in patients with spinal and central lesions. Electroenceph Clin Neurophysiol 60:407-416, 1985.

52. Diener HC, Bootz F, Dichgans J, Bruzek W. Variability of postural "reflexes" in humans. Exp Brain Res 52:423-428, 1983.

53. Diener HC, Dichgans J, Bootz F, Bacher M. Early stabilization of human posture after a sudden disturbance: Influence of rate and amplitude of displacement. Exp Brain Res 56:126-34, 1984.

54. Diener HC, Horak FB, Nashner LM. Influence of stimulus parameters on human postural responses. J Neurophysiol 59:1888-1905, 1988.

55. Diener HC, Horak FB, Stelmach G, Guschlbauer B, Dichgans J. Direction and amplitude precuing has no effect on automatic posture responses. Exp Brain Res 84:219-223, 1991.

56. Dietz V, Hortsmann GA, Trippel M, Gollhofer A. Human postural reflexes and gravity – an underwater simulation. Neurosci Let 106:350-355, 1989.

57. Do MC, Bussel B, Breniere Y. Influence of plantar cutaneous afferents of early compensatory reactions to forward fall. Exp Brain Res 79:319-324, 1994.

58. Donders, FC. On the speed of mental processes. In W. G. Koster (Ed. & Trans), Attention and performance II, North Holland, Amsterdam (Original work published in 1868), 1869.

59. Forgaard CJ, Maslovat D, Carlsen AN, Franks IM. Default motor preparation under conditions of response uncertainty. Exp Brain Res 215:235-245, 2011.

60. Freeman JH, Spencer CO, Skelton RW, Stanton ME. Ontogeny of eyeblink conditioning in the rat: effects of the US intensity and interstimulus interval on delay conditioning. Psychobiol 21:233-242, 1993.

61. Galvez R, Weible AP, Disterhoft JF. Cortical barrel lesions impair whisker-CS trace eyeblink conditioning. Learn Mem 14:94-100, 2007.

62. Gandevia SC, Wilson LR, Inglis JT, Burke D. Mental rehearsal of motor tasks recruits α-motoneurones but fails to recruit human fusimotor neurones selectively. J Physiol 505:259-266, 1997.

63. Garcia-Hoz V. Signalization and stimulus-substitution in Pavlov's theory of conditioning. Span J Psychol 6:168-176, 2003.

64. Gallistel CR, Gibbons J. Time, rate, and conditioning. Psychol Rev 107:289-344, 2000.

65. Grillon C, Ameli R, Woods SW, Merikangas K, Davis M. Fear-potentiated startle in humans: effects of anticipatory anxiety on the acoustic blink reflex. Psychophysiol 28:588-595, 1991.

66. Hageman PA, Leibowitz JM, Blanke D. Age and gender effects on postural control measures. Arch Phys Med Rehab 76:961-965, 1995.

67. Henry SM, Fung J, Horak FB. EMG responses to maintain stance during multidirectional surface translations. J Neurophysiol 80:1939-1950, 1998.

68. Hilgard ER. The nature of the conditioned response: I. the case for and against stimulus-substitution. Psychol Rev 43:366-385, 1936.

69. Holland PC. Conditioned stimulus as a determinant of the form of the Pavlovian conditioned response. J Exp Psych Anim Behav Proc 3:77-104, 1977.

70. Honeycutt CF, Gottschall JS, Nichols TR. Electromyographic responses from the hindlimb muscles of the decerebrate cat to horizontal support-surface perturbations. J Neurophysiol 6:2751-2761, 2009.

71. Honeycutt CF, Nichols TR. The decerebrate cat generates the essential features of the force constraint strategy. J Neurophysiol 6:3266-3273, 2010.

72. Horak FB, Diener HC, Nashner LM. Influence of central set on human postural responses. J Neurophysiol 62:841-853, 1989.

73. Horak FB, Nashner L. Central programming of postural movements: adaptation to altered support-surface configurations. J Neurophysiol 55:1369-1381, 1986.

74. Horak FB, Shupert CL, Dietz V, Horstmann G. Vestibular and somatosensory contributions to responses to head and body displacements in stance. Exp Brain Res 100:93-106, 1994.

75. Jacobs JV, Fujiwara K, Tomita H, Furune N, Kunita K, Horak FB. Changes in the activity of the cerebral cortex relate to postural response modification when warned of a perturbation. Clin Neurophysiol 119:1431-1442, 2008.

76. Jacobs JV, Horak FB. Cortical control of postural responses. J Neural Transm 114:1339-1348, 2007.

77. Johnson KO. The roles and functions of cutaneous mechanoreceptors. Curr Opin Neurobiol 11:455-461, 2001.

78. Kandel ER, Schwartz JH, Jessel TM. Principles of Neural Science. New York, New York: McGraw-Hill, 2004.

79. Kaulich T, Föhre W, Kutz DF, Gerwig M, Timmann D, Kolb FP. Differences in unconditioned and conditioned response of the human withdrawal reflex during stance: Muscles response and biomechanical data. Brain Res 1326:81-95, 2010.

80. Keele, SW, Summers, JJ. The structure of motor programs. In: G. E. Stelmach (Ed.), Motor Control: Issues and trends (pp. 109-141). New York, Academic Press, 1976.

81. Keshner EA, Allum JHJ, Pfaltz CR Postural coactivation and adaptation in the sway stabilizing responses of normals and patients with bilateral vestibular deficit. Exp Brain Res 69:77-92, 1987.

82. Keshner EA, Kenyon RV, Langston J. Postural responses exhibit multisensory dependencies with discordant visual and support-surface motion. J Vestib Res 14:307-319, 2004.

83. Keshner EA, Woollacott MH, Debu B. Neck, trunk and limb muscle responses during postural perturbation in humans. Exp Brain Res 71:455-466, 1988.

84. Kirsch I, Lynn SJ, Vigorito M, Miller RR. The role of cognition in classical and operant conditioning. J Clin Psychol 60:369-392, 2004.

85. Klapp ST. Motor response programming during simple and choice reaction time: the role of practice. J of Expl Psych: Hum Percep and Perf, 21:1015-1027, 1995.

86. Kolb FP, Irwin KB, Bloedel JR, Bracha V. Conditioned and unconditioned forelimb reflex systems in the cat: involvement of the intermediate cerebellum. Exp Brain Res 114:255-270, 1997.

87. Kolb FP, Lachauer S, Maschke M, Timmann D. Classical conditioning of postural reflexes. Eur J Physiol 445:224-237, 2002.

88. Kolb FP, Lachauer S, Maschke M, Timmann D. Classically conditioned postural reflex in cerebellar patients. Exp Brain Res 158:163-179, 2004.

89. Kosorok MR, Omenn GS, Diehr P, Koepsell TD, Patrick DL. Restricted activity days among older adults. Am J Public Health 82:1263-1267, 1992.

90. Küng UM, Horlings CGC, Honegger F, Allum JHJ. Incorporating voluntary unilateral knee flexion into balance corrections elicited by multi-directional perturbations to stance. Neuroscience 163:466-481, 2009.

91. Küng UM, Horlings CGC, Honegger F, Allum JHJ. The effect of voluntary lateral trunk bending on balance recovery following multi-directional stance perturbations. Exp Brain Res 202:851-865, 2010.

92. Kutas M, Donchin E. Preparation to respond as manifested by movement-related brain potentials. Brain Res 202:95-115, 1980.

93. Labyt E, Szurhaj W, Bourriez JL, Cassim F, Defebvre L, Destée A, Derambure P. Influence of aging on cortical activity associated with a visuo-motor task. Neurobiol Ageing 25:817-827, 2004.

94. Landis C, Hunt WA, Strauss H. The startle pattern. New York: Farrar & Rinehart, 1939.

95. Lavond DG, Kim JJ, Thompson RF. Mammalian brain substrates of aversive classical conditioning. Ann Rev Psych 44:317-342, 1993.

96. Lee DN, Lishman JR. Visual proprioceptive control of stance. J Hum Mov Sci 2:87-95, 1975.

97. Lestienne F, Soechting J, Berthoz A. Postural readjustments induced by linear motions of visual scenes. Exp Brain Res 28:363-384, 1977.

98. Leuthold H, Sommer W, Ulrich R. Partial advance information and response preparation: Inferences from the lateralized readiness potential. J Exp Psych: Gen 125:307-323, 1996.

99. Logan GD, Cowan WB, Davis KA. On the ability to inhibit simple and choice reaction time responses: A model and a method. J Exp Psych 10:276-291, 1984.

100. Mackey DC, Robinovitch SN. Mechanisms underlying age-related differences in ability to recover balance with the ankle strategy Gait Pos 23:59–68, 2006.

101. MacKinnon CD, Bissig D, Chiusano J, Miller E, Rudnick L, Jager C, Zhang Y, Mille ML, Rogers MW. Preparation of anticipatory postural adjustments prior to stepping. J Neurophysiol 97:4368-4379, 2007.

102. MacPherson JM, Everaert DG, Stapley PJ, Ting LH. Bilateral vestibular loss in cats leads to active destabilization of balance during pitch and roll rotations of the support-surface. J Neurophysiol 97:4357-4367, 2007.

103. MacPherson JM, Fung J. Weight support and balance during perturbed stance in the chronic spinal cat. J Neurophysiol 82:3088-3081, 1999.

104. Magnusson M, Enbom H, Johansson R, Pyykkö I. Significance of pressor input from the human feet in anterior-posterior postural control. Acta Otolaryngol (Stockh) 110:182-188, 1990.

105. Maki BE, Edmonstone MA, McIlroy WE. Age related differences in laterally-directed compensatory stepping behaviour. J Gerontol A Biol Sci Med Sci 55:M270-M277, 2000.

106. Maki BE, Holliday PJ, Fernie GR. Aging and postural control. A comparison of spontaneous- and induced-sway balance tests. J Am Geriatr Soc 38:1-9, 1990.

107. Maki BE, McIlroy WE. Cognitive demands and control of human balance-recovery reactions. J Neural Trans 114:1279-1296, 2007.

108. Manchester D, Woollacott MJ, Zederbauer-Hylton N, Marin O. Visual, vestibular and somatosensory contributions to balance control in the older adult. J Gerontol 44:M118-127, 1989.

109. Maschke M, Drepper J, Kinsvater J, Kolb FP, Diener HC, Timmann D. Fear conditioned potentiation of the acoustic blink reflex in patients with cerebellar lesions. J Neurol Neurosurg Psychi 68:358-364, 2000.

110. Maslovat D. Motor preparation changes with practice. circle.ubc.ca, 2010.

111. Maslovat D, Carlsen AN, Chua R, Franks IM. Response preparation changes during practice of an asynchronous bimanual movement. Exp Brain Res 195:383-392, 2009.

112. Maslovat D, Carlsen AN, Ishimoto R, Chua R, Franks IM. Response preparation changes following practice of an asymmetrical bimanual movement. Exp Brain Res 190:239-249, 2008.

113. Maslovat D, Hodges NJ, Chua R, Franks IM. Motor preparation and the effects of practice: Evidence from startle. Behav Neurosci 125:226-240 2011.

114. Maslovat D, Kennedy PM, Forgaard CJ, Chua R, Franks IM. The effects of pre-pulse inhibition on the startle reflex and reaction time. Neurosci Lett 513:243-247, 2012.

115. Massion J. Movement, posture and equilibrium: interaction and coordination. Prog Neurobiol 38:35-56, 1999.

116. McChesney JW, Sveistrup H, Woollacott MH. Influence of auditory precuing on automatic postural responses. Exp Brain Res 108:315-320, 1996.

117. McGarry T, Franks IM. A horse race between independent processes: Evidence for a phantom point of no return in the preparation of a speeded motor response. J Exp Psych 23:1533-1542, 1997.

118. McVea DA, Pearson KG. Long-lasting, context-dependent modification of stepping in the cat after repeated stumbling-corrective responses. J Neurophysiol 97:659-669, 2007.

119. Mittelstaedt H. Somatic graviception. Bio Psych 42:53-74, 1996.

120. Mochizuki G, Boe S, Marlin A, McIlroy WE. Perturbation-evoked cortical activity reflects both the context and consequence of postural instability. Neurosci 170:599-609, 2010.

121. Mochizuki G, Sibley KM, Esposito JG, Camilleri JM, McIlroy WE. Cortical responses associated with the preparation and reaction to full-body perturbations to upright stability. Clin Neurophysiol 119:1626-1637, 2008.

122. Müller MLTM, Jennings JR, Redfern MS, Furman JM. Effect of preparation on dual-task performance in postural control. J Mot Behav 36:137-146, 2004.

123. Müller MLTM, Redfern MS, Jennings JR. Postural prioritization defines the interaction between a reaction time task and postural perturbations. Exp Brain Res 183:447-456, 2007.

124. Nakata H, Yabe K. Automatic postural response systems in individuals with congenital total blindness. Gait Posture 14:36-43, 2001.

125. Nashner LM. Adapting reflexes controlling the human posture. Exp Brain Res 26:59–72, 1976.

126. Nashner LM. Fixed patterns of rapid postural responses among leg muscles during stance. Exp Brain Res 30:12-24, 1977.

127. Nashner LM. Analysis of movement control in man using the movable platform. Adv Neurol 39:607-619, 1983.

128. Nashner LM, Berthoz A. Visual contribution to rapid motor responses during postural control. Brain Res 2:403-407, 1978.

129. Nashner LM, Black FO, Wall C 3rd. Adaptation to altered support with visual conditions during stance: patients with vestibular deficits. J Neurosci 2:536-544, 1982.

130. Nashner LM, Cordo PJ. Relation of automatic postural responses and reaction-time voluntary movements of human leg muscles. Exp Brain Res 43:395-405, 1981.

131. Nickerson RS. Intersensory facilitation of reaction time: energy summation or preparation enhancement? Psychol Rev 80:489-509, 1973.

132. Nieuwenhuijzen PHJA, Schillings AM, van Galen GP, Duysens J. Modulation of the startle response during human gait. J Neurophysiol 84:65-74, 2000.

133. Oude Nijhuis LB, Allum JHJ, Borm GF, Honegger F, Overeem S, Bloem BR. Directional sensitivity of "First Trial" reactions in human postural control. J Neurophysiol 101:2802-2814, 2009.

134. Oude Nijhuis LB, Allum JHJ, Valls-Solé J, Overeem S, Bloem BR. First trial postural reactions to unexpected balance disturbances: A comparison with the acoustic startle reaction. J Neurophysiol 104:2704-2712, 2010.

135. Oude Nijhuis LB, Janssen L, Bloem BR, van Dijk JG, Gielen SC, Borm GF, Overeem S. Choice reaction time for human head rotations are shortened by startling acoustic stimuli, irrespective of stimulus direction. J Physiol 584:91-109, 2007.

136. Perry SD, McIlroy WE, Maki BE. The role of plantar cutaneous mechanoreceptors in the control of compensatory stepping reactions evoked by unpredictable, multi-directional perturbation. Brain Res 877:401-406, 2000.

137. Queralt A, Valls-Solé J, Castellote JM. The effects of a startle on the sit-to-stand manoeuvre. Exp Brain Res 185:603-609, 2008a.

138. Queralt A, Weerdesteyn V, van Duijnhoven HJR, Castellote JM, Valls-Solé J, Duysens J. The effects of an auditory startle on obstacle avoidance during walking. J Physiol 586:4453-4463, 2008b.

139. Quant S, Adkin AL, Staines WR, McIlroy WE. Cortical activation following a balance disturbance. Exp Brain Res 155:393-400, 2004.

140. Ravichandran VJ, Shemmell JB, Perreault EJ. Mechanical perturbations applied during impending movement evoke startle-like responses. Conf Proc IEEE Eng Med Biol Soc 2947-2950, 2009.

141. Redfern MS, Jennings JR, Martin C, Furman JM. Attention influences sensory integration for postural control in older adults. Gait Posture 14:211-216, 2001.

142. Redfern MS, Müller MLTM, Jennings JR, Furman JM. Attentional dynamics in postural control during perturbations in young and older adults. J Gerontol 57:B298-B303, 2002.

143. Reynolds B. The acquisition of a trace conditioned response as a function of the magnitude of the stimulus trace. J Exp Psych 35:15-30, 1945.

144. Rosenbaum DA. Human movement initiation: Specification of arm, direction, and extent. J Exp Psych: Gen 109:444-474, 1980.

145. Rossignol S. Startle responses recorded in the leg of man. Electroenceph Clin Neurophysiol 39:389-397, 1975.

146. Runge CF, Shupert CL, Horak FB, Zajac FE. Role of vestibular information in initiation of rapid postural responses. Exp Brain Res 122:403-412, 1998.

147. Schneiderman N, Gormezano I. Conditioning of the nictitating membrane of the rabbit as a function of CS-US interval. J Compar Physiol Psych 57:188-195, 1964.

148. Schwabe A, Drepper J, Maschke M, Diener HC, Timmann D. The role of the human cerebellum on short- and long-term habituation of postural responses. Gait Posture 19:16-23, 2004.

149. Schieppati M, Nardone A. Medium-latency stretch reflexes of foot and leg muscles analysed by cooling the lower limb in standing humans. J Physiol 503:691-698, 1997.

150. Schlich P. Risk table for discrimination tests. Food Qual Pref 4:141-151, 1993.

151. Schmidt RA, Lee TD. Motor control and learning: a behavioral emphasis (5^{th} ed.) Human Kinetics, Champagne (IL), 2011.

152. Sibley KM, Mochizuki G, Frank JS, McIlroy WE. The relationship between physiological arousal and cortical autonomic responses to postural instability. Exp Brain Res 203:533-540, 2010.

153. Siegmund GP, Blouin JS, Inglis JT. Does startle explain the exaggerated first response to a transient perturbation. Exerc Sport Sci Rev 36:76-82, 2008.

154. Siegmund GP, Inglis JT, Sanderson DJ. Startle response of human neck muscles sculpted by readiness to perform ballistic head movements. J Physiol 535:289-300, 2001.

155. Smith MC, Coleman SR, Gormezano I. Classical conditioning of the rabbit's nictitating membrane response at backward, simultaneous, and forward CS-US intervals. J Compar Physiol Psych 69:226-231, 1969.

156. Sundermier L, Woollacott MH. The influence of vision on the automatic postural muscle response of newly standing and newly walking infants. Exp Brain Res 120:537-540, 1998.

157. Stapley PJ, Drew T. The pontomedullary reticular formation contributes to the compensatory postural responses observed following removal of the support-surface in the standing cat. J Neurophysiol 101:1334-1350, 2009.

158. Stevens JA, Corso PS, Finkelstein EA, Miller TR. The costs of fatal and non-fatal falls among older adults. Injury Prevention. 12:290-5, 2006.

159. Sundermier L, Woollacott M. The influence of vision on automatic postural responses of newly standing and newly walking infants. Exp Brain Res 4:537-540, 1998.

160. Taub E, Bacon RC, Berman AJ. Acquisition of a trace-conditioned avoidance response after deafferentation of the responding limb. J Compar Physiol Psychol 59:275-279, 1965.

161. Taube W, Schubert M, Gruber M, Beck S, Faist M, Gollhofer A. Direct corticospinal pathways contribute to neuromuscular control of perturbed stance. J Appl Physiol 101:420-429, 2006.

162. Thompson RF. Neural mechanisms of classical conditioning in mammals. Phil Trans R Soc Lond B329:161-170, 1990.

163. Tinetti ME, Williams CS. Falls, injuries due to falls, and the risk of admission to a nursing home. New Eng J Med 337:1279-1284.

164. Ting LH, McKay JL. Neuromechanics of muscle synergies for posture and movement. Curr Opin Neurobiol 17:622-628, 2007.

165. Tokuno CD, Carpenter MG, Thorstensson A, Cresswell AG. The influence of natural body sway on neuromuscular responses to an unpredictable surface translation. Exp Brain Res 174:19-28, 2006.

166. Türker KS. Electromyography: some methodological problems and issues. Physical Therapy 73:698-710, 1993.

167. Valls-Solé J, Kofler M, Kumru H, Castellote JM, Sanegre MT. Startle-induced reaction time shortening is not modified by pre-pulse inhibition. Exp Brain Res 165:541-548, 2005.

168. Valls-Solé J, Rothwell JC, Goulart F, Cossu G, Munoz E. Patterned ballistic movements triggered by a startle in healthy humans. J Physiol 516:931-938, 1999.

169. Wadman WJ, Denier van der GON JJ, Geuze RH, Mol CR. Control of fast goal-directed arm movements. J Hum Mov Stud 5:3-17, 1979.

170. Winter DA. Human balance and posture during standing and walking. Gait Pos 3:193-214, 1995.

171. Wolpaw JR. The complex structure of a simply memory. Trends Neurosci 20:588-594, 1997.

172. Woodruff-Pak DS, Disterhoft JF. Where is the trace in trace conditioning? Trends Neurosci 31:105-112, 2008.

173. Woodruff-Pak DS, Jaeger ME, Gorman C, Wesnes KA. Relationships among age, conditioned stimulus–unconditioned stimulus interval, and neuropsychological test performance. Neuropsychol 13:90-102, 1999.

174. Woollacott M, Shumway-Cook A. Attention and the control of posture and gait: a review of an emerging area of research. Gait Posture 16:1-14, 2002.

175. Yardley L. Fear of falling: links between imbalance and anxiety. Rev Clin Gerontol 13:195-201, 2004.

176. Yeomans JS, Frankland PW. The acoustic startle reflex: neurons and connections. Brain Res Rev 21:301-314, 1996.

177. Zehr PR, Kido A. Neural control of rhythmic, cyclical human arm movement: task dependency, nerve specificity and phase modulation of cutaneous reflexes. J Physiol 537:1033-1045, 2001.

178. Zijlstra GA, Van Haastregt JC, Van Rossum E, Van Eijk JT, Yardley L, Kempen GI. Interventions to reduce fear of falling in community-living older people: a systematic review. J Am Geri Soc 55:603-15, 2006.