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RESEARCH ARTICLE

Effects of chronic ankle instability on cutaneous reflex modulation during walking

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Abstract

Chronic ankle instability (CAI) is characterized by persistent giving way at the ankle following an acute lateral ankle sprain and is associated with an early onset of osteoarthritis. Researchers have reported that the cutaneous afferent pathway from certain leg muscles is modified in people with CAI while in a seated position. However, we do not know if these reflex modulations persist during functional activities. The purpose of this study was to further explore sensorimotor function in patients with CAI by analyzing cutaneous reflex modulation during gait. CAI ($n=11$) and uninjured control ($n=11$) subjects walked on a treadmill at 4 km/h and received non-noxious sural nerve stimulations at eight different time points during the gait cycle. Net electromyographic responses from four lower leg muscles were quantified 80–120 ms after stimulation for each phase of the gait cycle and compared between groups. We found that cutaneous reflex responses between groups were largely similar from the late stance to late swing phases, but uninjured control subjects, and not CAI subjects, experienced significant suppression in the medial gastrocnemius and lateral gastrocnemius muscles during the early stance phase of the gait cycle. Our results indicate that people with CAI lack a protective unloading response in the triceps surae following high-intensity sural nerve stimulation during the early stance phase of the gait cycle. Evaluating cutaneous reflex modulations may help to identify neural alterations in the reflex pathways that contribute to functional deficits in those with CAI.

Keywords Cutaneous reflexes · Gait · Chronic ankle instability · Ankle sprain

Introduction

Approximately 30–40% of people who sustain an ankle sprain develop chronic ankle instability (CAI) (Itay et al. 1982; Soboroff et al. 1984). Patients with CAI report frequent "giving way" episodes in the ankle joint (Delahunt et al. 2010; Hertel 2002) reduced physical activity (Simon and Docherty 2014; Hubbard-Turner 2015) diminished physical function (Caffrey et al. 2009) recurrent ankle sprains (Doherty et al. 2014; Swenson et al. 2009) and an early onset of osteoarthritis (Golditz et al. 2014). While the exact cause of CAI is still unknown, this condition has frequently been defined as having a multi-factorial etiology. In other words, CAI develops due to a combination of mechanical and functional deficits that continue to persist following an acute ankle sprain (Docherty and Arnold 2008; Munn et al. 2003, 2010).

Recently, researchers have utilized reflex assessments to help investigate why subjects with CAI experience deficits in sensorimotor control. The majority of this research has focused on measuring the Ia-mediated reflex pathway (Donahue et al. 2014; Palmieri-Smith et al. 2009). Ia sensory afferents (originating from muscle spindles) communicate directly with alpha-motoneurons to produce the fastest reflex response in the parent muscle. Consequently, the body relies on this sensorimotor system to quickly correct sudden mechanical perturbations (Duysens and Levin

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2010). However, research has also found that following trap door perturbations, healthy adults show facilitatory reflex responses at a variety of latencies other than the Ia-mediated pathway (Grüneberg et al. 2003; Hopkins et al. 2007). These results seem to indicate that polysynaptic pathways, such as cutaneous afferents, may also play an important role in adjusting muscular activity following a sudden change in tactile environments.

Previous studies have been conducted to investigate sensory thresholds of cutaneous nerves in subjects with CAI. These results found that CAI subjects have an elevated cutaneous sensory threshold on the plantar surface of their feet (Hoch et al. 2012) and that it takes a greater amount of vibration for both the uninvolved and involved limbs to reach threshold compared to people with no history of ankle sprains (Bullock-Saxton 1994). Given this evidence for deficient afferent input in subjects with CAI, the idea that these signals would impact the ability of these individuals to modulate reflexive plasticity during gait seems plausible. Additionally, the results from previous ankle taping and bracing research provide further justification that altered cutaneous input likely contributes to ankle instability. Applying athletic tape or a semi-rigid brace to the skin significantly shortens the reaction time in peroneal muscles (Karlsson and Andreasson 1992) and increases peroneal H-reflex amplitude by approximately 10% (Nishikawa and Grabiner 1999). The authors attribute these results to the stimulation of skin receptors and a subsequent increase in afferent input to the central nervous system, although empirical data for this assumption are lacking. Despite previous studies focusing on cutaneous nerve integrity, there is a severe lack of research pertaining to cutaneous reflex function in subjects with CAI, especially during functional tasks.

In 2013, Futatsubashi et al. (2013) found that applying a non-noxious stimulus to the sural nerve (innervates the lateral aspect of the foot) caused a more pronounced suppressive EMG response in the peroneus longus and vastus lateralis muscles in the involved limb of subjects with unilateral CAI. This same group later reported that the suppressive cutaneous reflexes in the peroneus longus can be seen immediately after an ankle sprain and that this reflex modulation begins to recover over the course of rehabilitation (Futatsubashi et al. 2016).

The results from the Futatsubashi et al. (2013, 2016) studies suggest that measuring cutaneous reflex responses post-ankle sprain may help to identify subjects at risk for developing these chronic deficits. However, these data were collected while subjects performed muscle contractions in a seated position so it is unclear how these findings would change if collected during functional weight-bearing tasks. For example, neurologically intact adults modulate cutaneous reflexes according to the phase of the gait cycle and the intensity of the perturbation (Zehr et al. 1997). This modulation is likely accomplished by means of a central motor program that opens and closes polysynaptic pathways in the spinal cord in accordance with the task (Duysens et al. 1990). The sural nerve in particular elicits specific muscle responses depending on whether the nerve is stimulated during the stance phase or in the middle of the swing phase during the gait cycle (Zehr et al. 1998b). Since CAI subjects show suppressed cutaneous reflexes following sural stimulation while in a seated position, these patients may also have difficulty modulating cutaneous reflexes while performing functional tasks. Therefore, the purpose of this study was to determine whether subjects with CAI maintain an effective mechanism to modulate cutaneous reflexes while walking on a treadmill. Comparing cutaneous reflexes of adults with no history of ankle sprains to those with CAI will help to identify the lingering sensorimotor deficits that may contribute to the development of subjective symptoms and future, long-term sequelae.

Methods

Subjects

A total of 22 subjects (8 male, 14 female) were recruited to participate in this study. All subjects were physically active, which was defined as participating in at least 120 min of exercise at a moderate intensity each week. Subjects were excluded if they had a history of displaced fractures or surgeries in the lower extremity, had suffered an acute lower extremity injury in the past 6 weeks, or if they had been diagnosed with a neuromuscular condition such as multiple sclerosis or Parkinson's disease. Subjects were further separated into two different groups based on ankle sprain history: (1) unilateral CAI ($n=11$; 4 male, 7 female, 21.0 ± 3.6 years, 168.9 ± 9.8 cm, 67.4 ± 15.7 kg) and (2) uninjured control ($n=11$; 4 male, 7 female, 21.2 ± 1.5 years, 167.7 ± 9.7 cm, 67.1 ± 12.2 kg). Subjects were included in the control group if they had never sprained their ankle and scored a zero (0) on the Identification of Functional Ankle Instability questionnaire (IdFAI) for both limbs. The IdFAI is a self-reported questionnaire developed in 2012 to help identify individuals with ankle instability and symptoms of "giving way"(Simon et al. 2012). This questionnaire has been endorsed by the International Ankle Consortium as a valid ankle instability-specific questionnaire(Gribble et al. 2013) and has an overall accuracy rating of 89.6%.(Simon et al. 2012) An exploratory factor analysis identified that a cut-off score of 10.3 on this questionnaire identifies people who have the minimally accepted criteria for CAI (Simon et al. 2012). Therefore, for the present study, subjects were included into the unilateral CAI group only if one limb had a history of ankle sprains and the subjects scored an 11 or greater on the IdFAI, while

the contralateral limb had no history of ankle sprains and scored a zero on the IdFAI. The average IdFAI score for the involved limb of the CAI group was 21.8 ± 2.4 and the average number of ankle sprains was three. Before participating in the study, all subjects read and signed an informed consent form approved by the University's Institutional Review Board for the Protection of Human Subjects.

Experimental protocol

All qualified subjects reported to the research laboratory for a one-time data collection session. First, subjects completed the IdFAI and a brief health history questionnaire to identify inclusion/exclusion criteria and to assign group membership. Second, the subject's test limb was identified. The leg chosen for evaluation depended on the group. For CAI subjects, the test limb was the side identified as having CAI. However, the test limbs chosen for control subjects were matched to the CAI group according to limb dominance to ensure that the same number of dominant and non-dominant limbs was tested in each group. Limb dominance was determined by asking each subject which leg they would choose to kick a soccer ball. All of the CAI subjects were right limb dominant and three of the control subjects were left limb dominant. Seven of the 11 CAI subjects' involved limb was on the dominant side. Therefore, four subjects in each group ($n = 8$) were tested on the non-dominant limb.

Subjects were then instructed to walk continuously on a Series 2000 treadmill (Marquette Electronics, Milwaukee, WI) at a speed of 4.0 km per hour (km/h). This speed is consistent with previous cutaneous reflex studies (Baken et al. 2005, 2006; Zehr et al. 1998b) and allowed all subjects to complete the walking trial without fatigue or adopting observable gait abnormalities. Each subject walked for approximately 35 min. The first 5 min were used as a warm-up and to achieve a consistent gait cycle duration. For the remaining 30 min of the experiment, the sural nerve of the test limb was stimulated at different times throughout the gait cycle eliciting an ipsilateral reflex response measurable by offline electromyographic (EMG) analysis.

Electromyographic recordings

EMG signals were recorded continuously for the anterior tibialis (TA), peroneus longus (PL), medial gastrocnemius (MG), and lateral gastrocnemius (LG) using disposable Ag/AgCL surface electrodes with an inter-electrode distance of approximately 2 cm. Prior to electrode placement, the skin was shaved (if applicable), and lightly abraded using sterile gauze soaked in soapy water. Alcohol wipes were not used to prevent drying the skin and subsequently increasing impedance. The center of each muscle belly was identified by asking the patient to flex isometrically against resistance. The electrodes were then placed in the center of the muscle belly, parallel to the muscle fibers, in bipolar fashion. A ground electrode was placed over the tibial tuberosity. The electrode leads were firmly taped to the skin using loops to minimize unnecessary movement artifact. All EMG data were recorded using a Biopac MP150 (Biopac Systems, Inc., Goleta, CA) recording system with EMG100c electromyogram amplifiers. EMG amplifier gain was set to 1000 Hz with 10-Hz high-pass and 500-Hz low-pass filters. Sampling rate was set to 4000 Hz.

Electrical stimulation

A reusable, stimulating bar electrode (Ambu, Inc., Columbia, MD) with two, 10-mm stainless steel disks positioned 30 mm apart was taped to the outside of the ankle over the sural nerve as it runs posterior to the lateral malleolus. A DS7A constant current stimulator (Digitimer North America, LLC, Ft. Lauderdale, FL) and a Grass S88X stimulator (Natus Neurology, Middleton, WI) were used to elicit stimulation trains of 5 pulses at 200 Hz, with a pulse width of 1.0 ms.

Once the stimulating electrode was secured, subjects were exposed to a number of nerve stimulations while in a standing position to obtain the appropriate intensity level used for the remainder of the study. First, the perceptual threshold (PT) was identified by slowly increasing the amplitude from zero until the subject reported feeling any sensation around the foot and ankle. The PT amplitude was confirmed by increasing and decreasing the amplitude until a consistent value was obtained. Then, the stimulation amplitude was increased further causing the area of sensation to increase down the outside of the foot and up the lower leg. At some point, the amplitude reached a radiating threshold (RT) whereby subjects reported no change in sensation area and only an increase in stimulation intensity. The intensity was adjusted up and down from this point to confirm the RT amplitude. The final testing amplitude for sural nerve stimulation was obtained by adjusting the intensity either up or down until the subject reported feeling the strongest stimulation possible that was not painful and did not produce a withdrawal reflex. This procedure resulted in a stimulus intensity approximately 2.5 times RT for all subjects. Once the subject began to walk, they were asked routinely throughout data collection if the stimulations felt consistent. Two subjects (1 CAI and 1 Control) reported that the stimulation intensity became uncomfortable as they began to walk, which required a slight adjustment until the intensity was no longer painful. Table 1 shows the mean stimulation amplitudes of each group.

A custom-made insole equipped with heel and toe force sense resistors (Pololu Corp., Las Vegas, NV) was placed into the subject's shoe prior to walking on the treadmill. The

Table 1 Mean ± SD stimulation amplitudes for each group.

Group	Perceptual threshold (PT) (mA)	Radiating threshold (RT) (mA)	Final testing amplitude (mA)
Control	0.48 ± 0.25	3.55 ± 0.45	8.65 ± 0.95
CAI	0.60 ± 0.18	3.48 ± 0.55	8.29 ± 1.34

Final testing amplitude was approximately 2.5 times radiating threshold for all subjects

purpose of this insole was twofold. First, the force resistors helped to discern separate gait cycles. One gait cycle was defined as the time interval between subsequent heel strikes of the test limb. Second, the insole helped with timing the stimulation so that the sural nerve was stimulated at eight equal time points throughout the gait cycle. To determine these time points, the last 20 warm-up gait cycles were used to calculate an average gait cycle duration. The average gait cycle was further divided into eight equal time phases starting at zero, which were used as latency parameters for all subsequent nerve stimulations. The insole communicated with the Grass stimulator via a microcontroller board (Arduino Uno R3, Arduino, LLC, IT) programmed with custom written software. Depending on which of the eight phases the stimulation was to occur, the researcher set the appropriate latency on the Grass stimulator and pressed a toggle when the test limb was in swing phase. When the test limb made heel contact at the start of the next gait cycle, the stimulator was activated and the sural nerve was stimulated in the specific phase corresponding to the designated latency.

The sural nerve was stimulated randomly once every three to seven steps, and no more than one stimulus during each step cycle. Subjects were instructed to verbalize "yes" whenever they felt a stimulation to ensure that all trials were successful. The order of stimulation latency was randomized for all subjects, and the walking trial continued until ten stimulations were elicited in each of the eight phases.

Kinematic data

Ankle range of motion of the test limb was continuously monitored using a twin-axis electrogoniometer (SG110/A, Biometrics, Ltd, UK) secured to the medial aspect of the ankle with hook-and-loop fasteners and cohesive tape. The proximal end of the goniometer was aligned with the medial shaft of the tibia, while the distal base of the goniometer was secured below the medial subtalar joint at the level of the calcaneus. This position allowed for the twin-axis goniometer to measure changes in sagittal (plantar flexion–dorsiflexion) and frontal (eversion and inversion) plane kinematics. The goniometer data were digitized in real time using a Biopac UIM100C system (Biopac Systems, Inc., Goleta, CA) and sent to a computer for off-line analysis.

Data processing

Data processing was accomplished using AcqKnowledge 4.1 computer software (Biopac Systems, Inc., Goleta, CA). The raw EMG signal for each muscle was band pass filtered using a low-frequency cut-off of 50 Hz and a high-frequency cut-off of 500 Hz. The smoothed signals were then used to derive a root mean square EMG for each muscle. The stimulated gait cycles were labeled according to phase number and excluded if the stimulation timing was off by more than 1 ms, which was caused by pressing the stimulation button after the subject's heel had already touched the ground. This timing error was rare, and each stimulation phase had between eight to ten gait cycles for final data analysis. The computer software was then used to flag unstimulated gait cycles that were at least one full cycle away from any stimulation gait cycles. Using this selection criterion, between 250 and 300 unstimulated cycles were obtained for each subject and used as control trials for final reflex analysis.

EMG data were used to calculate the magnitude of the middle latency reflex (MLR; 80–120 ms post-stimulation) for each muscle at each phase, as the MLR has been shown to fluctuate depending on the phase of the gait cycle (Zehr et al. 1997). Any facilitation or inhibition of muscle activity following sural nerve stimulation was determined by comparing the stimulated MLR activity to the unstimulated gait cycles at the same time point of the gait cycle. The stimulated and unstimulated gait cycles were time locked with respect to the start of the gait cycle (according to the heel sensor data) and the stimulation latency. The mean EMG activity 80–120 ms after the first pulse of electrical stimulation was extracted for all gait cycles and averaged together. The mean EMG activity of the unstimulated gait cycles was subtracted from the corresponding values obtained from the eight phases of the stimulated step cycles, with a negative number indicating muscle inhibition and a positive number indicating facilitation. To enable accurate comparisons between subjects, the final EMG values were normalized as percentages of the peak EMG amplitude (averaged over a 20-ms time window) occurring across the subjects entire gait cycle.

Kinematic data were low-pass filtered at 100 Hz and compared between stimulated and unstimulated gait cycles to determine if significant EMG activity resulted in altered foot mechanics in either the frontal or sagittal planes of motion. Given the electromechanical delay between EMG activity and muscle force production, maximum kinematic data points were extracted from a time window of 140–220 ms after the first pulse of electrical stimulation. Similar to the EMG analysis, stimulated and unstimulated gait cycles were aligned with respect to the stimulation timing and the average kinematic data from the unstimulated trials were subtracted from the corresponding stimulated trials. The final

data were normalized as a percent of the maximum range of motion occurring throughout the entire step cycle for each subject.

Statistics

Statistical analyses were first completed using the normalized EMG data from the eight phases of the unstimulated gait cycles. Four separate two-way mixed factor ANOVA's (one for each muscle) with one between-subject factor (group at two levels: CAI and Control) and one within-subject factor (phase number at eight levels) were performed to determine whether average EMG activity throughout the unstimulated gait cycles differed between groups. Then, we used one-sample *t*-tests to compare each group's mean difference values to zero for both the EMG and kinematic data. Any values statistically different from zero would, therefore, indicate a significant net reflex effect following sural nerve stimulation. This statistical analysis does not directly compare reflex amplitudes between the two groups for each phase of the gait cycle. Rather, the analysis generates a reflex modulation pattern for each group and identifies points in the gait cycle where significant reflexes occur or do not occur. Additionally, previous studies measuring sural nerve reflexes in neurologically intact adults have generated similar reflex modulation patterns using one-sample *t* tests (Zehr et al. 1998b; Duysens et al. 1992). Using the same statistical procedures allowed us to compare our control group's modulation pattern to these studies. All statistical analyses were conducted using IBM SPSS Version 24 and significance was set a priori at $p \leq 0.05$.

Results

Unstimulated EMG analyses

Figure 1 shows group EMG activity for all four muscles at each phase of the unstimulated gait cycle. Before conducting any inferential analyses, the EMG activity data from the unstimulated gait cycles were assessed for normality and the presence of outliers. Observation of box plots found no extreme outliers, and the EMG activity for each muscle and group was found to be normally distributed by visually inspecting histograms and Q–Q plots. Levene's test of homogeneity of variances revealed the variance within each population was equal ($p > 0.05$), and Box's test for equality of covariance matrices found that the homogeneity of within-group covariance matrices was not violated.

For all four ANOVAs, the main effect of phase showed a statistically significant difference in mean EMG activity between different points of the gait cycle ($p < 0.05$), but the main effect for group showed that there was no difference in EMG activity between the control and CAI subjects ($p > 0.05$). Finally, there was no statistically significant interaction between the stage of the gait cycle (phase) and group on EMG activity for the TA ($F_{(2.470,\ 49.397)} = 0.323$, $p = 0.770$, partial $\eta^2 = 0.016$, $\varepsilon = 0.353$), PL ($F_{(3.987,\ 79.750)} = 1.824$, $p = 0.133$, partial $\eta^2 = 0.084$, $\varepsilon = 0.570$), LG ($F_{(2.345,\ 46.893)} = 1.82$, $p = 0.168$, partial $\eta^2 = 0.083$, $\varepsilon = 0.335$), or MG ($F_{(2.95,\ 59.007)} = 1.428$, $p = 0.244$, partial $\eta^2 = 0.067$, $\varepsilon = 0.421$).

Net reflex analyses: EMG

Figure 2 provides subtracted reflex traces from one healthy control subject. Figure 3 shows group reflex amplitudes for each muscle throughout the gait cycle and indicates the specific phases in which significant reflex responses from zero were observed. Both groups produced similar overall responses for the TA and PL muscles following sural nerve stimulation. For the TA, no significant reflex responses were observed during the early part of the stance phase. However, stimulation of the sural nerve within phases 5, 6, and 7 resulted in a large facilitation (≈10–30%) of TA activity. At phase 8, both groups demonstrated a reversal of TA activity resulting in an average TA suppression of − 21.5% for the CAI group (95% CI [− 31.3, − 11.7]) and − 21.0% for the control group (95% CI [− 31.7, − 10.3]). For the PL, significant facilitation was observed throughout the entire gait cycle with the exception of phase 8, in which no significant reflex response was identified.

During the early stages of the stance phase (phases 2–4), control subjects showed large suppressive responses in the MG and LG muscles following sural nerve stimulation. In contrast, subjects in the CAI group demonstrated a delayed suppressive response in these same muscles. More specifically, CAI subjects showed no significant suppression in muscle activity until phase 4 for the LG muscle (− 21.0%, 95% CI [− 30.4, − 11.5]) and phase 3 for the MG muscle (− 13.0%, 95% CI [− 24.0, − 2.0]). The control subjects produced a consistent reflex response in the LG when the sural nerve was stimulated at phase 3, with an average suppression of − 18.6% and a 95% CI [− 26.3, − 10.9]. However, the CAI group did not exhibit a significant reflex response in the LG at this stage of the gait cycle (− 3.6%, 95% CI [− 14.9, 7.75]). To further illustrate group reflex responses of the LG at the third phase of the gait cycle, Fig. 4 shows a reflex trace from one control subject and one CAI subject. Both groups showed a reversal of reflex activity in the LG and MG muscles from phase 5 to phase 6, as nerve stimulation during this part of the gait cycle resulted in a significant facilitation of MG and LG activity. No significant responses in the MG or LG were observed during the late swing phase for both groups.

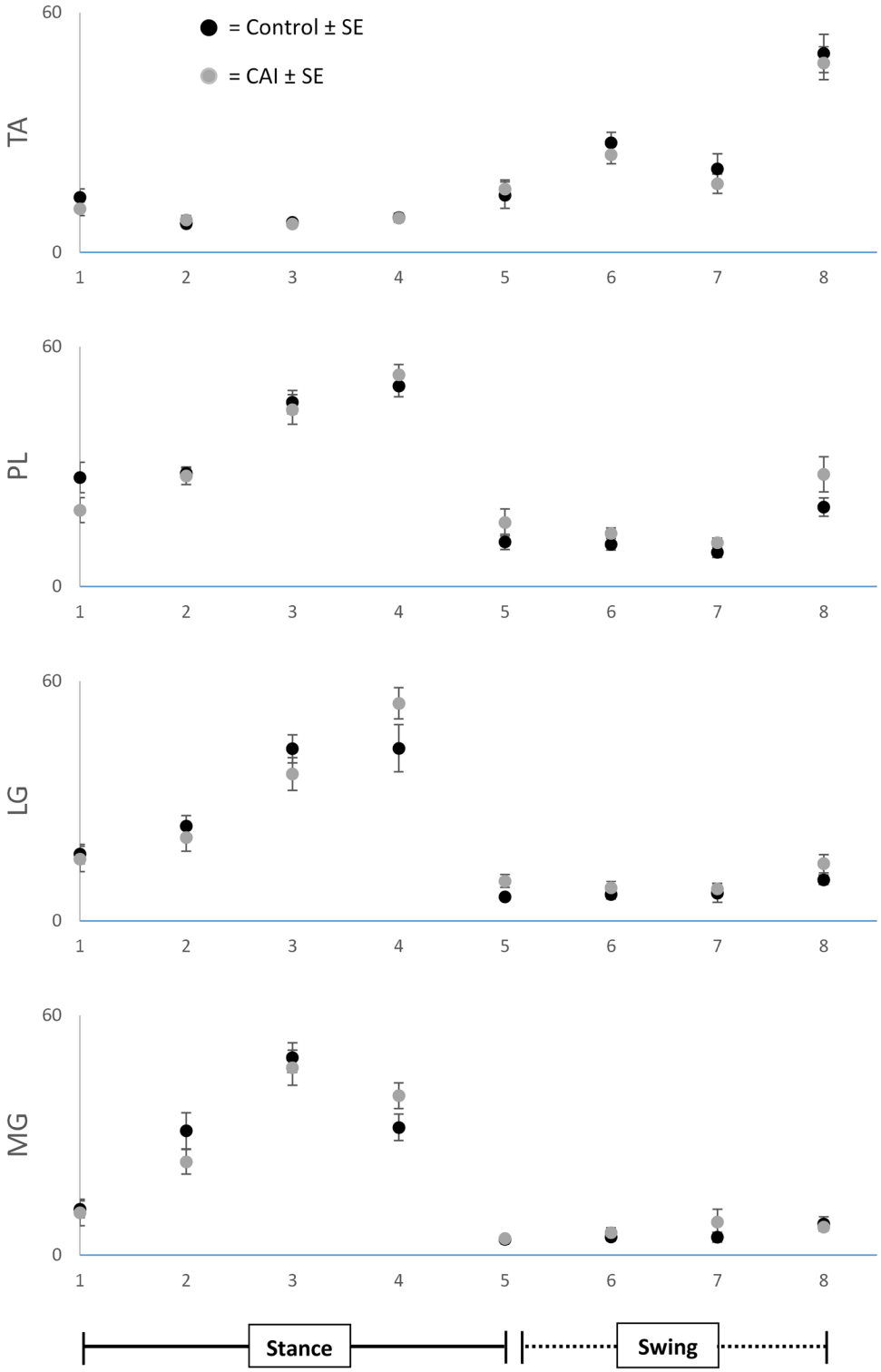

Fig. 1 Average muscle activation patterns at each stage of the gait cycle observed during unstimulated gait cycles. All values are normalized to peak muscle activation recorded throughout each subject's step cycle. Error bars indicated standard error of the mean. The solid and dashed lines located below the figure show how the different phases were typically divided with respect to test limb's position. Phases 1–5 were located predominantly in the stance phase, phases 7 and 8 were located in the swing phase, and phase 6 represented the transition period between toe off and swing phase

Net reflex analyses: kinematics

In the sagittal plane, both groups experienced a significant increase in dorsiflexion at phases 4, 5, 6, and 7 as a result of sural nerve stimulation (Fig. 5). However, there was no change in frontal plane ankle kinematics throughout the entire gait cycle following electrical stimulation for both groups.

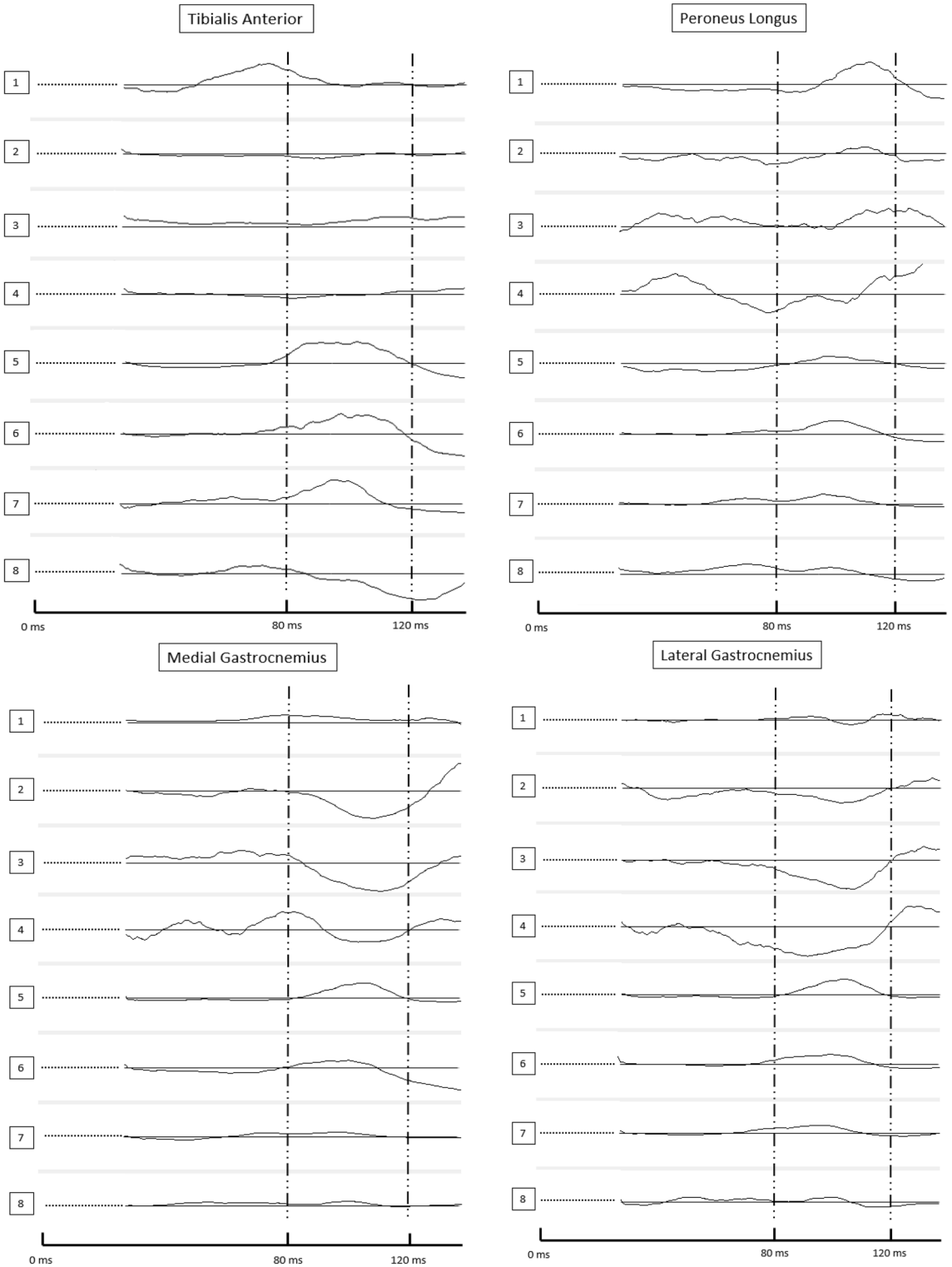

Fig. 2 Subtracted reflex traces obtained from one control subject for all four muscles. Each interval of the gait cycle is aligned by point of stimulation and labeled accordingly on the *y*-axis. The subsequent signal noise from each stimulation was replaced with a horizontal dotted line. The two vertical bars in each graph represent the MLR period in which net reflex responses were obtained (80–120 ms)

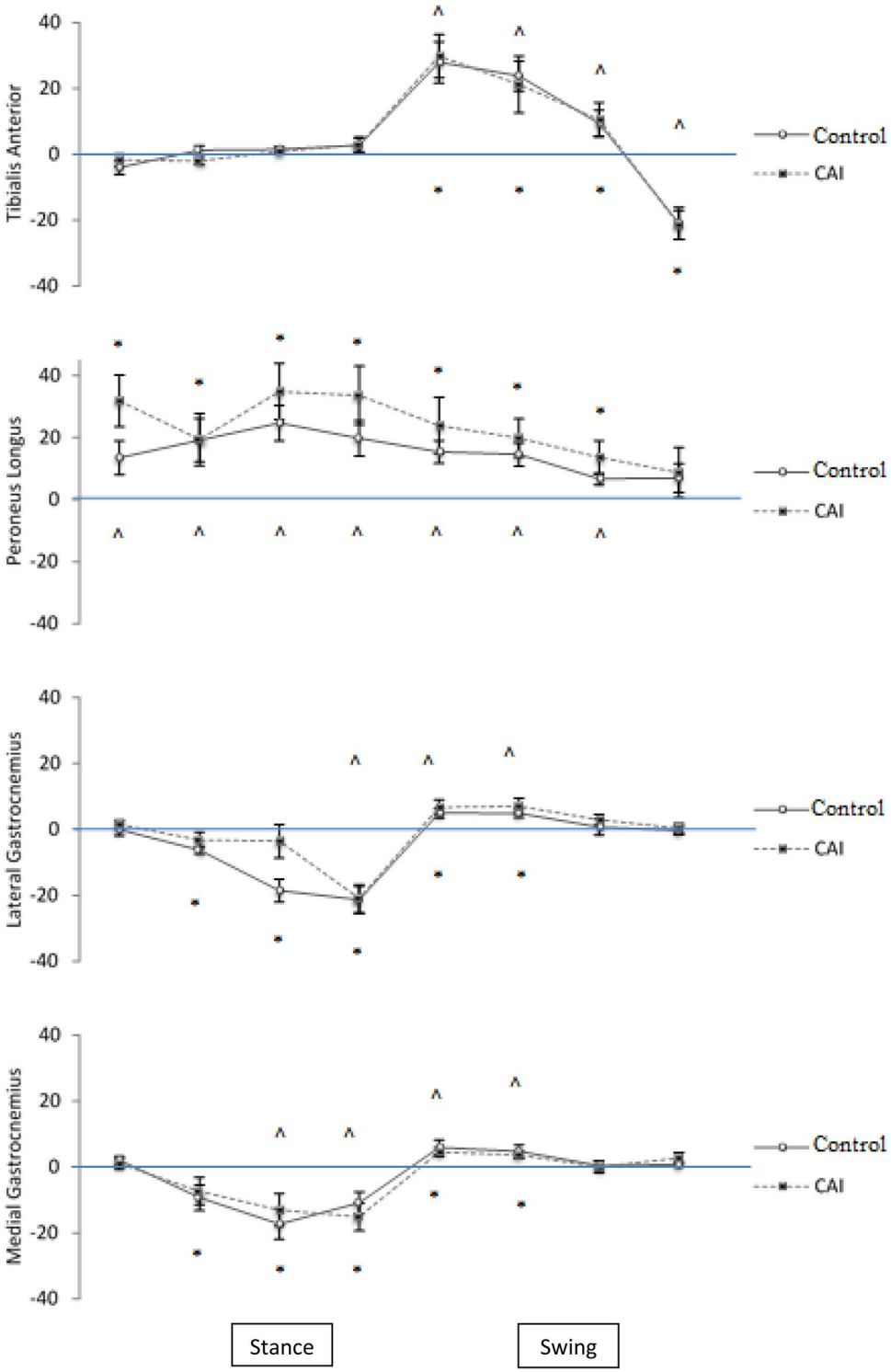

Fig. 3 Modulation of net cutaneous reflex responses between 80 and 120 ms post-stimulation for both groups. Error bars represent standard error of the mean. Significant differences from zero at $p < 0.05$ are labeled with * for control subjects and ^ for CAI subjects

Discussion

Many research studies have been conducted over the past 30 years to further our understanding of cutaneous reflexes and their specific function during human gait. From this work, there is a general consensus that cutaneous reflexes in healthy, neurologically intact adults can vary considerably in amplitude throughout the gait cycle and may also reverse from facilitation to inhibition at separate time points, a reflex response often referred to as phase-dependent modulation (Baken et al. 2005; Duysens et al. 1990). The functional purpose of cutaneous modulation remains

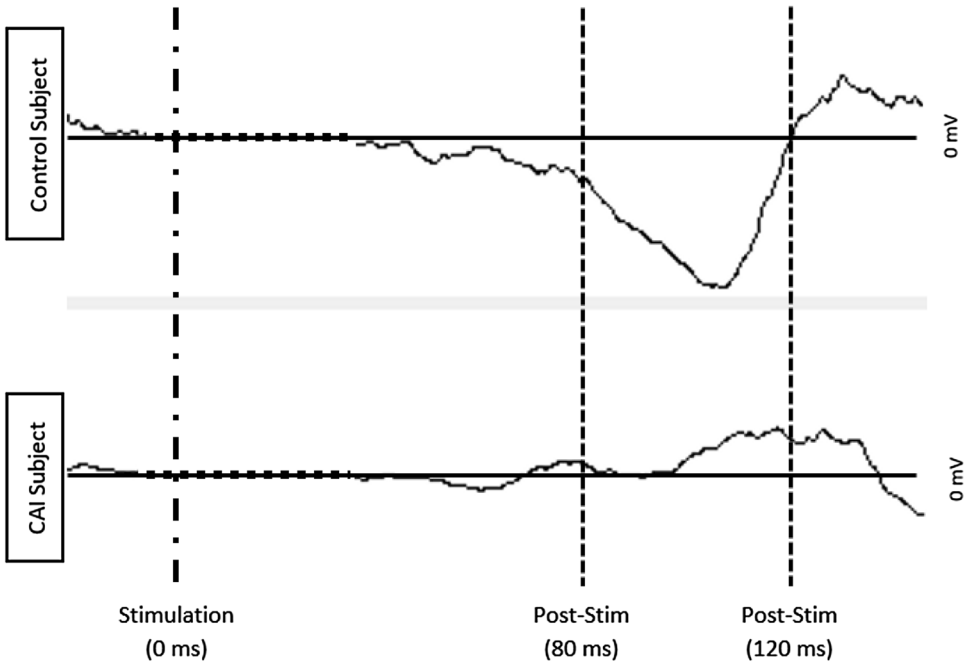

Fig. 4 Subtracted reflex traces for the lateral gastrocnemius muscle at the third phase of the gate cycle for one control subject and one CAI subject. The traces are aligned by point of stimulation and extend from 20 ms prior to the stimulation to 150 ms after the stimulation. Stimulation artifact was removed and replaced by horizontal dotted lines. The vertical dash bars at 80 and 120 ms post-stimulation represent the MLR period in which net reflex responses were obtained

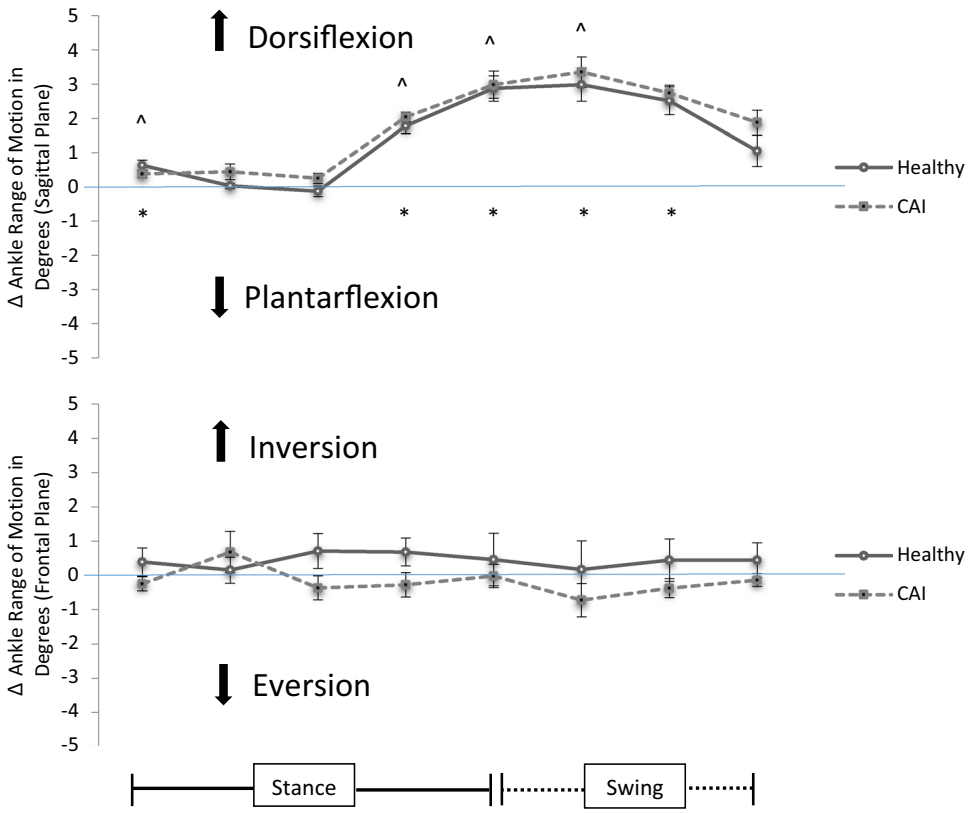

Fig. 5 Net kinematic changes 140–220 ms post-stimulation. Error bars represent standard error of the mean. Significant differences from zero at $p < 0.05$ are labeled with * for control subjects and ^ for CAI subjects

speculative, but research suggests that parallel inhibitory and excitatory pathways, which relay sensory information from cutaneous afferents to alpha-motoneurons, open and close by means of a central pattern generator for locomotion (Duysens et al. 1992; Zehr and Stein 1999). The body relies on this neural mechanism to help alter reflex responses and prevent stumbling no matter where in the gait cycle the foot experiences tactile perturbations. Therefore, observing cutaneous reflexes throughout the gait cycle in patients with chronic ankle instability will help further our understanding

of how repetitive injury to the ankle complex may alter neural organization within the spinal cord and minimize future compensatory reflex responses.

In terms of EMG patterns during gait, we observed that both groups demonstrated similar EMG amplitudes across the entire gait cycle for all four muscles. This is an important finding as a muscle's reflex pattern will fluctuate depending on its level of activation at the time of cutaneous stimulation (Buford and Smith 1990; Duysens et al. 1996). Since the main purpose of this study was to assess the underlying mechanisms that control phase-dependent cutaneous modulation, we can safely infer that any variations in reflex profiles from one experimental group to the other are the result of improper gaiting of polysynaptic reflex responses rather than differences in the levels of muscle activation.

Although our group EMG amplitudes were statistically similar, there is one aspect of the EMG profiles that is worth discussing. Figure 1 shows that the CAI group is trending toward a higher PL activation immediately prior to heel contact (phase 8). Although the PL amplitude at this phase was not statistically different between groups, the trend is consistent with previous studies conducted by Feger et al. (2015) and Delahunt et al. (2006) who reported that subjects with CAI exhibit an early activation of the PL immediately before heel contact. This early PL activation directly effects the dynamic stability of the ankle because the muscle spindles within the PL are more relaxed and in a suboptimal position to detect quick changes in muscle length when an inversion perturbation is experienced at heel strike. While this finding does not directly affect the interpretation of our cutaneous reflex profiles, we believe it helps substantiate previous research in this field.

The general reflex responses observed in this present study appear to coincide with results published in earlier work. That is, we found phase-dependent modulation of discrete net muscle activity occurring within a latency of 80–120 ms post-stimulation (Baken et al. 2006; Zehr and Stein 1999). For clarification purposes, the reflex responses observed in both experimental groups will be discussed in three separate sections relating to the stage of the gait cycle in which the sural nerve was stimulated.

Late stance phase/early swing phase

Given that the cutaneous receptive field of the sural nerve extends to portions of the lateral sole of the foot, stimulating the nerve as the test limb transitions from stance phase to swing phase likely mimics prolonged foot contact with the ground. Previous research found that cutaneous reflexes at this stage of the gait cycle function in withdraw reactions to help maintain balance and cadence (Zehr and Stein 1999). More specifically, stimulating the sural nerve at toe off produces significant facilitation of the TA which assists the foot to dorsiflex up and away from unwanted ground contact (Duysens et al. 1990; Zehr et al. 1998b). Our results coincide with these previous observations. During the late stance phase, both groups experienced a large facilitation response ($\approx$ 30%) in the ipsilateral TA muscle. While this TA response was found to be most prominent at phase 5, the facilitation remained statistically significant through phases 6 and 7 until the test limb reached the end of the swing phase, suggesting a prolonged influence on the TA motor pool. Further analysis of the ankle kinematic data shows that both groups experienced a significant increase in ankle dorsiflexion during the late stance and early swing phase of the gait cycle, suggesting a behavioral outcome as a result of this TA facilitation.

While our results show some similarities with previous literature, there are some notable discrepancies. First, the stimulation intensity we used to evoke TA facilitation was considered high (2.5 $\times$ RT), but yet we saw responses more typical of medium stimulation intensities. For example, earlier studies observed TA facilitation using a medium-intensity level ($\approx$1.9 $\times$ RT), while a higher intensity ($\approx$2.3 $\times$ RT) caused only small, insignificant net effects in that muscle (Duysens et al. 1992; Zehr et al. 1998b). Only one previous study reported late stance phase TA facilitation following high-intensity (2.8 $\times$ RT) sural nerve stimulation (Duysens et al. 1990). The reason for this discrepancy is unknown. One possibility is that ankle movement during the swing phase caused the skin mounted stimulating electrode to shift further away from the nerve compared to when the foot was planted. We attempted to combat this potential issue using sufficient tape to secure the electrode in place and by routinely asking the subjects if the stimulus felt similar throughout the gait cycle.

Second, our results show that stimulating the sural nerve at the late stance phase (phases 5 and 6) also evoked a small, but statistically significant facilitation of the LG and MG muscles in both groups that was not evident during the swing phase. This finding appears to be novel when compared with previous literature. One study reported a facilitatory response in the MG during the late stance phase, but this response was only elicited using a medium-intensity stimulation (Zehr et al. 1998b). Additionally, the simultaneous MG and TA facilitation was highly correlated with an increase in ankle eversion and dorsiflexion (Zehr et al. 1998b). Our results found no changes in sagittal plane ankle kinematics throughout the entire gait cycle for both groups. Perhaps the additional facilitation of the LG observed in our subject pool worked in concert with the MG to stabilize the subtalar joint and guide the withdrawn foot straight into dorsiflexion.

Late swing phase

As was the case throughout the early swing phase, both groups produced similar reflex patterns during the late swing phase. Stimulating the sural nerve before the heel contacted the ground caused a phase-dependent reflex reversal. In other words, the TA response went from significant facilitation in the early- and mid-swing phases to significant suppression in the late swing phase, which again is consistent with previous research (Duysens et al. 1990, 1992; Tax et al. 1995; Van Wezel et al. 1997; Yang and Stein 1990). While some studies have shown an increase in plantar flexion following this late TA suppression (Duysens et al. 1992) our kinematic data show no difference in ankle movement compared to unstimulated gait cycles. However, it is worth noting that phase 8 is the only stage of the swing phase in which a significant increase in dorsiflexion compared to unstimulated cycles was not observed 140–220 ms following stimulation. These data suggest that, instead of a withdrawal reflex, stimulating the sural nerve as the limb begins to transition from swing to stance produces a type of placing reflex that enables the foot to move toward the ground and prepare for weight distribution (Zehr and Stein 1999). This reflex response is only possible by closing TA facilitatory pathways and opening TA suppressive pathways, and our results suggest that subjects with chronic ankle instability appear to mediate this process via central pattern generators.

Early/mid stance phase

Throughout the early- and mid-stance phases, both groups showed a lack of net reflex activity in the ankle flexors, which is a normal response according to previous research (Tax et al. 1995; Van Wezel et al. 1997; Zehr et al. 1998b). Perhaps, the most notable variation in phase-dependent reflex patterns was that the control subjects showed a significant net suppression in the MG and LG at phases 2, 3, and 4 while CAI subjects showed no significant reflex effect in the LG and MG until phase 4 and phase 3, respectively. According to previous literature, suppressive EMG activity in the MG and LG is a normal reflex response following high-intensity stimulations of the sural nerve during the stance phase of gait, and can also be observed in the MG after stimulation of the superficial peroneal nerve (Zehr et al. 1997, 1998a). Some studies have reported a correlation between this suppressive ankle extensor reflex and a reduction in plantar flexion during mid stance (Zehr et al. 1998b) but our results failed to identify changes in ankle joint kinematics.

The purpose of this suppressive reflex response in the ipsilateral triceps surae during the stance phase is likely to reduce plantar pressure in the stance foot should weight need to be transferred to the contralateral limb in the event of a stumble (Haridas and Zehr 2003). Our results indicate that subjects with CAI produce this protective reflex response much later in the stance phase compared with the control subjects, suggesting that persons with CAI have difficulty modulating cutaneous reflexes within the spinal cord across the entire gait cycle. Additionally, 5 of the 11 CAI subjects actually showed facilitation in the LG and MG during this stance phase, which is a response more typical following low stimulation of the sural nerve. Low-intensity stimulations produce neural signals reflective of walking on uneven ground. The reflex response in this instance activates muscles on both sides of the ankle to stabilize the entire joint instead of withdrawing or unloading to the contralateral limb to avoid an injurious obstacle (Zehr et al. 1998b). Perhaps, injury to the ankle affects the central mechanism responsible for alternating between these two different reflex outputs when appropriate.

Cutaneous reflex response of the peroneus longus

Another result that must be discussed is that both groups showed significant facilitation in the PL across the entire gait cycle with the exception of the late swing phase. This result is surprising for two reasons. First, one previous study using healthy subjects found that cutaneous reflexes during the stance phase induced a predominantly suppressive response in the PL (Baken et al. 2006). However, this study used a significantly lower stimulation intensity, which may account for the differences in the present study. Aniss et al. (1992) is the only other group to measure PL activity following stimulation of the sural nerve in a healthy, neurologically intact population. They found that healthy adults exhibit pronounced facilitation in PL, but the subjects were stimulated while in a standing, stationary position and not during the functional tasks of locomotion (Aniss et al. 1992).

Second, we found no evidence of an increased suppression in the PL among people with CAI as suggested by previous studies (Futatsubashi et al. 2013, 2016). Our results provide evidence that the neural mechanisms that may appear deficient while CAI subjects are in a seated or standing position do not persist while walking. Other studies utilizing H-reflex techniques have found that subjects with CAI experience decreased alpha-motoneuron pool excitability, or arthrogenic muscle inhibition, in ankle stabilizing muscles such as the peroneus longus and soleus (McVey et al. 2005; Palmieri-Smith et al. 2009). However, this decreased alpha-motoneuron pool excitability has only been reported while CAI subjects are in a non-functional position and does not directly correlate with functional deficits while walking (Palmieri-Smith et al. 2009).

Limitations and future research

The first limitation of our study was the decision to use an electrogoniometer to measure ankle kinematics. Although this instrument has been deemed reliable (Bronner et al. 2010), the ideal placement of the electrogoniometer is on the lateral side of the ankle. For the present study, we had to secure the electrogoniometer to the medial side of the ankle due to the location of our stimulating electrode, which may have prevented the coil from detecting precise changes in subtalar joint kinematics between the unstimulated and stimulated gait cycles. However, no net movement of the ankle joint in the sagittal plane following reflexive EMG patterns does not necessarily mean our electrogoniometer was insufficient at capturing frontal plane kinematics. Instead, the suppressive and facilitatory muscle responses in the lower leg may reflect balance strategies of the trunk or more proximal limbs such as the knee or hip (Tax et al. 1995). Regardless, future research should make three important alterations to the study design to further investigate group differences in frontal plane kinematics following sural nerve stimulation. First, utilize camera-based motion analysis systems. Second, increase the walking speed, as previous research has identified differences in frontal plane kinematics between healthy and CAI subjects as the gait cycle approaches a jogging speed (Chinn et al. 2013). Third, increase the sample size to improve statistical power when comparing frontal plane kinematics.

The second limitation of our study was that we only measured muscle activity in the lower leg and did not include proximal muscles such as the biceps femoris (BF) and vastus lateralis (VL). Previous research of healthy, neurologically intact adults has found that sural nerve stimulation causes facilitatory responses in the BF and VL during the stance phase, which increases knee stiffness and prevents undesired movement up the kinetic chain to reduce the chances of stumbling following a tactile stimulation (Zehr et al. 1998b). We believe future CAI studies would benefit from measuring cutaneous reflex activity in the BF and VL to establish a more complete understanding of the lower limb modulation patterns following sural nerve stimulation. Finally, our study investigated how subjects with CAI respond to sural nerve stimulations at a high intensity. We recommend future research investigate how subjects with CAI modulate reflex outputs as the stimulation intensity decreases to explore the threshold sensitivity of responses in this cohort.

Conclusion

The results of this study indicate that people with CAI have a unique phase-dependent modulation pattern of cutaneous evoked responses during walking. The lack of a protective unloading response in the gastrocnemius during the early stance phase may lead to chronic insufficiencies during gait, and may be useful in identifying those individuals predisposed to CAI. For those patients with CAI, this activation pattern may be useful as a biomarker for recovery from injury.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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