Restoring Sensation After Lower-Limb Amputations to Improve Balance and Gait

by

Bailey Anne Petersen

BS, The Pennsylvania State University, 2013

MS, The Pennsylvania State University, 2015

DPT, University of Pittsburgh, 2018

Submitted to the Graduate Faculty of the

Swanson School of Engineering in partial fulfillment

of the requirements for the degree of

Doctor of Philosophy

University of Pittsburgh

2022

UNIVERSITY OF PITTSBURGH

SWANSON SCHOOL OF ENGINEERING

This dissertation was presented

by

Bailey Petersen

It was defended on

August 26, 2022

and approved by

Patrick J. Sparto, Ph. D, PT, Professor, Department of Physical Therapy, School of Health and Rehabilitation Sciences

April J. Chambers, Ph. D, Associate Professor, Department of Health and Human Development, School of Education

Roberta Klatzky, Ph. D, Professor, Department of Psychology, Carnegie Mellon University

Marco Capogrosso, Ph. D, Assistant Professor, Department of Neurological Surgery, School of Medicine

Dissertation Director: Lee E. Fisher, Ph. D, Associate Professor, Department of Physical Medicine and Rehabilitation, School of Medicine

Restoring Sensation After Lower-Limb Amputations to Improve Balance and Gait

Bailey Petersen, PhD, DPT, MS

University of Pittsburgh, 2022

People with a lower-limb amputation rely heavily on vision for balance to compensate for the lack of sensory feedback from their missing limb. As sensory feedback from the foot activates neural pathways that maintain stability and promote healthy gait patterns, restoring this sensation is vital to improve function and reduce fall risk in people with an amputation. Sensory neuroprostheses stimulate the remaining afferent pathways, in either the periphery or the spinal cord, to evoke sensations in the missing limb in real-time during balance and gait. While studies using peripheral nerve stimulation have set the precedent for these devices, these approaches are not easily translatable to clinical practice. Regardless of the stimulation approach, determining the effectiveness of sensory neuroprostheses has remained a significant challenge. Because standard clinical outcome measures used to assess balance and gait in this population are intended to provide an overall picture of an individual's functional status, they fail to isolate more specific aspects of balance or gait, such as reliance on sensation. Additionally, more intuitive sensations over unnatural sensations have shown promise in providing additional functional benefits, however we lack reliable measures of intuitiveness or multi-sensory integration of stimuli to systematically evaluate these advancements.

In this dissertation, I address some key challenges and barriers to advancement of sensory neuroprosthetics. Here we will use a lumbosacral spinal cord stimulation, a clinically available stimulation method for sensory neuroprostheses, which will provide an additional pathway of clinical translation. Furthermore, we will evaluate the relationship between sensation and standard clinical measures, the effects of sensory neuroprostheses on both standard clinical

measures and more robust measures of balance and gait, and evaluate a measure of multi-sensory integration of sensory stimuli for future studies to evaluate the functional effects of different stimulus types.

Table of Contents

List of Tables

List of Figures

Preface

I would like to express my utmost gratitude to the many, many people who have supported me on my journey as both a researcher and a physical therapist. First and foremost, I would like to recognize my advisor, Dr. Lee Fisher. Few have made as profound an impact on my academic career as Lee and he is truly, as he would gladly tell anyone, a pretty big deal. Lee is an amazing mentor and I am so thankful for his unwavering support throughout the last six years. I also want to especially recognize Dr. Patrick Sparto, who is not only a member of my committee, but also my DPT advisor throughout the dual-degree program. I would not be where I am today without Dr. Sparto's support and continued guidance along the way.

I would also like to thank my other committee members who have all helped me tremendously in various aspects of this project. Dr. Chambers, I am incredibly grateful for your mentorship as a committee member and co-sponsor for my fellowship. Your advice on gait analysis and biomechanics was a huge help to our continued experiments. Dr. Klatzky, I have always come out of our meetings more inspired, which I attribute mostly to your creative problem solving and vast knowledge of cognitive psychology. Dr. Capogrosso, your advice on how to effectively communicate our research has been incredibly valuable and I will miss hearing your thoughts on why so many things are "not a thing."

I am so grateful to everyone at RNEL for always being a great support system and an open environment. Thanks to Stephanie Rigot, Rohit Bose, Maria Jantz and Julie Rekant for being great friends and always being up for going for a midday coffee, no matter how busy you were at the time. To Ameya Nanivadekar, Tyler Madonna, Devapratim Sarma, Santosh Chandrasekaran, and Ashley Dalrymple, thank you for guidance when I had no idea what I was

doing, for being there with a laugh when we all needed it, and for your incredible work ethic and patience as we dealt with the unavoidable setbacks in new experiments. I have learned so much from each of you. Thank you to our staff, including Debbie Harrington, Bree Bigelow, Casey Konopisos, Alayna Schwerer, Sam Ward, and Adelaide Edgett, without whom this work would not be possible.

A huge thank you to my friends and family, for keeping me sane for the last six years and for being the best support system I could ask for. Mom and Dad, thank you for the much needed weekly if not daily phone calls about plants, work, and life in general. Drew, thanks for being an amazing brother, for bringing research talks to our family dinners, and for inspiring my physical therapy career when I was back in undergraduate school. And mostly, a huge thank you to my amazing husband, Nick. Words cannot describe how much your love and support mean to me. Thank you for keeping me going through it all, I truly couldn't have done this without you.

1.0 Introduction

Individuals with a lower-limb amputation can experience a wide range of functional impairments and, critically, a substantially higher risk of falls than the average population. In fact, over 50% of community dwelling adults with lower-limb amputation reported at least one fall within the past year, nearly double the normal fall risk for able-bodied adults.[1,2] To alleviate the impairments caused by lack of muscular power to progress the prosthetic leg, motorized prosthetics have made significant advances that can aid in reducing energy consumption and functional impairments in some tasks, however these solutions address only the efferent activity lost with an amputation. Afferent activity, or sensory feedback from the foot, is critical for stability, facilitates gait phase transitions and can trigger a host of coordinated reflexive responses to perturbations to prevent falls.[3–5] Following amputation, the lack of sensation coming from the missing limb likely substantially contributes to these functional impairments and fall risk. Thus, restoring this sensory feedback from the missing foot has the potential to provide functional benefits to individuals with a lower-limb amputation. Sensory neuroprostheses stimulate the intact afferent fibers in the residual limb or spinal cord to evoke sensations in the missing limb. While significant advancements have been made in developing these devices, several barriers exist to successful clinical translation. Critically, we lack robust functional measures to evaluate the effectiveness of sensory neuroprostheses and the multi-sensory integration of this feedback, which may provide additional functional benefits. The purpose of this dissertation is to evaluate the functional effects of a sensory neuroprostheses in individuals with a lower-limb amputation. To that end, we will assess (1) the relationship between sensation and standard clinical measures of balance and gait in this population, (2) the functional effects of

restoring sensory feedback through spinal cord stimulation (SCS) on both standard clinical measures, as well as newer, more robust measures that may be more sensitive to differences in somatosensory function, and (3) validate a measure of multi-sensory integration for future studies to be able to evaluate the functional effects of different stimulus types.

1.1 Role of Sensation in Balance and Gait

Plantar sensation usually plays a critical role in balance and gait, as shown in both humans and animal models.[6] Individuals with amputation, however, lack this sensory feedback from the missing foot. Though biomechanical constraints of using a prosthetic leg can lead to functional impairments, the lack of sensation and neuromuscular control also play a role. A recent study demonstrated common gait deviations across groups with different levels of amputation and different types of prosthetics.[7] If biomechanical differences alone were the cause for functional impairments after an amputation, we would expect to see dramatically different gait patterns across these groups. Instead, the consistency of gait deficits across populations may indicate that biomechanical constraints are only partly responsible for observed impairments.[7]

In able-bodied individuals, somatosensory inputs play a vital role in maintaining balance. These inputs from the plantar aspect of the feet drive gait phase transitions, provide protective responses to prevent falls, and contribute to healthy biomechanical patterns and muscle activation.[3–5] Conversely, individuals with sensory loss exhibit many of these same functional impairments as those with lower-limb amputation, including a five-fold increase in fall risk.[8–10] In both individuals with neuropathy and able-bodied individuals with experimentally dulled plantar sensation, studies show a direct correlation between sensory integrity and more severe

balance and gait impairments.[11–13] Additionally, a more recent study found that older adults with sensory loss have higher prefrontal cortical activity during walking.[14] Higher prefrontal cortical activity during gait indicates these individuals need to focus on walking, which can lead to a greater risk of falls in more complex, attentionally-demanding functional tasks.[15] Furthermore, in a small, preliminary study of individuals with a lower-limb amputation, subjects with more severe sensory loss had worse balance performance.[16] Taken together, these findings indicate the critical functional role of sensation in both populations with intact limbs and with amputation.

1.2 Sensory Feedback to Improve Function

Given the critical role of sensation in able-bodied individuals, interventions that restore sensation to the prosthetic are likely to improve function. Sensory substitution methods, which provide feedback of a different modality or location during gait to compensate for the lacking sensation, has been widely used to improve task performance in individuals with an amputation. Vibratory feedback applied to the residual limb, as well as auditory and visual feedback, have all been found to improve function in simple balance or gait tasks.[17–20] Based on these functional improvements, restoring or even supplementing sensation may be beneficial in this population. However, the best way to provide this sensory feedback is still under debate. While sensory substitution has provided benefits in simple tasks, it may not be suitable for more challenging tasks, such as walking with reduced visual feedback or responding to postural perturbations. In these more complex tasks, in which falls are more likely, this less intuitive feedback has been shown to increase cognitive load required for the task.[21] An increased cognitive load and the learning required to effectively utilize sensory substitution may be a detriment in more

challenging environments and dynamic activities that require higher levels of motor control. Ideally, to effectively improve balance and gait, sensory feedback should be easily incorporated into one's neural schema to minimize the cognitive load during complex tasks. Additionally, many of these sensory substitution or biofeedback methods are intended for short-term training to improve function in a rehabilitation setting, not for long-term use.[22]

In an effort to provide feedback with greater functional benefits for long-term use, sensory restoration (stimulating the intact nervous system afferents to provide feedback of the same modality and location as the missing sensation) has recently gained popularity. Most of these studies have created neural interfaces at the peripheral nerve in the residual limb to stimulate the afferent fibers and evoke sensations that appear to emanate from the missing limb (Figure 1.1).[23–27] These sensations can be remarkably focal in different regions of the foot or even individual toes. Not surprisingly, these techniques have been able to improve function on challenging tasks when using stimulation to evoke real-time sensory feedback in those with lower-limb amputation.[23–27] Charkhkar et al. implanted epineural composite flat interface nerve electrodes (C-FINEs) on the remaining peripheral nerves of the residual limb in individuals with transtibial amputation.[28] They found improvements in balance on challenging balance conditions and improvements in foot placement on an ambulatory searching ladder task without visual feedback (Figure 1.1).[25,26] In addition, Shell et al. demonstrated that these participants, over a year after implantation, were able to make small postural adjustments in quiet standing balance in response to sensory stimulation.[27] Using a different type of electrodes, Valle et al. studied the use of transverse intrafasicular multichannel electrodes (TIMEs) implanted in the tibial nerve of the residual limb in individuals with a transfemoral amputation.[29] They encoded both plantar pressure and knee angle as stimulus parameters to provide real-time tactile and proprioceptive

feedback during gait. With feedback, participants improved in speed and balance confidence on overground walking without visual feedback and on a stair task. The same group found improvements with stimulation in walking speed and confidence on a challenging figure-of-eight task, as well as decreases in oxygen consumption during walking and reductions in phantom limb pain (PLP, Figure 1.2).[23]

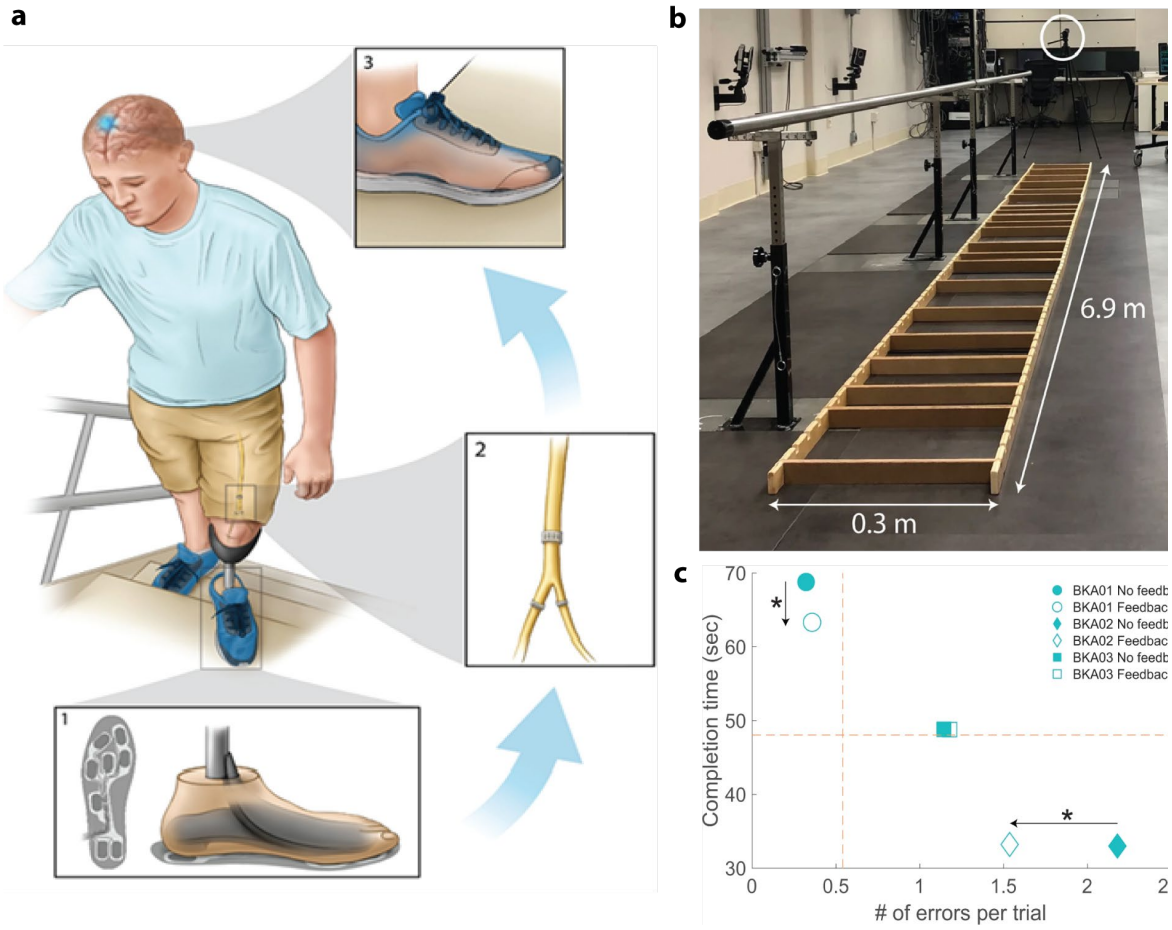


Figure 1.1 Use of peripheral nerve stimulation as real-time sensory feedback in a functional task.[28] (a) Epineural nerve cuff electrodes on the sciatic and/or tibial and peroneal nerves are stimulated to evoke sensations in the missing foot. Plantar pressure from a sensorized insole triggers stimulation of the percept localized to that region of the foot. (b) Subjects ambulate down the rungs of the ladder blindfolded with support from the handrail. The completion time and accuracy (c) are recorded for each trial. Two subjects demonstrated improvements either in completion time or in accuracy with sensory feedback. Adapted with permission from [a].

[a] Adapted from Scientific Reports, 10, Christie, B.P., Charkhkar, H., Shell, C.E. et al*, Ambulatory searching task reveals importance of somatosensation for lower-limb amputees*, 10216, Copyright (2020) under Creative Commons Attribution License.

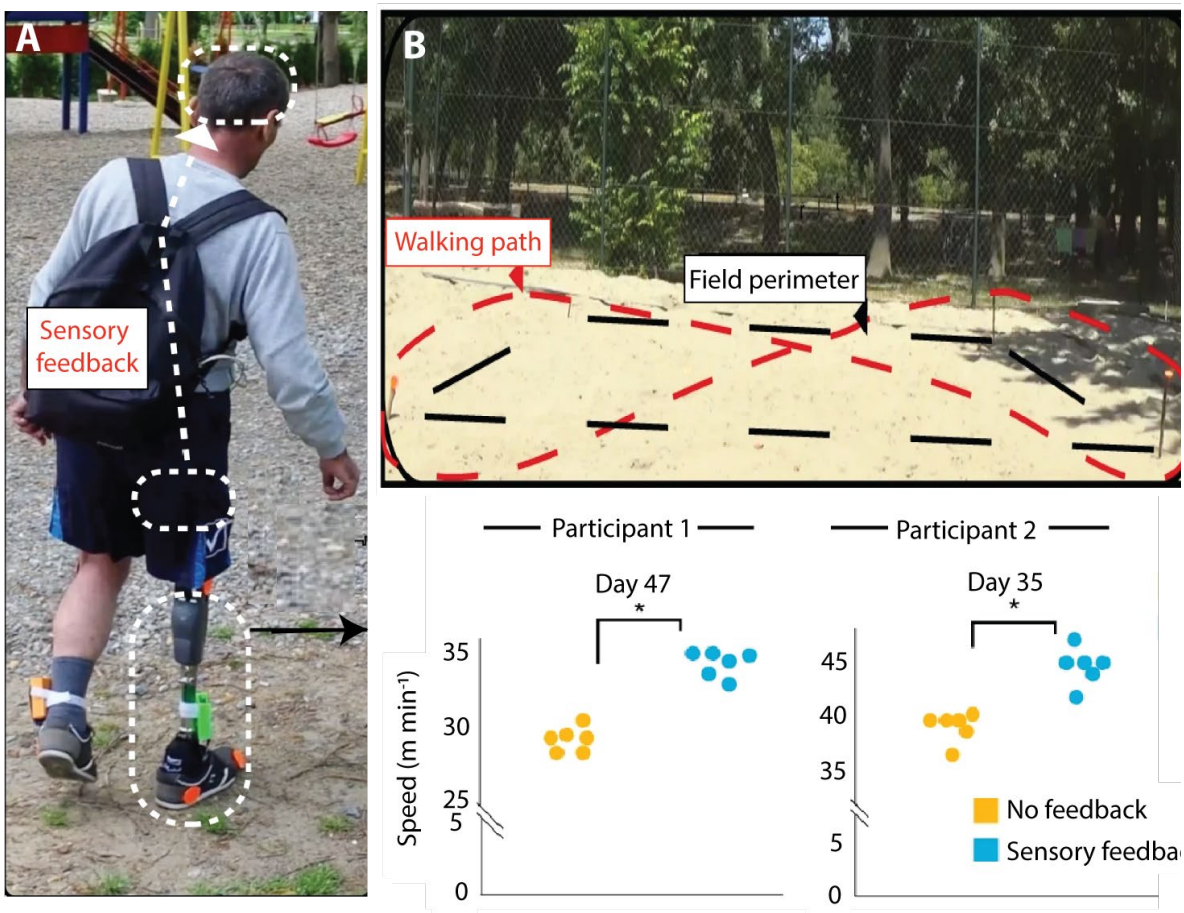


Figure 1.2. Sensory feedback in people with a transfemoral amputation during a walking task.[23] (A) Knee angle and plantar pressure are encoded and mapped to sensations evoked with stimulation. Stimulation is delievered via transverse intrafasicular multichannel electrodes (TIMEs) implanted in the tibial nerve of the residual limb. (B) The task is a timed figure-of-eight pattern on sandy terrain. Both subjects saw statistically singificant improvements in gait speed with sensory feedback. Adapted with permission from [b].

[b] Adapted by permission from Springer Nature: Nature Medicine (Sensory feedback restoration in leg amputees improves walking speed, metabolic cost and phantom pain, Petrini FM et al.) Copyright (2019)

1.3 Standard Measures of Balance and Gait for Evaluating Sensory Feedback

While sensory neuroprostheses have achieved promising demonstrations of functional improvements, finding outcome measures to demonstrate these effects has proven a challenging task. Selecting appropriate outcome measures to assess these changes is a key component of neuroprosthetic development and is vital to the success of these projects. However, the many complex physiological and neurological variables that contribute to balance and gait make detecting differences with sensory feedback extremely difficult. Furthermore, the traditional outcome measures that are used to clinically evaluate this population may lack the sensitivity to detect the functional changes that occur with the restoration of sensory feedback. For example, the current clinical standard for measuring reliance on sensory systems for balance is the Sensory Organization Test (SOT). Though the SOT has been validated clinically in individuals with lower-limb amputation, there is conflicting evidence on its sensitivity to differences in sensation across this population.[30–32] If these measures cannot detect even large differences in sensation, they will likely not be able to detect the more specific changes in function we see with the addition of sensory feedback.

Part of the difficulty in selecting outcome measures lies in the wide range in mobility levels across individuals with a lower-limb amputation. This population is extremely diverse, including individuals that need assistive devices to ambulate for even short distances and others that have additional prostheses they use for high-impact activities, such as running. As these sensory restoration studies are still in early stages of clinical research, most of the handful of participants included so far have been very active individuals with traumatic amputations. In these studies, the outcome measures had to be challenging enough to avoid the ceiling effects

that occur in many standard clinical tests. Because of this, labs studying these devices have developed new tasks for these studies to specifically evaluate somatosensory ability, including a ladder ambulation searching task without visual feedback (Figure 1.1) and a timed figure-of-eight task on sand (Figure 1.2). While these tasks detect changes with the addition of sensory feedback, they are too challenging to be completed by much of the population with a lower-limb amputation. As 44% of individuals with a lower-limb amputation use an assistive device to ambulate, those individuals would not be able to complete these challenging assessments without a device.[33] Thus, outcome measures used to quantify changes in function with sensory neuroprostheses should be able to both (1) accommodate the wide range of functional abilities and (2) detect a change in sensory ability across this population. To this end, we will determine the ability of standard clinical outcome measures to detect changes in sensation across a wide range of individuals with an amputation and determine the effects of sensory neuroprostheses using these standard measures.

1.4 More Robust Outcome Measures for Evaluating the Effects of Neuroprostheses

Newer tasks used in both clinics and gait laboratories may be able to better discern changes in somatosensory ability, while still accommodating the variability of baseline functional status in this population. These more robust measures include challenging tasks for the vast majority of individuals with an amputation: balance on the prosthetic limb, balance without vision, walking with a narrow base of support and walking on uneven surfaces. The Narrowing Beam Walking Test (NBWT) was developed as a more robust outcome measure for this population that avoids floor and ceiling effects.[34,35] Additionally, walking on an irregular surface

has been shown to detect differences across individuals with dysvascular and traumatic amputations (which are diagnoses often used as a proxy for somatosensory ability).[36] Though these tasks are difficult, studies have demonstrated that they can be performed by individuals across the spectrum of functional abilities and therefore have potential as outcome measures for neuroprosthetics studies.

1.5 Spinal Cord Stimulation to Restore Sensation

In lieu of peripheral nerve interfaces, studies in our lab have investigated the use of commercially available SCS to evoke similar sensations in the missing limb.[37] The use of pre-approved devices will facilitate and expedite the transition of these interventions into clinic practice. SCS is a common outpatient clinical procedure performed in over 50,000 people annually for lower-back and chronic pain syndromes.[38] Under fluoroscopic guidance, leads are percutaneously implanted into the dorsal epidural space in order to stimulate the dorsal roots that project to the lower-limbs.[38] Studies using cervical SCS in individuals after an upper-limb amputation have reliably evoked sensations in the missing hand and arm even years after amputation.[37] Furthermore, these evoked sensations have been used in a closed-loop task using a robotic hand to demonstrate functional improvements with SCS-evoked sensory feedback.[39] As SCS has proved to be a viable alternative for evoking sensations in missing limbs, we will be using SCS for sensory restoration purposes in this dissertation.

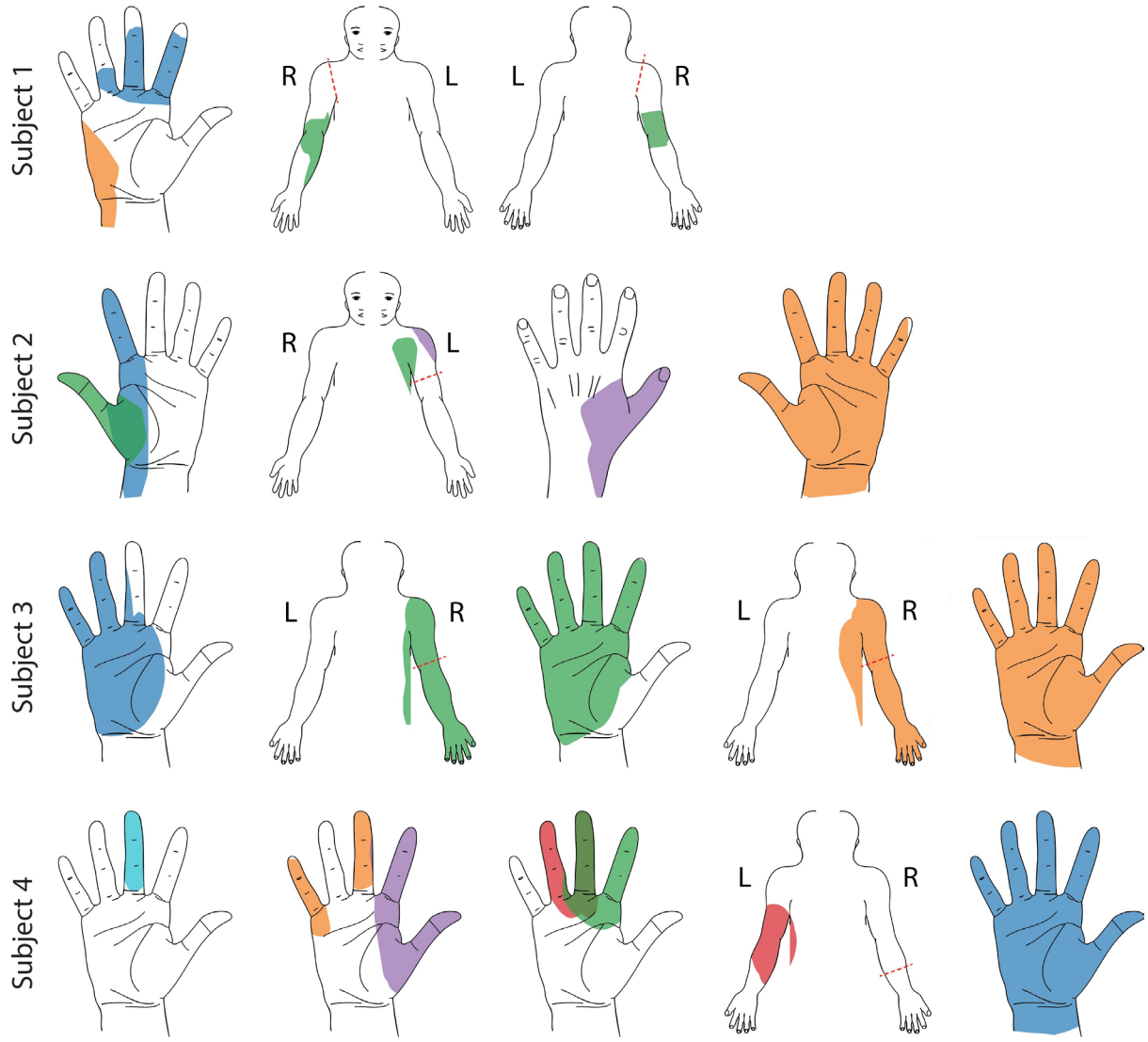


Figure 1.3. Cervical SCS reliably evokes sensations in the missing limb of four individuals with upper-limb amputation.[37] Colors represent an indiviudal sensations that were evoked for at least two weeks in the 29-day study. Reprinted with permission from [c].

[c] Reprinted from eLife, 9, Chandrasekaran S, Nanivadekar AC, McKernan G, Helm ER, Boninger ML, Collinger JL, Gaunt RA, Fisher LE, *Sensory restoration by epidural stimulation of the lateral spinal cord in upper-limb amputees,* e54349, Copyright (2020) with permission under the Creative Commons Attribution License.

1.6 Sensorimotor Integration of Evoked Sensations

In both spinal cord and peripheral nerve stimulation sensory restoration studies, most often stimulation has been applied at constant frequency. Stimulating at a constant frequency and amplitude recruits a host of afferent fibers simultaneously, which is not how the nervous system usually fires.[40] Perhaps unsurprisingly, these stimulation patterns often evoke tingling, vibratory or buzzing sensations.[37] However, the importance of intuitiveness of these evoked sensations for a functional task remains unclear. As our goal is to achieve the best possible functional benefits with sensory restoration, determining the extent to which the intuitiveness of the evoked sensation is critical. Biomimetic stimulation, or stimulation that more closely follows the natural firing patterns of afferent fibers, has been proposed as a solution to provide more intuitive sensations. To study these hypotheses, however, we currently rely on subjective ratings of naturalness, which are unreliable across subjects and even within subjects across sessions. We do not yet have a reliable scientific measure of intuitiveness, or how well these percepts integrate into our neural schema, to properly determine how stimulus patterns affect function.

In perceptual and cognitive neuroscience experiments, the cross-modal congruency task has long been used to quantify multi-sensory integration of stimuli.[41–47] Generally, these studies focus on the interaction of tactile and visual stimuli and, in more recent years, the cross-modal congruency task has been used in rubber hand illusions and adapted as a proxy for intuitiveness of a sensation.[46] This task quantifies the multi-sensory integration of sensory feedback by evaluating one's ability to attend to a specific sensory stimulus while ignoring a distractor visual stimulus (Figure 1.4). If the stimulus and distractor occur at the same location (congruent trials), the response time is very quick, however this response slows if they occur at different locations

(incongruent trials). The slowing of these responses on incongruent trials is labeled the cross-modal congruency effect (CCE).

When Blustein et al. used this task for evaluating different types of sensory stimuli, they found that more intuitive stimuli (touch, vibration) have a higher CCE than less intuitive stimuli (constant frequency electrical stimulation).[46,47] The authors suggest more intuitive stimuli are better integrated with the neural schema and therefore harder to separate and ignore the distractor in those trials.[46] To ultimately use this task to evaluate different types of sensory feedback in individual with lower-limb amputation, to the task must first be adapted for the lower extremity and validated in able-bodied individuals. The validation of the cross-modal congruency task in the lower extremity has the potential to provide a reliable measure of the multi-sensory integration of different types of stimulus trains used in somatosensory neuroprostheses, which is a critical step in evaluating the effect of stimulus quality on function.

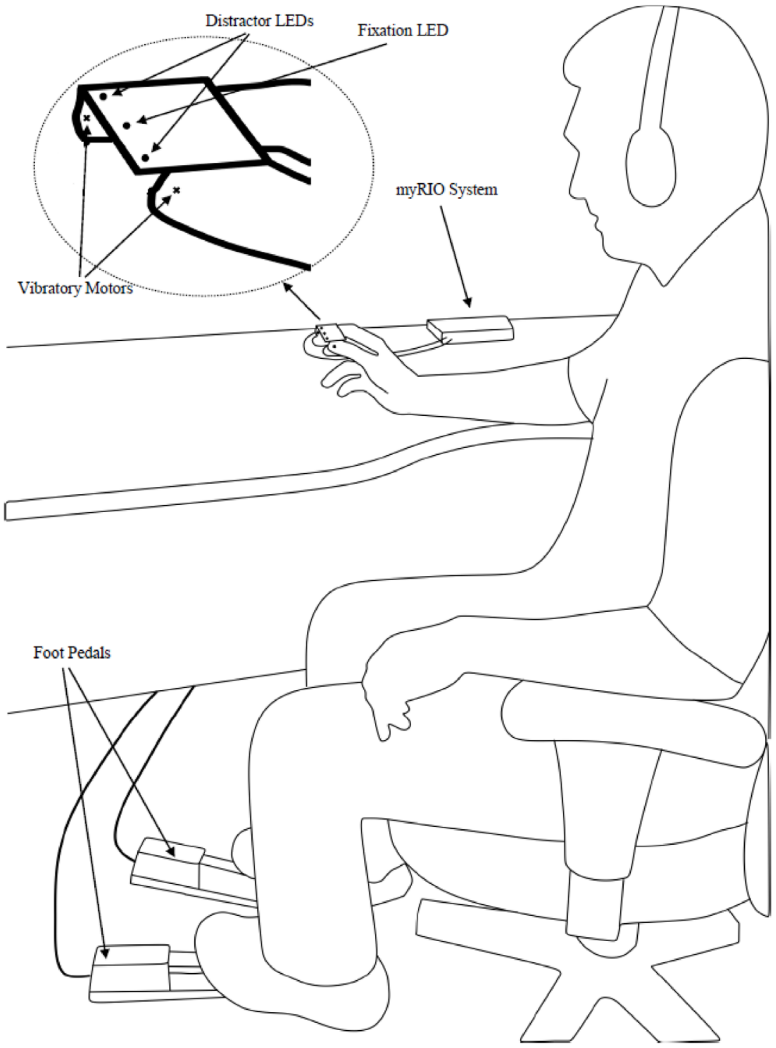


Figure 1.4. Cross-modal congruency task as a measure of integration of sensory feedback.[47] In this task, individuals must respond with a foot pedal to discriminate between stimulation location of the vibratory stimulus. This occurs with distracts either at the same location (congruent trials) or the opposite location (incongruent trials). The slowing of responses for incongruent trials is considered the cross-modal congruency effect (CCE). Figure reprinted from [d].

[d] Reprinted from PeerJ, 7, Blustein D, Gill S, Wilson A, Sensinger J, *Crossmodal congruency effect scores decrease with repeat test exposure.* E6976, Copyright (2019) with permission under the Creative Commons Attribution License.

1.7 Problem Statement and Aims

Sensory feedback from the foot is critical for maintaining stability.[3–5] Consequently, loss of this sensation leads to severe balance and gait impairments.[6] Augmenting or providing additional feedback to individuals lacking sensation, including people with an amputation, can improve balance and gait, however these solutions are often unintuitive and designed for short-term use.[17–20] For long-term use, recent neuroprosthetic studies have shown that stimulation in the peripheral nerves or the spinal cord evokes sensory percepts from the missing limb and that these percepts can be used in functional tasks as sensory feedback.[23–27,39] However, some significant challenges remain in developing an effective sensory neuroprosthesis for clinical use.

When assessing the effectiveness of these devices, we do not yet know how sensitive our current clinical outcome measures are to changes in sensation. Sensory inputs are finely tuned to provide salient information to the nervous system and trigger monosynaptic reflexes. On the other hand, clinical outcome measures are generally designed to be a quick, crude assessments of an individual's overall functional mobility and often combine multiple aspects of mobility (static balance, walking, transfers). The usual clinical outcome measures for individuals with an amputation are not designed with respect to sensation and may not be able to detect changes in sensation or with the addition of sensory feedback. A lack of sensitive outcome measures poses a problem when developing or improving sensory neuroprostheses for people with a lower-limb amputation.

Furthermore, we do not yet know to what extent the intuitiveness of the sensory feedback matters to improve balance and gait. Much of the peripheral nerve stimulation and SCS has used constant frequency stimulation patterns, which often evoke unintuitive sensations. Biomimetic stimulation patterns, that match the firing of the nervous system in response to sensory inputs,

can evoke more intuitive percepts in peripheral nerve stimulation studies. However, we lack a scientific measure of integration of these percepts into the neural schema. A task to quantify the multi-sensory integration of a stimulus would provide a framework for analyzing the effects of stimulation quality on balance and gait performance with a sensory neuroprosthetic.

The experiments discussed in this dissertation are the first studies to demonstrate SCS sensory neuroprostheses in people with a lower-limb amputation. We will perform experiments to evaluate (1) the relationship of sensory impairments to standard clinical measures of balance and gait, (2) the effects of sensory feedback on both the standard and more robust measures of balance and gait, and (3) evaluate differences in multi-sensory integration of various sensory stimuli using the cross-modal congruency task for the lower-limb. To achieve these goals, we will explore the following aims:

Aim 1: Evaluate the relationship of sensory impairments to current clinical measures of balance and gait.

Individuals with lower-limb amputation often have impaired balance responses and severe gait dysfunction.[48–50] Additionally, up to 80% of lower-limb amputations occur because of advanced dysvascular disease, which leads to additional sensory impairments in the contralateral intact limb.[51] While the literature has extensively demonstrated balance and gait impairments and a reliance on visual feedback in lieu of somatosensory feedback, we do not yet know if the standard clinical outcome measures are sensitive to sensory impairments in these individuals. In this study, we will evaluate the relationship between measures of sensation (light touch, protective sensation, proprioception, and monofilament testing) and clinical outcome measures (Sensory Organization Test [SOT] and Motor Control Test [MCT]) and gait (Functional Gait Assessment [FGA], step width variability).

Hypothesis 1: Individuals with lower-limb amputation and more severe sensory impairment (e.g. higher monofilament threshold) will have worse balance and gait performance than those with less severe sensory loss.

Aim 2: Determine the functional effects of sensory feedback with a sensory neuroprosthesis.

The lack of sensory feedback from the missing limb can lead to functional deficits, such as balance impairments and gait asymmetry. Thus, restoring the missing feedback using evoked sensations in the missing foot may improve balance and gait. In this aim, we will explore the effects of using evoked stimuli as sensory feedback in a sensory neuroprosthesis using SCS. We will study the effects of using a sensory neuroprosthesis on both standard clinical measures (SOT, FGA, gait kinematics) and more robust measures designed for individuals with amputation (balance on prosthetic leg, visual feedback test [VFT], NBWT, and walking on uneven surfaces).

Hypothesis 2.1: The addition of sensory feedback in a bi-direction prosthesis will result in an improvement in function in standard clinical measures of balance (SOT, FGA, gait kinematics).

Hypothesis 2.2: The addition of sensory feedback will result in a significant functional improvement in more robust measures of balance and gait (a clinically meaningful change or a statistically significant difference from baseline in balance on the prosthetic limb, VFT, NBWT, walking on uneven surfaces).

Aim 3: Use the cross-modal congruency task to determine the sensorimotor integration of evoked sensations.

Studies with sensory neuroprostheses typically stimulate using constant frequency and amplitude stimulus trains that do not replicate the natural firing patterns of the nervous system and evoke

unnatural percepts such as buzzing and tingling.[52–54] Recent studies suggest a biomimetic approach, which more closely matches patterns of neural activity, could evoke more intuitive percepts and potentially maximize functional improvements with sensory feedback.[40,55] However, the extent to which these patterns are better integrated into the neural schema is unclear and we often rely on unreliable "naturalness" ratings. Thus, to determine if a more intuitive percept would provide a greater functional benefit, we need a measure of the multi-sensory integration of sensory stimuli. In this aim, we will validate the use of the cross-modal congruency task to determine the integration of evoked sensations in able-bodied participants for both tactile and electrical stimuli.

Hypothesis 3.1: More intuitive stimuli (pneumatic tactile feedback) will have a higher CCE score than less intuitive stimuli (electrical stimulation).

1.8 Overall Impact

Through this dissertation, we aim to address several key challenges in developing sensory neuroprostheses. In these experiments, we will assess how clinical outcome measure relate to sensation, restore sensation for a somatosensory neuroprosthesis with lumbosacral SCS in individuals with a lower-limb amputation and analyze a method of quantifying multi-sensory integration of somatosensory stimuli. These studies can provide a framework for evaluating functional effects of sensory neuroprostheses and for further evaluating the effects of stimulus type on function, a significant step to making necessary advancements in these devices.

2.0 Clinical Measures of Balance and Gait Cannot Differentiate Somatosensory Impairments in People with Lower-Limb Amputation.

The contents of this chapter are published as: *Petersen BA, Sparto PJ, Fisher LE. (2022) Clinical measures of balance and gait cannot differentiate somatosensory impairments in people with lower-limb amputation. In Press. Gait & Posture.*

2.1 Introduction

By the year 2050, an estimated 3.6 million Americans will be living with limb loss, with lower-limb amputations accounting for approximately 65% of this population.[56] People with lower-limb amputation can suffer from a range of functional impairments and have a substantially higher risk of falls and fear of falling than the average adult.[2,57] Over 50% of community-dwelling adults with lower-limb amputation reported at least one fall within the past year.[2] In comparison, only 27.5% of able-bodied, community-dwelling adults over 65 years old reported a fall last in the last year.[58] In addition, people with lower-limb amputation can exhibit a wide range of gait and balance impairments compared to their able-bodied counterparts.[59] Determining the factors that influence functional impairments is critical to designing and implementing interventions to improve mobility. Studies suggest that impairments in gait and balance may be due to lack of sensory feedback from the prosthetic limb, but the extent to which sensation relates to various functional measures is not fully understood.[60]

In animal models and humans, tactile and proprioceptive inputs to the spinal cord drive gait phase transitions and contribute to healthy balance mechanics and muscle activation.[3,4] Further, individuals with sensory loss exhibit a wide array of functional deficits, including balance and gait impairments. For example, diabetic peripheral neuropathy is associated with a five-fold increase in fall risk.[8,9] Studies have found direct correlations between measures of sensory loss and balance impairments, suggesting that sensation in individuals with intact limbs is crucial for balance.[11,12] In a small sample (n=4) of individuals with an amputation, sensory integrity was correlated with a functional reach task.[16] However, the nature of any relationship between sensory integrity and functional outcome measures and assessments has not been established in people with lower-limb amputation.

Studies have consistently shown that individuals with a lower-limb amputation rely more heavily on visual feedback for static balance than able-bodied controls, likely as a compensatory mechanism for a lack of sensory feedback.[61] For example, a review conducted on the relative contributions of the amputated and intact limbs to balance control after amputation found that the intact limb contributed more to postural stability in quiet standing.[61] The authors postulated that this is likely due to disruption of the cutaneous and proprioceptive systems that occurs with an amputation.[61] More recently, another study on the contributions of sensory feedback in each limb found that individuals with transfemoral amputation relied more heavily on proprioceptive feedback in the intact limb for balance.[60] Notably, the participants in this study also did not have any dysvascular disorders that affected the intact side. Without visual feedback, the reliance on the intact limb was notably increased.[60] These findings suggest that individuals with an amputation rely more on their contralateral limb with intact sensation and compensate using vision for balance in lieu of sensory feedback. Again, these studies did not evaluate those with

sensory impairments on their intact limb, excluding the large group of people with dysvascular amputations and concomitant contralateral sensory impairments.

With existing outcome measures and assessment, there is conflicting evidence on whether individuals with dysvascular amputations (i.e. those with sensory impairments that often affect the intact limb) have more severe functional impairments than individuals with traumatic amputations. In individuals with a transtibial amputation, Jayakaran et al. used the Sensory Organization Test (SOT) to study postural control in individuals with traumatic and dysvascular transtibial amputations compared to controls with and without dysvascular conditions.[30] The SOT is a widely used clinical standard for measuring reliance of visual, somatosensory and vestibular systems for balance.[62] In the SOT, participants stand on a force platform while either visual (eyes closed, sway-referenced surround rotation) or somatosensory feedback (sway-referenced platform rotation) are altered across six conditions. By altering visual or somatosensory feedback, the SOT forces the participant to rely on their other systems for balance. In that study, there were no significant differences based on cause of amputation (i.e. traumatic vs. dysvascular), with both AMP groups showing less anteroposterior stability than able-bodied groups.[30] Similar studies have been performed evaluating reactive balance. Impaired somatosensation likely plays a role in altered balance responses to these perturbations, given the reflexive pathways based on tactile and proprioceptive inputs that mediate these postural corrections and gait stability.[63] Molina-Rueda et al. evaluated reactive balance using the motor control test (MCT) across groups with traumatic and dysvascular transtibial amputations. The MCT is a test of involuntary, reactive balance in response to anteroposterior surface translations of the support surface. The MCT evaluates reaction latency, amplitude, and symmetry to assess subjects' ability to respond to an external translation. They found that the dysvascular group had

slower responses on their sound limb than the traumatic group. Additionally, several studies have shown key differences in gait kinematics and kinetics in dysvascular versus traumatic amputations, although there is evidence that these differences may be due primarily to differences in gait speed.[64] Though both of these studies included participants with sensory loss, they both excluded individuals with phantom limb sensation or pain, which may include up to 80% of individuals with an amputation.[65] Thus, determining the impact of sensation across the full spectrum of individuals with an amputation is critical to evaluating whether current clinical outcome measures can detect functional differences due to sensory impairments.

The relationship between clinical measures of somatosensation and clinical measures of function in individuals with a lower-limb amputation needs clarification. The purpose of this study is to determine whether some current clinical outcome measures can detect a relationship between sensory impairments and function. To test this, we evaluated the correlation between measures of somatosensory integrity to measures of static, reactive and dynamic balance, and gait stability across a wide range of individuals with a lower-limb amputation, regardless of level or nature of amputation. The relationship of quantitative measures of somatosensation to these outcomes can elucidate how well these outcome measures can detect differences in function due to sensation.

2.2 Methods

We collected measures of balance, gait, and sensation from 20 individuals with lower-limb amputation (AMP) and 20 age- and gender-matched able-bodied individuals (CON). The CON group was added to confirm these metrics were confirming previously established

differences between CON and AMP groups in this population. Inclusion criteria for individuals with an amputation included: (1) amputation of one lower-limb, (2) between the ages of 18 and 70, (3) ability to stand unassisted for 10 minutes, and (4) ability to ambulate. To evaluate these metrics across a wide range of individuals, our exclusion criteria were intentionally broad and included both transtibial and transfemoral amputation. Participants were excluded if they had a known balance disorder or were pregnant. All experiments were performed under the supervision of the University of Pittsburgh's Institutional Review Board. For more detail on specific outcome measures, see Appendix A.

2.2.1 Sensory Measures

Measures of sensory integrity included somatosensory monofilament pressure thresholds, light touch sensation, protective sensation, lower extremity reflexes, proprioception, and vibration sense. All sensory tests were performed with the participant's eyes closed. Somatosensory pressure thresholds were assessed using Semmes-Weinstein monofilaments, which include varying grades of monofilament thickness, ranging from 0.01 g to 300g. The filament was applied perpendicular to the plantar aspect of the feet until the filament bent, three times per site. The plantar aspect of the hallux, first metatarsal head, fifth metatarsal head, and heel were tested. If the subject reported sensation for at least 2 of 3 trials, the next monofilament was tested, until the subject could no longer detect the filament. For the residual limb (AMP only), we tested the distal-most aspect of the residual limb with the limb stabilized to avoid excessive skin movement. Light touch, protective (pin prick), reflexes, proprioception and vibration sense were assessed bilaterally, as well (Appendix A).

2.2.2 Performance Measures

To quantify static, reactive, and dynamic balance and gait, we used the Sensory Organization Test (SOT), Motor Control Test (MCT), Functional Gait Assessment (FGA), and gait kinematics, respectively. These clinical assessments were selected because they have been used to assess changes with somatosensory feedback.[25,66] The SOT and MCT were both performed using the NeuroCom Equitest system (Appendix A). By altering the visual or somatosensory feedback participants receive, the test provides a method for measuring the reliance on the somatosensory, visual, and vestibular systems to maintain balance. Three, 20-second trials were completed per condition. Center of pressure (COP) traces were recorded from the force plates (100 Hz), filtered with a low-pass fourth-order Butterworth filter, and analyzed for standard measures of posturography, in addition to clinical measures. Standard posturography measures were calculated, including excursion, sway velocity, 95% confidence interval ellipse of sway area, sample, and approximate entropy (Appendix A). Clinical measures, including equilibrium scores and somatosensory ability were also recorded. Equilibrium scores indicate a participant's ability to stay within a normative 12.5° anteroposterior sway envelope (Appendix A). Somatosensory ability (ratio of equilibrium scores in static conditions without vision, condition 2, to equilibrium scores with normal vision, condition 1) indicates a participant's ability to utilize somatosensation for balance when vision is impaired.

In the MCT, participants must maintain balance in the Equitest system following translational perturbations in both anterior and posterior directions. The perturbations in this task included three grades (small, medium, large) with random time delays ranging 1-3 seconds. The medium and large translational trials were assessed for latency of onset of active response and symmetry of strength of responses (Appendix A). For the AMP group, the active response was

typically too small to be detected on the prosthetic side, so the latency and strength of responses was determined only on the intact limb.[67]

Dynamic balance was assessed using the FGA, a clinical gait and dynamic balance assessment that involves ambulating 6 meters down a hallway.[68] There are 10 items which are scored from 0 (severe impairment) to 3 (no impairment). This gait assessment has been validated in community-dwelling adults and individuals with neurological and balance disorders.[69,70]

Gait kinematics during walking on a level surface were recorded using a 16-camera OptiTrack motion analysis system (Natural Point, OR, USA). Participants were instructed to walk at their self-selected speed for six trials across a 6-meter walkway. Sixteen reflective markers were placed on anatomical landmarks according to the OptiTrack "Conventional Lower Body" model.[71] Kinematic marker data was collected at 100 Hz and filtered using a 4th order low-pass Butterworth filter at 12 Hz. Step length asymmetry (normalized to stride length, SLA), step length variability, and step width variability (standard deviation and coefficient of variation) were calculated as measures of gait stability (Appendix A). Gait assessments were only collected from 12 of the 20 AMP participants, as our motion capture lab was only available midway through data collection.

2.2.3 Statistical Analysis

A Pearson correlation was performed between all measures of balance and clinical sensory scores. However, because we observed a bimodal distribution of sensory impairment in individuals with AMP (Figure 1), monofilament threshold was categorized as intact (<10 g threshold, n=10) or impaired sensation (>10 g threshold, n=10) based on the clinical standard for diagnosing peripheral neuropathy.[72] Due to this distribution of sensory loss, the non-parametric

Mann Whitney U test was used to determine significant differences between the participants with intact and impaired sensation. Comparisons between AMP and CON groups were completed using the Wilcoxon signed rank test for pair-wise comparisons. Significance level was set at 0.01 for all tests because of the large number of comparisons completed. Effect sizes as partial eta squared (η^2) are reported.

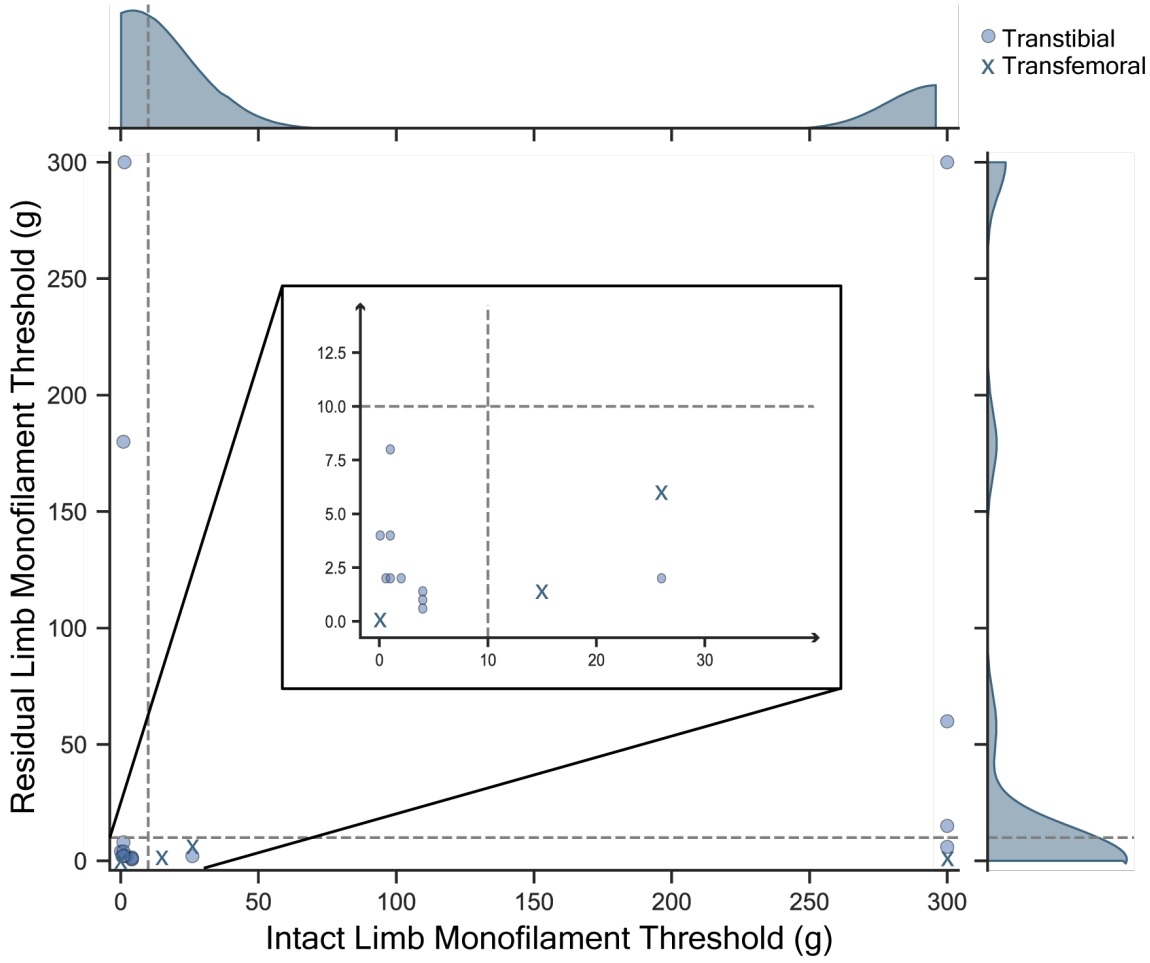


Figure 2.1. Distribution of sensory loss in individuals with amputation (AMP). Bimodal distribution of sensory loss seen in both the intact and residual limb monofilament threshold. Sensory loss was then categorized into impaired sensation (>10g monofilament threshold, dotted line). Individuals with transfemoral amputation are identified (X) versus transtibial amputation (open circle).

2.3 Results

2.3.1 Participant Characteristics

Twenty people with limb amputation and twenty age- and gender-matched controls were included in the study. Age and gender across both AMP and CON groups are comparable (Table 1). The majority of amputations were transtibial (80%) and the average use of the prosthesis exceeded 12 hours per day. In addition, 10 participants had full sensation bilaterally, while 10 participants had impaired sensation (3 had impaired sensation bilaterally, 5 had impaired sensation on the contralateral limb only and 2 participants had impaired residual limb sensation only). In comparison to the CON group, the AMP group had a significantly slower self-selected gait speed (0.88±0.18 m/s AMP, 1.12±0.18 m/s CON, $p<0.001$).

Table 1. Participant characteristics of amputation group (AMP) and able-bodied controls (CON). Mean ± standard deviation of age, time since amputation, and time spent wearing prosthesis per day are reported. Sensory impairments defined as >10g monofilament threshold on 1st metatarsal (intact limb) and distalmost residual limb. (TT=transtibial, TF=transfemoral, * indicates statistically significant difference, $p<0.001$)

Group	AMP	CON
Gender (M/F)	16/4	16/4
Age (years)	53.5 ± 10.2	53.5 ± 11.0
Amputation level (TT/TF)	16/4	---
Time since amputation (months)	114±173	---
Daily prosthesis wear (hours)	12.5±4.2	---
Participants with impaired sensation (bilateral/contralateral only/ipsilateral only/none)	3/5/2/10	---
Self-selected gait speed (m/s)*	0.88 ± 0.18	1.12 ± 0.18

2.3.2 Static Balance

The AMP group had a greater increase in sway area in the condition without vision than the CON group **(**Figure 2.2A**,** 16.05±19.77 cm^2 AMP, 3.03±3.05 cm^2 CON, $p<0.001$, $\eta^2= 0.878$), with no significant differences in sway area between AMP group based on sensation ($p>0.01$, Figure 2.2B). Similar, the CON group had significantly greater SOT somatosensory ability, the ratio of anteroposterior sway in the static condition without vision to the condition with vision, than the AMP group (Figure 2.2C, 0.90±0.08 AMP, 0.95±0.03 CON, $p<0.005$, $\eta^2=0.213$). However, SOT somatosensory ability was not significantly different between individuals with an amputation with full or impaired sensation ($p>0.01$). The distribution of scores in the impaired sensation group is larger and more skewed than the full sensation group for change in area without vision or SOT somatosensory ability (Figure 2.2).

There were no significant differences in equilibrium scores in any conditions or in composite equilibrium scores between AMP groups with full or impaired sensation. No significant differences were found across groups based on other sensory measures (proprioception, reflexes, vibration), as well ($p>0.01$).

Within the CON group, there were no significant relationships ($p>0.01$) between monofilament thresholds or other sensory measures and clinical or posturography measures of balance across all conditions of the SOT.

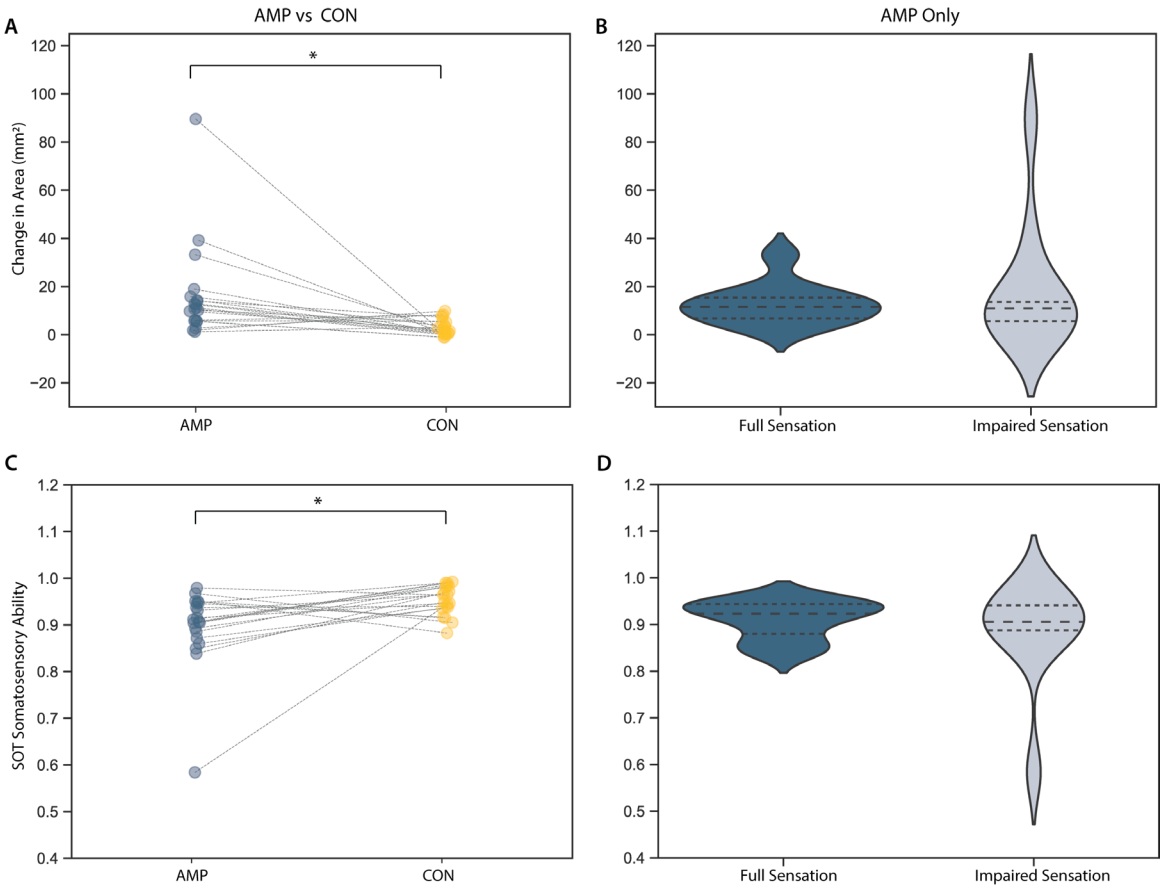


Figure 2.2. Static balance performance in individuals with an amputation versus controls and individuals with amputation separated by somatosensory impairments. (A,B) Change in area from static conditions with vision to static conditions without vision. In both groups, sway increases without vision, however this increase is significantly larger in individuals with an amputation (AMP, blue) than controls (CON, yellow). (C,D) No significant differences are seen with sensory impairment (MDC=0.05) between individuals with full sensation (dark blue) and individuals with impaired sensation (light blue). Lines in A and C connect age- and gender-matched AMP and CON subjects. MDC=Minimum Detectable Change, SOT=Sensory Organization Test.

2.3.3 Reactive Balance

The latencies of responses on the intact limb in the AMP group were significantly slower than for the dominant limb in the CON group (150±18 ms AMP,132±12 ms CON, $p<0.001$, $\eta^2=0.82$, Figure 2.3A), however latencies on the intact limb were not significantly different based on sensation ($p>0.01$, Figure 2.3B). The AMP group were significantly less symmetrical than the CON group, bearing more weight on the intact limb (-16.54±13.68 AMP, -0.01±6.55 CON, $p<0.001$, $\eta^2=0.150$, Figure 2.3C), with no significant differences based on sensation ($p>0.01$, Figure 2.3D).

2.3.4 Dynamic Balance and Gait Stability

Total FGA score was significantly lower in the AMP group compared to controls (19±5 AMP, 29±2 CON, $p<0.001$, $\eta^2=0.0213$, Figure 2.4A). The FGA showed no significant differences between AMP participants with full or impaired sensation on either residual or contralateral limbs ($p>0.01$, Figure 2.4B). There were no significant differences in SLA between AMP and CON groups or between full and impaired sensation groups (Figure 2.4C-D) or with step width variability (Figure 2.4E-F).

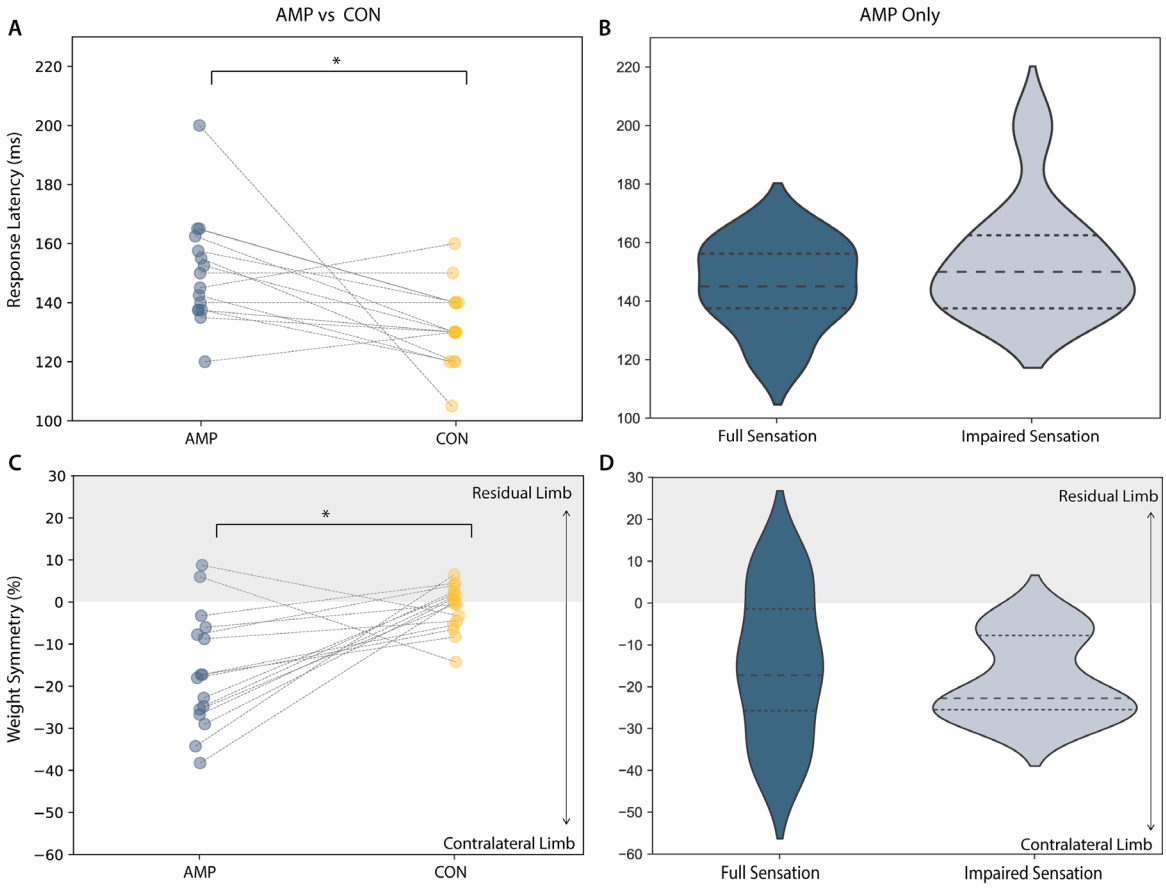


Figure 2.3. Reactive balance performance in individuals with an amputation and according to somatosensory impairments. (A,B) Latency of response to perturbations (MDC=12.3 ms) and (C,D) weight symmetry prior to perturbation showed significant differences between AMP (blue) and CON (yellow) groups ($p<0.01$), with AMP participants bearing more weight through their contralateral (intact) limb. However, these assessments demonstrated no significant differences by sensory impairment in individuals with full sensation (dark blue) and individuals with impaired sensation (light blue). Lines in A and C connect age- and gender-matched AMP and CON subjects. MDC=Minimum Detectable Change.

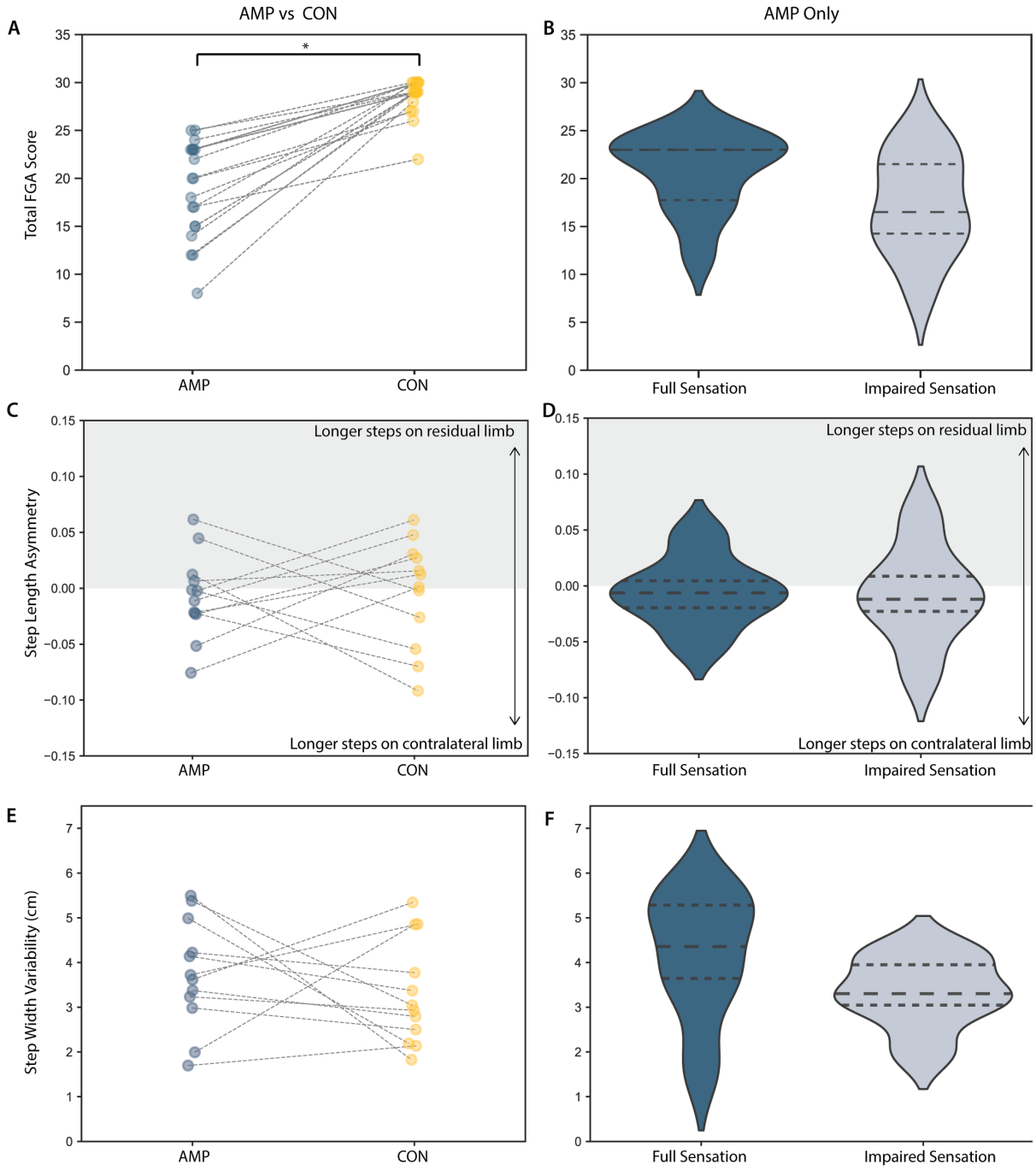


Figure 2.4. Dynamic gait stability according to somatosensory impairments. (A,B) Total Functional Gait Assessment (FGA, MCID= 4 pts) showed significant differences between AMP (blue) and CON (yellow) groups. (C,D) SLA and (E,F) step width variability showed no significant differences between individuals with full sensation (dark blue) and impaired sensation (light blue). Lines in A, C, and E connect age- and gender-matched AMP and CON subjects. MCID= Minimum Clinically Important Difference.

2.4 Discussion

In this study, we explored the relationship between sensory impairments in the residual and contralateral limbs and performance on a variety of clinical outcome measures for people with lower-limb amputation. Consistent with previous literature, the SOT, MCT, and FGA can detect differences in functional abilities between individuals with a lower-limb amputation and able-bodied individuals. However, these measures are not able to detect even substantial differences in somatosensory integrity within populations with a lower-limb amputation. These findings are surprising, given the critical role sensation and spinal reflexes play in balance and gait. While one possible interpretation of this result is that somatosensory impairments do not make a difference in function, the reflexive pathways and role of tactile feedback in balance and gait have been characterized extensively[3,4,73] and preliminary evidence in a small sample of individuals with an amputation (n=4) has suggested plantar sensation plays a significant role in balance control.[16] Thus, these disturbances in somatosensory input in the AMP group still likely have a functional impact. Instead, these findings more likely suggest that the current battery of tests we have for this population are not able to distinguish between these differences in somatosensory integrity.

The lack of significant differences within the AMP group for the SOT are consistent with those seen by Jayakaran, who found no difference in SOT measures between groups with dysvascular and traumatic amputations.[30] Our reactive balance findings are inconsistent with previous studies, which found significant differences in latencies between individuals with dysvascular and traumatic amputations.[67] Again, these responses were only measured for the intact limb and an alternative measure of reactive balance utilizing the residual limb may be necessary to detect differences in sensation for the amputated side.

The lack of significant differences between AMP and CON groups for kinematic measures of gait stability has also been reported in previous literature. Keklicek et al. found that despite differences in step length variability, individuals with transtibial amputation demonstrated step lengths on both intact and residual limbs, though not normalized, similar to those in able-bodied subjects.[74] These findings differ from studies reporting SLA in individuals with amputation, however these studies did not normalize SLA to stride length[75], which is now recommended in use of SLA to avoid accentuating asymmetries in individuals with shorter stride lengths.[76] The dilemma posed here is that these gait measures (FGA, gait kinematics) are used clinically across the full range of individuals with an amputation. However, our results indicate that these outcomes should not be used for many subgroups of individuals with amputations. Together these findings suggest that more challenging and robust measures of gait analysis are necessary to capture differences between groups across the wide variety of impairments seen in this population. Newer measures are being studied to address this issue. For example, Thies et al. found that walking on an irregular surface can detect differences across subgroups of individuals with amputations and Sawers et al. developed the narrowing beam walking test (NBWT) as a more robust outcome measure for this population.[34,36] Future work should explore whether these measures can detect differences in somatosensory function among people with limb amputation.

Notably, the measures of sensation used in this study are crude measures designed to be used in clinics. This may account for the inability to characterize the more mild-moderate range of somatosensory impairments. Thus, future studies with more robust measures of sensation may further elucidate these findings. In addition, this was a small sample of 20 individuals with an amputation, only four of which had a transfemoral amputation. A larger sample size would be necessary to perform multiple regression to determine how factors like level of amputation,

prosthetic usage, or time since amputation impact functional measures, in addition to sensation. Additionally, assessing fall risk more directly, as well as balance confidence, phantom pain or prosthesis comfort should be evaluated in future studies to determine what other effects sensation may have for this population and what other factors are driving the differences in performance for this population when compared to healthy controls.

In conclusion, while these clinical measures detect differences between able-bodied individuals and individuals with an amputation, they are not able to distinguish between levels of somatosensory impairments within groups with an amputation. These findings, in addition to other recent work evaluating current outcomes for this population, suggest that more challenging and robust metrics are necessary to evaluate the role of sensation and other factors on functional impairments in people with lower-limb amputation.

3.0 Spinal Cord Stimulation Restores Sensation, Improves Function, and Reduces Phantom Pain After Transtibial Amputation.

3.1 Introduction

Every year, approximately 150,000 people in the United States undergo amputation of a lower-limb.[1] Loss of a lower-limb leads to chronic challenges including major mobility impairments and emergence of chronic pain that appears to emanate from the missing limb (i.e. phantom limb pain [PLP]). Current clinical practice involves prescribing a passive prosthetic limb to improve mobility and opioids or other pharmaceuticals to treat PLP. Even with these interventions, people with lower-limb amputation exhibit a high rate of falls, a lack of confidence during gait, and persistent PLP. All of these problems have been associated with the disruption of somatosensory feedback from the missing limb when the peripheral nerve is severed. First, tactile feedback from the sole of the foot is critical for maintaining balance and postural stability.[6,11] Second, the loss of somatosensory feedback after an amputation causes a sensorimotor mismatch that has been implicated in the development and maintenance of PLP. Therefore, to effectively address the sequela of lower-limb amputation, we seek to develop approaches that restore somatosensation in the missing limb, improve functional outcomes, and reduce PLP.

Previous studies have demonstrated that electrical stimulation of peripheral nerves in the residual limb can evoke sensations in the missing hand or foot.[24,29,77] Tactile feedback via peripheral nerve stimulation has been shown to enhance control of the prosthesis and improve functional outcome measures related to balance and gait.[23,25,52,78,79] Additionally, anecdotal

evidence suggests that chronic peripheral nerve stimulation reduces PLP.[23,54,80,81] To date, most studies to restore sensory feedback from the missing limb have relied on invasive and complex surgical techniques to implant devices inside or around peripheral nerves or to reroute those nerves to other regions on the residual limb. While these approaches clearly demonstrated the promise of electrical stimulation for restoring somatosensation, improving prosthetic function, and treating PLP, their surgical complexity remains a substantial barrier to widespread clinical adoption. There may also be challenges with evoking sensations via peripheral nerve stimulation in individuals with severe peripheral neuropathy, which is a common co-morbidity for people with dysvascular amputations secondary to diabetes, which account for up to 82% of lower-limb amputations.[51] To our knowledge, no study to date has demonstrated restored somatosensation in the amputated foot for people with diabetic amputation.

Here, we aimed to address these challenges by relying on spinal cord stimulation (SCS), rather than peripheral nerve stimulation, to restore somatosensory feedback from the missing lower-limb. SCS is an existing clinical technology that is implanted in as many as 50,000 people each year to treat chronic pain.[82] The surgical procedures involved in the implantation of these devices and the associated risks are well understood, and most major medical centers throughout the US have physicians that routinely perform SCS implants.[83] Recently, we have shown that cervical SCS can be used to restore somatosensation from the missing hand in people with upper-limb amputation.[37] Our goal in this study was to demonstrate that lumbar SCS could evoke sensations in the missing foot, and that the restored somatosensory feedback could improve functional use of the prosthesis and reduce PLP. Importantly, we aimed to demonstrate that we could achieve these effects regardless of whether the amputation was traumatic or dysvascular, which substantially increases the potential pool of people that might benefit from these devices.

3.2 Results

In three people with below-knee amputation (Table 2), we implanted commercially available SCS leads in the thoracolumbar epidural space to stimulate the lateral lumbar spinal cord. We identified electrode contacts that evoked sensation experienced on the missing foot and performed psychophysical assessments to characterize those sensations. We developed a closed-loop system (Figure 3.1) where SCS was modulated by pressure signals wirelessly recorded from an insole in the shoe under the prosthetic limb. Using this system to deliver real-time somatosensory feedback, we characterized functional outcome measures of balance and gait, as well as changes in PLP over the duration of the multi-week implantation period. Our results indicate that lumbar SCS is a promising intervention to restore sensations, improve function, and reduce PLP in people with a lower-limb amputation.

Table 2. SCS participant demographics and amputation data.

Subject	Age	Gender	Ambulation Level	Years since amputation	Side of amputation	Nature of amputation	Implant Duration (days)
1	56	M	Limited community	3	Left	Dysvascular	28
2	56	M	Active	7	Left	Traumatic	28
3	65	F	Limited community	5	Left	Dysvascular	84

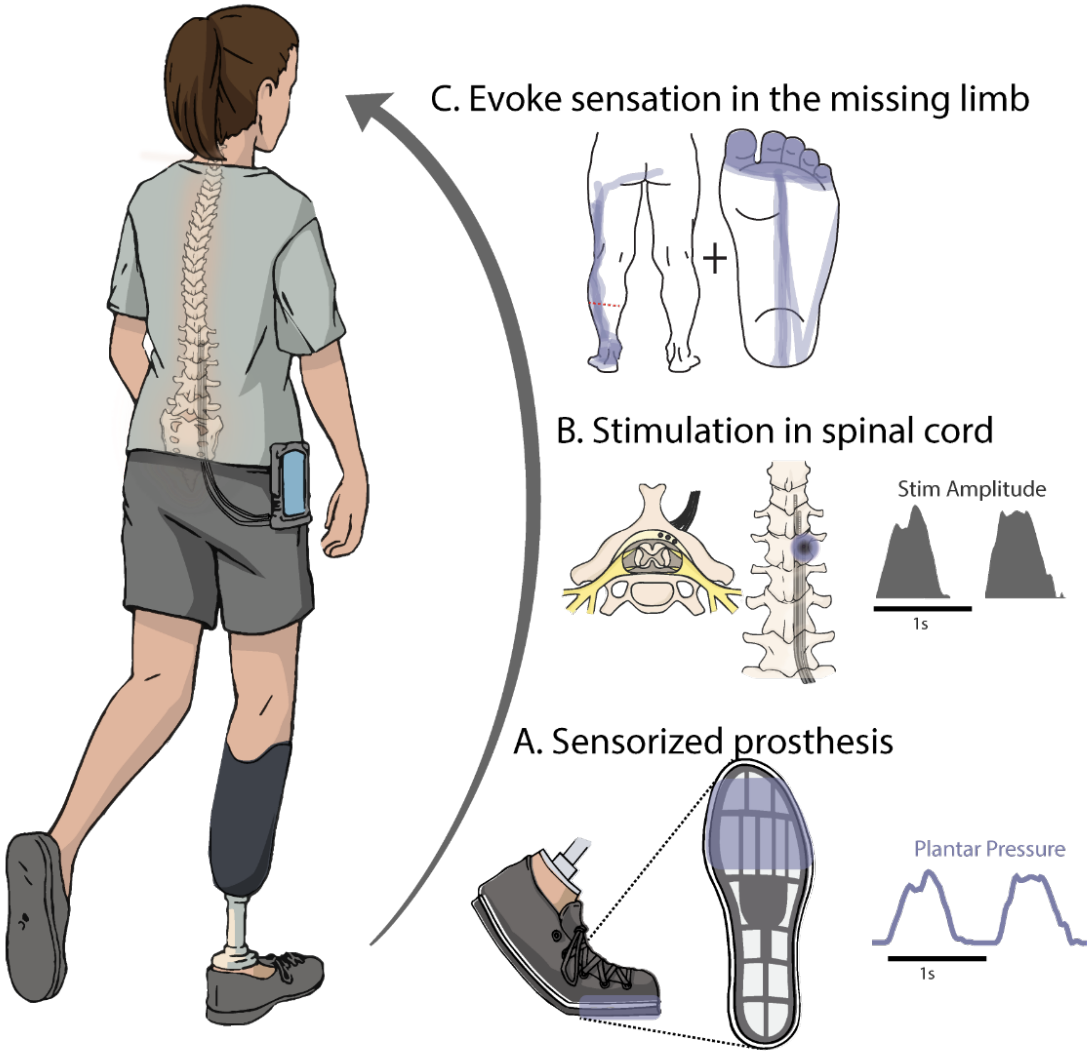


Figure 3.1: Schematic of the closed-loop SCS system used in this study. Electrical stimulation was delivered to the spinal cord via three 8- or 16-contact leads implanted percutaneously near the lateral lumbosacral spinal cord. The leads were tunneled through the skin and connected to an external stimulation system. A sensorized insole was inserted into the shoe to measure pressure under the prosthesis, and signals from this insole were used to modulate stimulation amplitude.

3.2.1 Spinal Cord Stimulation Evokes Sensation in the Missing Foot

A primary goal of this study was to characterize the location and perceptual qualities of sensations evoked by lumbosacral SCS. To map the location of evoked sensations, we delivered 1-sec long stimulation trains and asked the subjects to draw the location of the perceived sensations on a schematic of the foot and legs. For all three subjects, SCS evoked sensations in the missing limb, including the toes and heel (Figure 3.2A), though these were absent in the first two weeks of the study and emerged gradually thereafter (Figure 3.2C and Supplementary Figure 5). The sensations in the missing limb were always accompanied by sensations in the residual limb, and higher stimulation amplitudes were required to evoke sensation in the missing limb than in the residual limb (Supplementary Figure 3). The rostral-caudal arrangement of the electrodes across different levels of the spinal cord elicited sensations that corresponded to the physiological dermatomal distribution (Figure 3.2B and Supplementary Figure 4).[84] The elicited sensations were stable across trials within a session.

The subjects also reported the perceived quality of the sensations using a predefined list of descriptors based on previous literature.[85] For analytical purposes, we grouped these descriptors as sensations that subjects might experience commonly in their daily life (naturalistic) or rare, less familiar sensations (paresthetic). All subjects reported a combination of natural and paresthetic descriptors in different proportions (Supplementary Figure 6).

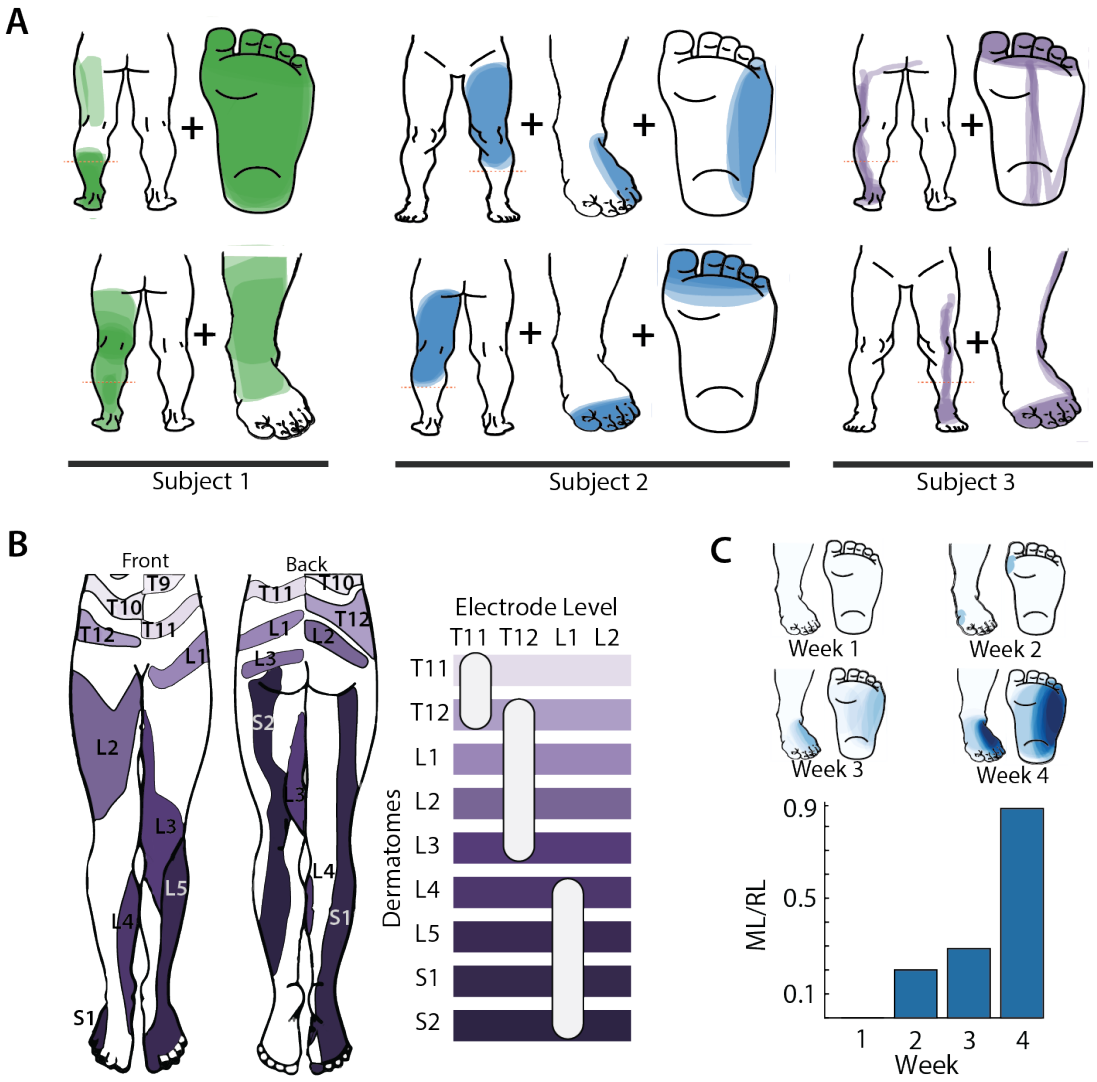


Figure 3.2: SCS evokes percepts in the missing limb. (A) Examples of percepts evoked in the missing limb from one session for each subject. Two different sensations (from two different electrodes) are shown for each subject. (B) Dermatome activation by electrodes located at different vertebrae levels for Subject 3. Expected dermatomal innervation in the leg (left), adapted from [84]. In the right, the horizontal bars indicate different dermatomes and the vertical columns indicate the approximate electrode position with respect to the vertebrae level. (C) Rate of occurrence of sensations in the missing limb across weeks from one electrode in Subject 2 and example of percepts evoked in the foot (top) and the ratio of the frequency of sensations in the missing limb (ML) to the frequency of sensations only in the residual limb (RL, bottom).

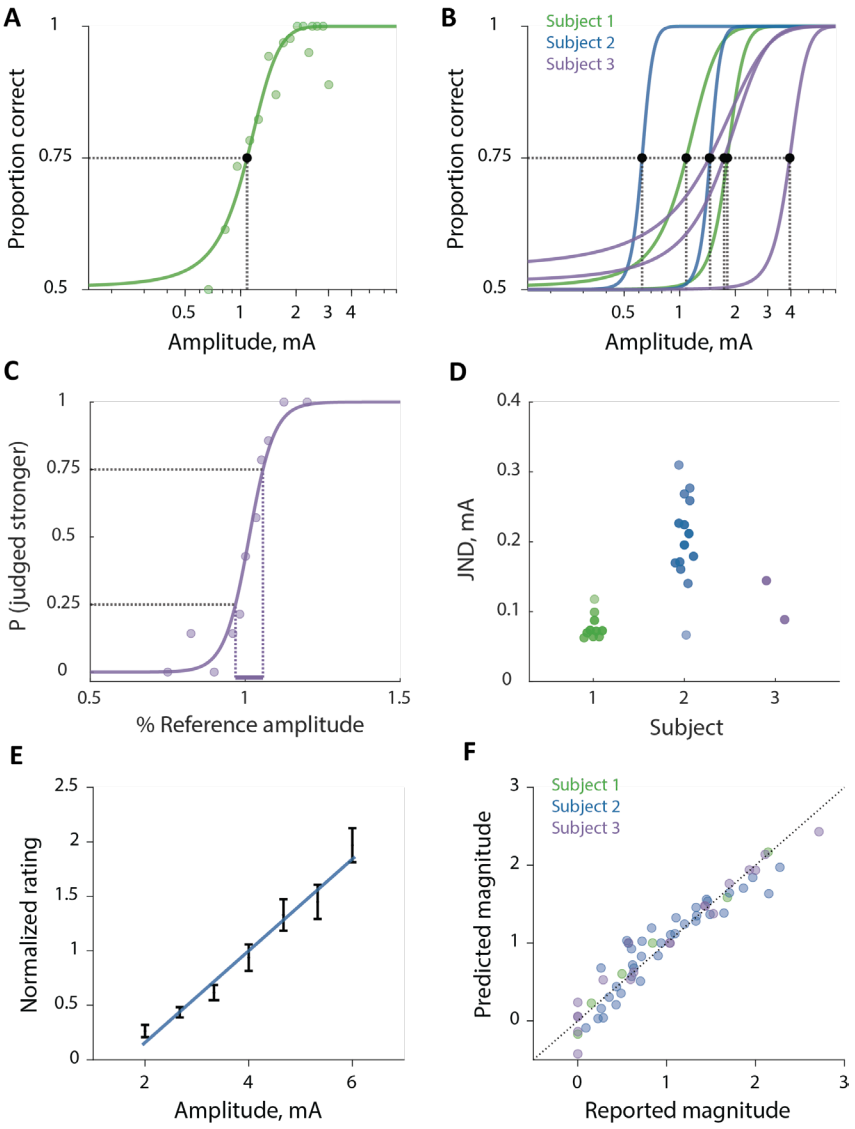


Figure 3.3 Psychophysical assesment of evoked sensations. (A) Performance of Subject 1 on the detection task for one electrode. (B) Fitted psychometric functions from all subjects and electrodes. Different colors for denote different subjects. The dashed lines indicate the detection threshold for each electrode. (C) Performance of Subject 3 on the amplitude discrimination task with a standard amplitude of 2 mA on one electrode. The dashed line indicates the just-noticeable difference (JND). (D) Distribution of JNDs across the three subjects. (E) Magnitude ratings as a function of amplitude for one electrode for Subject 2. The error bar denotes the standard deviation across repeated presentations of the same stimulus. (F) Intensity ratings, normalized to the mean rating obtained from each subject and electrode. The dashed line shows unity.

3.2.2 Sensory Magnitude Can Be Systematically Manipulated by Varying Stimulation Amplitude

A key step in designing a sensory prosthesis is to assess the dependence of the sensation on stimulation parameters. With this in mind, we first established the stimulation intensity required to evoke a conscious percept. To this end, we had the subjects perform a detection task in a two-alternative forced choice paradigm. In brief, a 1-sec stimulation train at one of 5 to 10 amplitudes, determined in preliminary experiments to be peri-liminal, was presented in one of two visually cued stimulus intervals, and the subject's task was to report which interval contained the stimulus. Each stimulus was presented at least 4 times and we tallied the proportion of times the subject correctly identified the interval containing the stimulus for each amplitude (Figure 1A). The detection threshold was the amplitude (estimated from the fitted psychometric function, a cumulative normal distribution) at which the subject would correctly identify the stimulus interval 75% of the time. Detection thresholds varied across electrodes and subjects from 0.6 to 4 mA but there were no large and systematic differences across subjects (Figure 3.3B).

Next, we measured the subjects' sensitivity to changes in stimulation amplitude. To achieve this, we had them perform an amplitude discrimination task. On each trial, the subject was presented with two stimuli: (1) a standard whose amplitude was fixed within the block and (2) a comparison, whose value varied from trial to trial. After both presentations, the subject reported which of the two felt stronger (Figure 3.3C). For each electrode and subject, we fitted a psychometric function and computed the just noticeable difference (JND), the change in amplitude required for the subject to correctly identify the more intense stimulus 75% of the time. JNDs varied from 0.05 to 0.3 mA across subjects and electrodes (Figure 3.3D). The range

of JNDs overlapped across subjects, though one subject tended to have higher JNDs than the other two.

Finally, we wished to explicitly measure the relationship between stimulation amplitude and perceived magnitude. To this end, we delivered stimuli that spanned a range of intensities and had the subject report how intense the stimulus felt with the following instructions: (1) If they did not feel the stimulus; they ascribed to it a rating of 0; (2) If one stimulus felt twice as strong as another, it was to be ascribed a number that was twice as high (other examples were also provided); (3) They could any scale they wanted, and were encouraged to use fractions and decimals, if necessary. Perceived magnitude increased nearly linearly with stimulation amplitude for all subjects and electrodes (Figure 3.3E,F), as has been previously found with stimulation of the peripheral nerves[86–89] and of somatosensory cortex.[90]

3.2.3 Spinal Cord Stimulation Improves Functional Use of a Prosthesis

One of the main goals of this study was to demonstrate that restored somatosensation can improve functional use of a prosthetic limb. To restore somatosensation during functional tasks, such as standing and gait, we placed a wireless pressure-sensing insole (Moticon Insole 3, Munich, Germany) under the prosthetic foot and used the output from that insole to drive stimulation in real-time. In Subjects 2 and 3, we selected an SCS electrode that reliably evoked sensation on the plantar surface of the missing foot and used the pressure signal from the same location under the prosthetic foot to control stimulation amplitude (Figure 3.1A). Because of time constraints in testing, we did not perform similar experiments in Subject 1. We used clinical measures of balance and gait to compare postural stability with and without restored somatosensory feedback.

3.2.4 Spinal Cord Stimulation Improves Standing Balance

To assess standing balance with and without sensory feedback, we used the Sensory Organization Test (SOT), a clinical outcome measure that quantifies reliance on visual, vestibular, or somatosensory feedback to maintain balance control. The SOT requires the subject to maintain balance (Figure 3.4A) while standing within a visual surround that can sway, providing erroneous visual information, and standing on a support surface that can sway, providing erroneous somatosensory information. To characterize reliance on visual, vestibular, and somatosensory feedback, the SOT comprises six different conditions, which each obscure different combinations the relevant sensory feedback modalities. With somatosensory feedback restored via SCS, both Subjects 2 and 3 achieved improvements in SOT scores (Figure 3.4C), with greater improvements in the most challenging conditions (platform sway with eyes closed and platform sway with visual surround sway, Supplementary Figure 7). Notably, both participants experienced at least one "fall" without stimulation, but neither subject fell with stimulation (Figure 3.4D). A "fall" denotes a failure to complete the trial due to taking a step, falling in the harness, or grabbing the walls for support. Performance was slightly worse with stimulation during the least challenging conditions (i.e. no visual or support surface sway) with eyes open and eyes closed (Supplemental Figure 5), although this difference was smaller than the MCID (Supplementary Figure 7). Biomechanical analyses of center of pressure traces (Figure 3.4D) revealed that both participants had statistically significant decreases in sway area (indicating greater stability) with eyes closed and an unstable support surface (Subject 2 decreased by 19.34 cm^2, Subject 3 decreased by 39.04 cm^2, $p<0.001$, Figure 3.4D).

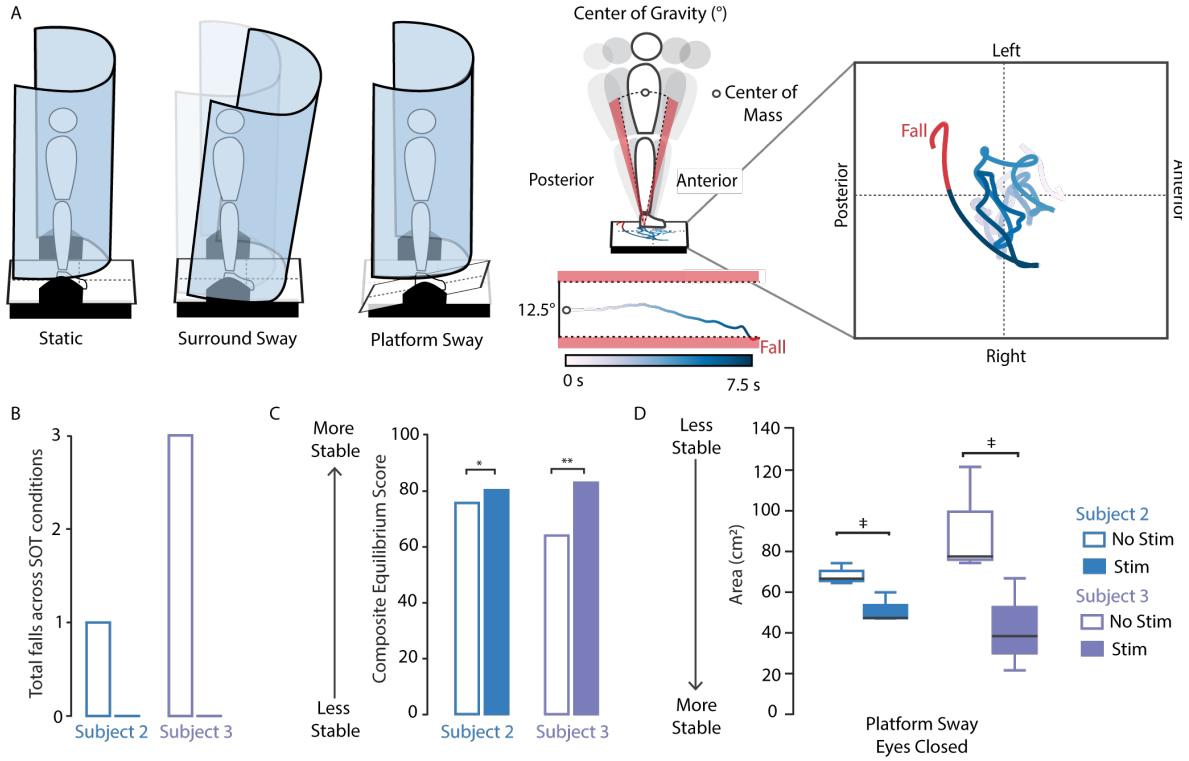


Figure 3.4. **Closed-loop sensory feedback improves postural stability.** (A) The SOT has six conditions, over which the platform or surround can sway. (B) The center of gravity (COG) is a projection of the pressure traces to indicate their center of mass (COM) movement throughout a trial. The equilibrium score indicates how well subjects maintain their COG within a normative 12.5° limit of anteroposterior sway. Beyond these limits, a fall can occur (red). (C) Falls occurred only without stim for both subjects (left) and both subjects had an improvement in composite equilibrium score (right), above the minimum detectable change for Subject 2 (*) and above the threshold for a clinically meaningful difference in Subject 3 (**). (D) Both subjects had a statistically significant decrease in sway area, indicating greater stability, with stimulation (ǂ).

3.2.5 Spinal Cord Stimulation Improves Gait Stability

To assess stability during gait, participants performed the Functional Gait Assessment (FGA), a clinical measure of dynamic balance, commonly applied to detect reliance on visual and somatosensory systems for maintaining balance during walking.[66,69] This task consists of ten

items, including walking with eyes closed, walking with a narrow base of support, and walking over obstacles. Restored somatosensation led to a clinically meaningful improvement (>4 points) in FGA score for Subject 3, but not Subject 2 (Figure 3.5A-B). Notably, Subject 3 had worse baseline performance on the FGA than Subject 2. Subject 2 demonstrated baseline performance 3.9 points below age-matched able-bodied controls, whereas the baseline score for Subject 3 was 13.5 points below age-matched normative data.[69]

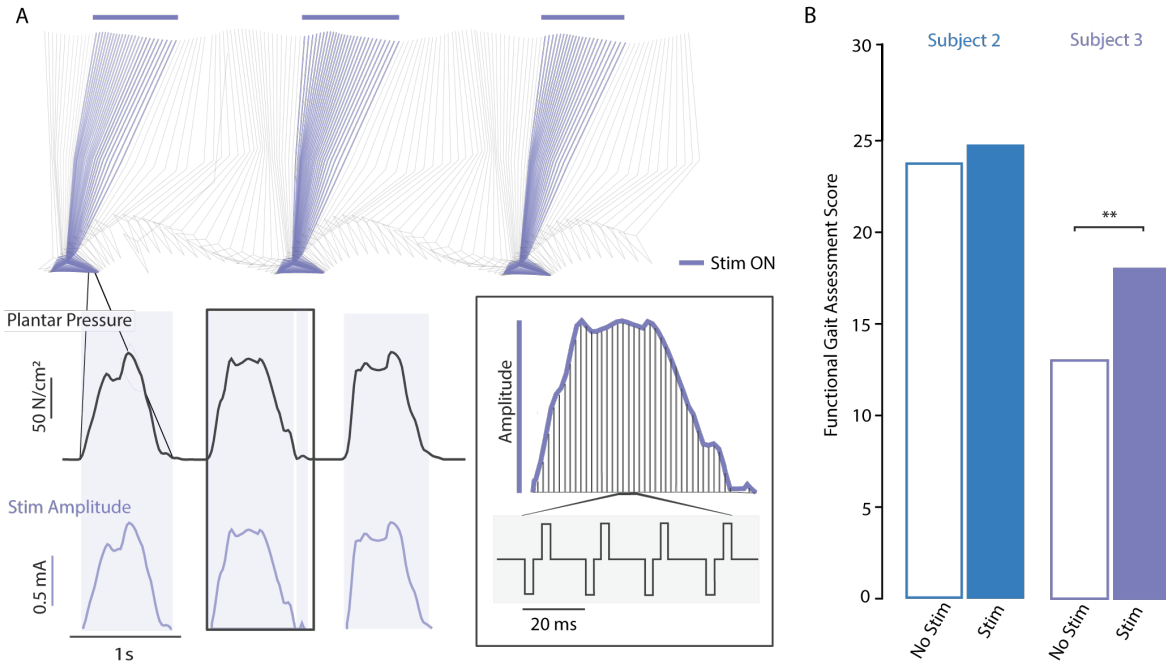


Figure 3.5: **Closed-loop sensory feedback improves gait stability.** (A) Example of amplitude modulation with plantahyr pressure throughout the gait cycle. Stimulation was triggered above a threshold for the metatarsals (purple shading) and amplitude was modulated linearly with pressure signals at a constant frequency. (B) The Functional Gait Assessment (FGA) increased in both subjects, but increased beyond the minimal clinically important difference (MCID, 4 points) for Subject 3, with the lower baseline score.[21]

3.2.6 Spinal Cord Stimulation Reduces PLP

To assess the impact of stimulation on PLP, we examined subjects' reports of their current pain level on a visual-analog scale. As the study progressed, we observed a clinically meaningful decrease in PLP score (defined as a 50% reduction from the baseline pain score) for Subjects 1 and 3 (Figure 3.6). While the PLP score from Subject 2 also decreased by more than 50% (0.73 from 1.2 points), this improvement is considered sub-clinical because it is less than 1 point. For both Subjects 1 and 3, the first clinically meaningful decrease in PLP coincided with the emergence of electrically evoked sensations in the missing limb (i.e. week 3 and week 2, respectively). For Subject 3, experiments were suspended over a one-week holiday (week 11), at which time pain scores increased sharply (3.65 times greater than week 10), consistent with the hypothesis that SCS relieves PLP.

We also conducted the McGill Pain Questionnaire[91] once per week, instructing subjects to rate their pain over the most recent week of the study. We observed a clinically meaningful decrease (>5 point decrease) for Subject 1 (28 points) and for Subject 2 (10 points) in the McGill Pain scores across the 4-week implant phase. For Subject 3, across the 12-week implant, there was a reduction in the pain scores until week 8 (15 points) followed by an increase from week 9 onwards, along with the break in testing during week 11. However, Subject 3 anecdotally reported a substantial reduction in PLP episodes.

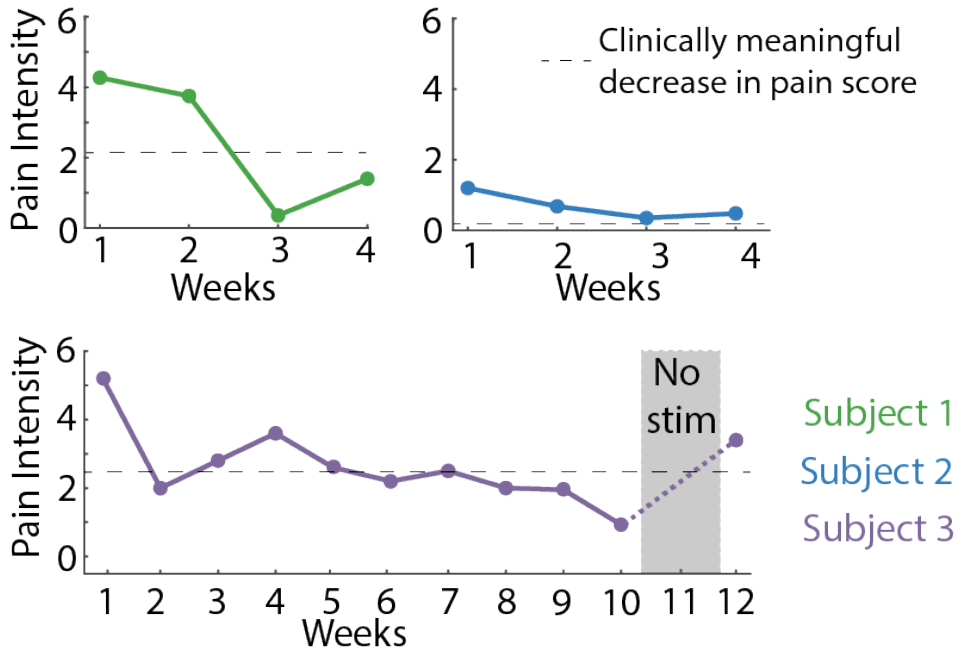


Figure 3.6: Phantom Limb Pain across weeks. The top panel shows the phantom limb pain intensity for Subject 1 and 2 (implanted for 4 weeks) and bottom panel for Subject 3 (implanted for 12 weeks). The dashed line indicates a clinically meaningful decrease in the pain score. For Subject 3, no experiment was conducted in week 11 (marked with a gray box).

3.3 Discussion

In this study, we demonstrate that lateral lumbosacral SCS evokes sensations in the missing limb in people with transtibial amputation, and that this restored somatosensation can improve balance control, gait stability, and reduce PLP. Importantly, we demonstrate these effects across a variety of subjects, including people whose amputations occurred long before enrollment in the study (up to 7 years), people with both traumatic and dysvascular amputations,

and people with different levels of mobility. Critically, the implantable electrodes used in this study were commercially available devices that are currently implanted in more than 50,000 people each year for the treatment of pain. The devices were implanted via a minimally invasive approach in an outpatient procedure, and future development and translation of our approach can leverage the vast infrastructure of clinicians and surgical techniques that already exist for SCS. While translation will still require substantial technical and clinical development, this study demonstrates the feasibility of using SCS to restore somatosensation from the missing foot to improve quality of life for people with a lower-limb amputation.

In all subjects, we found that multiple SCS electrodes evoked sensations in the missing limb, and each subject reported more than one sensation in different locations on the missing limb. However, we also found that the sensations evoked in the missing limb always co-existed with sensations in the residual limb. Subject 1 and 2 reported co-occurring sensations distinct areas of the residual and missing limb, whereas Subject 3 reported contiguous sensations spanning the residual and missing limb. In a previous study on people with upper-limb amputation, we observed similar coincidence of SCS-evoked sensations on the residual and missing limb in only two out of four subjects, and the sensations on the residual limb tended to be more focal in the arm than in the leg.[37] This difference may reflect anatomical differences between the cervical and lumbar regions of the spinal cord. Indeed, sensory neurons enter the cervical spinal cord at a shallow angle, nearly perpendicular to the rostrocaudal axis, whereas they travel parallel to the rostrocaudal axis for several segments in the lumbar cord before entering the spinal cord.[92] Accordingly, afferents are more densely packed in the lumbar region than in the cervical regions. Therefore, delivering charge in the epidural space using the large

commercially available SCS electrodes likely recruits more sensory afferents in the lumbar cord, increasing the likelihood of activating neurons projecting from both missing and residual limb.

The sensations reported in this study were also generally larger than those reported in the missing hand in our previous study. Additionally, in all three subjects, monopolar stimulation was sufficient to evoke sensations in the missing foot, while multipolar combinations of cathodes and anodes were typically required to evoke sensations in the missing hand. The receptive fields of tactile sensory afferents innervating the feed are larger and sparser than are those innervating the hand.[93] Stimulating the same number of neurons in the lumbar and cervical spinal cord is thus likely to evoke sensations in a larger area of the foot than the hand. Moving forward, designing SCS leads with smaller and more densely packed electrodes may allow us to achieve more selective stimulation and consequently, more focal sensations in the missing limb.

We also found that subjects did not report sensations in the missing limb until the second or third week of the study. During the first 1-2 weeks, a majority of reported sensations were diffuse and limited to the residual limb. Following this period, subjects would report consistent sensations in the missing limb. Other studies using peripheral nerve stimulation to evoke sensation in the missing foot have also reported that the incidence of sensations in the missing limb increases with time.[28] This delayed emergence of sensations stands in contrast to our previous study, in which subjects frequently reported sensations in the missing limb during intraoperative testing of cervical SCS.

We find that the magnitude of electrically evoked sensations can be systematically manipulated by modulating the stimulation amplitude, as has been previously found across a variety of stimulation modalities.[37,87] JNDs ranged from around 0.05 to 0.3 across subjects and leads (mean ≈ 1mA) and were independent of the reference amplitude, in violation of Weber's

law as has been found both for peripheral nerve stimulation[87] and for intracortical microstimulation.[94] Together, these results suggest that an average of around 20 discriminable steps can be achieved from threshold (typically less than 2 mA) to maximum amplitude (4-6 mA). The dynamic range of SCS stimulation-based tactile feedback is thus comparable to or wider than its counterparts in the peripheral nerve.[87]

A primary goal of this study was to demonstrate that restored somatosensation could improve standing balance and gait stability. We found that SCS-evoked somatosensory feedback improved standing balance, particularly in the more challenging conditions (in which visual and somatosensory feedback were altered), consistent using peripheral nerve stimulation.[25] Furthermore, the reduction of falls in the SOT with stimulation constitutes a critical improvement in balance control. Note that these substantial and clinically meaningful improvements in balance were observed despite the fact that evoked sensations extended from the missing limb onto the residual limb. While evoking focal sensations in the missing limb is likely to further improve balance, our results suggest even non-focal sensations projecting from both the missing and residual limb may be sufficient to improve function.

During gait, we saw a clinically meaningful improvement in the FGA for Subject 3. Notably, Subject 3 had a lower baseline FGA score, allowing us to see greater improvements with stimulation. Because this clinical assessment serves as a relatively crude measure of dynamic balance control during ambulation, it may not be sensitive to changes with sensory feedback and, like many other clinical measures, is subject to ceiling effects. Additionally, we have recently demonstrated that spatiotemporal analysis of level walking is insensitive to large differences in somatosensory ability across individuals with an amputation, and is similarly unlikely to be able to detect finer differences with a sensory neuroprosthesis.[95] These findings

indicate that, while we see improvements with stimulation, we should identify more sensitive and more challenging outcome measures to be able to detect finer changes in function with restored somatosensory feedback. Additionally, evaluating fall risk over a longer time period will be critical in future studies to demonstrate the clinical importance of restored somatosensation after amputation.

As SCS targets dorsal sensory afferents, the mechanism of these functional gains is likely a result of reflexive EMG responses evoked with stimulation or voluntary supraspinal modulation of these pathways.[96–98] In preliminary analyses of reflexive activity during gait on level ground, we did not observe significant changes in reflexive activity with SCS, which coincides with prior literature that shows cutaneous activity is not the primary driver of normal locomotion. Evaluating reflexive activity in more physically demanding tasks, in which afferent feedback is essential to maintaining fine motor control, would shed light on the mechanism of functional improvements with SCS.[99]

In addition to functional improvements, we also found evidence that stimulation reduced PLP. For Subjects 1 and 3, a clinically significant decrease in PLP was observed during the week in which they first reported experiencing evoked sensations in the missing limb. This observation is similar to the gradual decrease in PLP reported in other studies involving individuals with a lower-limb amputation and suggests neuroplastic changes in the brain may follow evoked sensations in the missing limb.[86] Furthermore, traditional SCS for treatment of chronic pain involves generating a paresthesia that overlaps with the location of pain. It is possible that treatment of PLP also requires evoked sensations in the missing limb. Subject 3 also reported that PLP increased when testing was paused for a week. The recurrence of PLP within a week of stopping SCS aligns with anecdotal evidence from traditional SCS studies which report that the

wash-in and wash-out period of SCS can be 3-7 days.[100] Our observations support growing evidence that somatosensory neuroprosthetic systems are associated with a decrease in PLP.[23,54,79–81] Future studies should explore the impact of stimulation dosing and the nature of evoked sensations on the time dynamics of pain relief.

This study marks an important step towards clinical translation of somatosensory neuroprosthetics for people with lower-limb amputation. There are, however, several important limitations that should be addressed in future studies. The subject pool in this study was small and heterogenous, including two people with dysvascular amputation and substantial mobility limitations and a third person with a traumatic amputation and a high degree of active mobility. Further, the study was not blinded to either the subject or the investigators. The goal of this study was to demonstrate initial feasibility of our approach, but in the future, larger randomized controlled trials will be critical for demonstrating that SCS can improve function and reduce phantom pain after lower-limb amputation. Additionally, the intervention in this study involved a percutaneously implanted device tested over 29 or 90 days in a lab-based setting. Future studies should include a fully implanted system, including an implantable pulse generator wirelessly communicating with external sensors on the prosthesis, as well as long-term testing of performance in real-world settings. Finally, the stimulation delivered during this study involved simple, constant frequency trains in which amplitude was modulated based pressure signals from an insole under the prosthetic foot. Importantly, almost none of the sensations evoked in this study were described as proprioceptive. More complex trains of stimuli, such as biomimetic patterns that more closely match the naturalistic patterns of activity in somatosensory afferents, may produce more naturalistic sensations including proprioceptive percepts, possibly with stronger effects on function and pain.

3.4 Methods

3.4.1 Subjects

Three subjects with transtibial amputation (Table 3) participated in this study. Two subjects had dysvascular amputations, while one subject had a traumatic amputation. All subjects were active users of a passive prosthetic limb before beginning the study. Two were limited community ambulators and one was an active ambulator (exceeding community ambulation skills, Subject 2). The study was approved by the University of Pittsburgh Institutional Review Board under an Investigational Device Exemption from the U.S. Food and Drug Administration and is registered at ClinicalTrials.gov (NCT04547582). Subjects provided informed consent prior to participation.

3.4.2 Electrode Implant

SCS leads were implanted percutaneously via a minimally invasive procedure, under local and/or twilight anesthesia. Subjects were in the prone position while leads were inserted using into the dorsal epidural space using a 14-guage Tuohy needle, and the leads were steered laterally using a stylet under fluoroscopic guidance. In Subject 1, two 16-contact leads (Infinion, Boston Scientific) were implanted near the T12-L2 vertebral levels and a third 16-contact lead was inserted through the sacral hiatus to target the cauda equina. The third lead did not produce useful sensations in the missing limb, so this type of insertion was not repeated in subsequent subjects. In Subject 2, two 16-contact leads (Infinion, Boston Scientific) were inserted near the T12-L2 vertebral levels. In Subject 3, three 8-contact leads (Octrode, Abbott Medical) were

inserted near the T12-L2 levels. Lead migration was monitored by comparing intraoperative fluoroscopic images to weekly X-rays for the first 4 weeks and then bi-monthly X-rays for the following weeks in Subject 3. In Subject 1, leads were anchored to the superficial layers of skin at the exit sites with sutures, and all three leads demonstrated substantial significant caudal lead migration across weeks during the implant. Therefore, to better stabilize the electrode placements, in Subjects 2 and 3 the leads were anchored to the subcutaneous fascia via a small incision. With this procedure, we saw minimal lead migration across weeks. At the end of the 29- or 90-day implant period, a similar procedure was performed to remove all leads from the body.

3.4.3 Mapping Evoked Sensations

To map the location of evoked sensations, we stimulated each electrode contact using a 1-second-long charge-balanced bi-phasic pulse trains. We stimulated with amplitudes from 0.5 mA to 6 mA and frequencies from 1 Hz to 1000 Hz. Pulse width was fixed at 200 μs and the interphase interval was set to 60 μs. Stimulation was delivered using a custom-built circuit board and three 32-channel stimulators (Nano 2+Stim; Ripple, Inc) as described in Chandrasekaran et. al.[37]

After each stimulation train, the subjects reported the location and quality of the sensation using our previously developed interface.[101] The quality of the sensations was described using a pre-defined list of descriptors developed specifically for characterizing sensations evoked by electrical stimulation[85] and included descriptors of mechanical, movement, tingle, and temperature sensations. For analytical purposes, we grouped these descriptors as sensations that subjects might experience commonly in their daily life (naturalistic) or rare, less familiar

sensations (paresthetic). In total, 13 descriptors were used for naturalistic modalities (pulsing, pressure, touch, sharp, tap, urge to move, vibration, flutter, itch, tickle, prick, cool and warm) and 5 descriptors were used for paresthetic modalities (electric current, tingle, buzz, shock and numb).

3.4.4 Psychophysical Analysis: Detection Threshold Estimation

We used a two-alternative forced choice task where the subjects were presented with two 1-second-long blocks with a variable delay period: one with stimulation and one without stimulation, assigned randomly. The subjects were instructed to select the block they felt a sensation. The stimulus amplitudes were centered around the rough detection threshold we observed from the mapping trials in the specific day. Overall, stimulus amplitudes ranged from 0.5 to 6 mA and each amplitude was repeated 4-10 times. The stimulation frequency remained constant at 50 Hz for all stimuli. For each stimulus amplitude, we calculated the number of times the subject responded correctly (accuracy rate). For electrodes with dense amplitude sampling, the values were re-binned with 0.1 mA steps and the amplitudes that fall in the same interval were replaced by their mean. Constrained logistic psychometric curve, with a minimum at 0.5, was fit to the accuracy rate and the stimulus amplitude corresponding to 75% accuracy rate was selected as the detection threshold. Electrodes with insufficient repetitions per condition (<5) or poor logistic fit (goodness of fit of the model is insignificant at 10%) were excluded from analysis.

3.4.5 Psychophysical Analysis: Just-Noticeable Differences

We used a similar two-alternative forced choice task like the detection threshold experiments. On each trial, two stimuli were presented, and the subject's task was to report which of the two felt more intense. Each pair consisted of a reference pulse train with fixed amplitude throughout the set and a test train with amplitude ranging from 50 to 150% of that reference amplitude. Reference amplitudes on different sets ranged from 1-3.5 mA for Subject 1, 1.2-4.55 mA for Subject 2 and 2-4.74 mA for Subject 3. The frequency and pulse width remained constant (50 Hz, 0.2 ms) for all stimuli. In each set, each stimulus pair was presented at least 5 times, and both the order of stimuli within the pair and the order of the pairs were varied pseudo randomly.

Generalized linear model with logit link function was fit to the percentage of times the test interval was selected by the subject to obtain psychometric curves for each reference amplitude. Then, just noticeable difference (JND) was calculated as the change in amplitude that led to 75% accuracy according to the psychometric curve. Sets with poor psychometric fits (goodness of fit of the model is insignificant at 10%) were omitted from the analysis.

3.4.6 Psychophysical Analysis: Perceived Stimulation Intensity

To understand the relationship between the stimulus amplitude and the perceived intensity, we conducted a magnitude estimation experiment. On each trial, a 1s-long pulse train was delivered, and the subject's task was to state a number whose magnitude corresponded to the magnitude of the evoked sensation. Subject were instructed to use their own scale including fractions. If a stimulus was imperceptible, it was ascribed the number 0. If one stimulus felt

twice as intense as another, it was given a number that was twice as large. The tested amplitudes ranged from 0.5 to 6 mA and were restricted to above detection threshold for each channel. The maximum stimulus delivered was below the pain threshold for all the subjects. Each test amplitude was presented at least 5 times.

Ratings were normalized by the mean rating on their respective set and linear regression was fit to the observed data for each channel separately. The residuals of regression models were tested for normality with Kolmogorov-Smirnov test to justify linear fit.

3.4.7 Closed-loop Stimulation

To use these evoked sensations for real-time feedback in a functional task, like gait or balance, wireless plantar pressure sensing insoles (Moticon Insole 3, Munich, Germany) triggered stimulation in real-time. Plantar pressure in the same region as the mapped sensation triggers stimulation above a certain threshold. For Subject 2, when testing in free-standing (without the standing frame), he experienced sustained quadriceps contractions above 2.5 mA. Because of the small range of stimulation amplitudes available (2.25-2.5 mA), we utilized constant amplitude stimulation for this subject, in which stimulation turned on above the pressure threshold and turned off below threshold. For Subject 3, plantar pressure linearly modulated stimulation amplitude, as she put more weight on her metatarsals, she felt a more intense sensation (Figure 3.1). Stimulation was kept at constant frequency (50 Hz Subject 1, 90 Hz Subject 2) and pulse width (200 us) and amplitude was updated at 50 Hz.

3.4.8 Functional Tasks

For gait tasks, the Functional Gait Assessment (FGA) and kinematics of walking on a level surface were evaluated. The FGA is a 10-item test of dynamic balance, including challenging items like walking with eyes closed, walking with a narrow base of support, and walking backwards. Each item is scored 0 to 3 points, where 3 indicates no impairment and 0 indicates an inability to complete the task. Gait kinematics were recorded with a 16-camera OptiTrack motion analysis system (Natural Point, OR, USA). Participants were instructed to walk at their self-selected speed across a 6-meter walkway. For Subject 3, 14 trials of walking without stimulation and with stimulation were analyzed. For Subject 4, 28 trials without stimulation and with stimulation were compared across two sessions. Sixteen reflective markers were placed on anatomical landmarks according to the OptiTrack "Conventional Lower Body" model. Data was collected at 100 Hz and filtered using a 4th order low-pass Butterworth filter at 12 Hz.

The Sensory Organization Test (SOT) was used to determine changes in balance ability using the NeuroCom Equitest system (Figure 3.4A). The SOT is a clinical measure of reliance on visual, vestibular and somatosensory systems for balance using six conditions where either the surround or platform sway. Three, 20-second trials were completed per condition. The SOT was completed pre-implant without stimulation and again with stimulation. Center of pressure (COP) traces was recorded at 100 Hz, filtered with a low-pass fourth-order Butterworth filter, and analyzed for biomechanical and clinical measures of posturography. Standard posturography measures were calculated, including excursion, sway velocity, 95% confidence interval ellipse of sway area, sample, and approximate entropy. The primary clinical outcome measure for each

condition is the equilibrium score, a measure of the participant's ability to stay within a normative 12.5° of anteroposterior sway (Figure 3.4B).

For clinical measures, published standards for clinically meaningful difference (CMD) or MDC were used to compare baseline and stimulation trials.[68,102,103] For biomechanical measures of balance and gait, comparisons between outcomes were performed using permutation testing, a non-parametric method often used for smaller sample sizes. We completed 10,000 permutations of both baseline and with stimulation groups and the difference in means was determined. The p-value in permutation testing is the count of permutations in which the observed difference in means is above the actual difference in means. An alpha of 0.05 was used for all statistical analyses.

4.0 Spinal Cord Stimulation Improves Balance and Gait in Individuals with Lower-Limb Amputation: A Case Study.

4.1 Introduction

Most individuals with lower-limb loss struggle with gait and balance impairments, likely due to a lack of sensory feedback from the missing limb. Though a large body of work has focused on improving myoelectric control of a prosthesis, prosthetic control cannot be complete without sensory feedback from the prosthetic limb.[100,101] Many biofeedback systems that provide sensory feedback through visual or auditory cues can improve mobility, however these systems are focused on short-term training and not long-term use.[21,104] Sensory neuroprostheses are designed as a long-term treatment and have shown improvements in balance and gait, mostly in challenging conditions.[23,25,26] This is in large part due to the lack of appropriate functional outcome measures to evaluate the effectiveness of sensory neuroprostheses. Standard outcome measures for people with an amputation are not sensitive to changes in sensation and are not challenging enough for the patient populations in these studies.[105] However, new tasks in both clinics and clinical research labs may be able to better discern changes with the addition of somatosensory feedback. The aim of this study is to evaluate the functional effects of using a somatosensory neuroprosthesis in these more robust measures of balance and gait.

Sensory feedback from the foot is critical for maintaining static balance and dynamic stability with walking.[4,11] Both tactile sensation (sense of touch) and proprioception (sense of joint movement) improve motor control during balance and facilitate healthy gait mechanics and

muscle activation.[4,11,73,106] Furthermore, sensory neurons trigger monosynaptic reflexes that recruit coordinated groups of muscles in both legs to protect from falls.[4,107,108]

A lower-limb amputation removes tactile receptors in the plantar foot and proprioceptive receptors in the ankle results in a lack of sensory feedback coming from the missing limb. Not surprisingly, individuals with a lower-limb amputation rely heavily on vision to maintain their balance to compensate for the lack of somatosensation.[61] Relying on vision puts these individuals at a substantially higher risk of falls in situations with low vision (i.e. low lighting environments).[1] Additionally, slips and trips are a common cause of falls in people with an amputation, likely due to the lack of sensory inputs to trigger protective reflexive pathways.[2,109,110] Restoring sensory feedback is necessary to improve balance and gait stability in individuals with a lower-limb amputation.

To restore sensory feedback, sensory neuroprostheses leverage the intact afferent pathways, usually activated by plantar sensation, by electrically stimulating either the peripheral nerves or the spinal cord. In neuroprostheses that use peripheral nerve stimulation, evoking sensations in the missing limb in real-time during gait can improve function.[24–26,29] Similarly, SCS-evoked sensory feedback can improve function on the more physically demanding aspects of traditional clinical measures of balance and gait (Chapter 3.0).

Notably, improvements with the use of somatosensory neuroprosthetics are almost exclusively seen in challenging functional tasks, not the standard clinical outcome measures for individuals with a lower-limb amputation. For example, studies using peripheral nerve stimulation have used a figure-of-eight test walking on sand and an ambulatory searching task, in which participants walk across a horizontal ladder blind-folded.[26,29] The participants in these studies were individuals with traumatic amputations and very high mobility levels, beyond the

usual community ambulation. Though these tasks challenge sensory systems, individuals with less mobility (e.g. limited community ambulators) would likely not be capable of performing these tasks. While most individuals with an amputation can perform the standard clinical measures, such as the Sensory Organization Test, we have shown that these outcomes are not able to detect even large differences in sensation across individuals with an amputation (see Chapter 2.0). Consequently, these standard measures are unlikely to be able to detect differences in function with the addition of sensory feedback. Thus, here we use more robust measures to detect meaningful functional effects of sensory feedback.

The usual clinical measures for people with a lower-limb amputation are designed to provide an overall assessment of functional status. These measures are not intended to evaluate the impact of sensory feedback. New tasks developed for both clinics and clinical research labs may be able to better discern changes with the addition of somatosensory feedback. The aim of this study is to evaluate the functional effects of using a somatosensory neuroprosthesis using these more robust measures of balance and gait.

In most balance tasks, individuals with an amputation often compensate for a lack of somatosensory feedback from the feet by relying on vision or the intact limb to maintain stability.60,109 Thus, we need tasks that restrict potential compensation for these deficits to determine the effects of sensory neuroprostheses. We will target balance on the prosthetic side by using single leg stance (SLS) time and target the somatosensory system by manipulating balance with and without visual feedback (visual feedback test, VFT).

To evaluate the effects of sensory feedback on gait, more challenging tasks are being used for individuals with an amputation to differentiate abilities within the group. The Narrowing Beam Walking Test (NBWT) is a novel gait task designed specifically for individuals with a

lower-limb amputation (Appendix C). The participant must walk on a beam with their arms crossed across their chest as the beam tapers from a wide beam (18.6 cm across) to a very narrow beam (2 cm across). This task was specifically designed to evaluate walking with a narrow base of support, as this is a situation that individuals with an amputation can have difficulty with day-to-day. Individuals with an amputation also often have difficulty walking across uneven surfaces (Appendix C). Thies et al. designed a standardized uneven surface to simulate walking over more challenging surfaces, like grass or gravel.[36,111] This assessment has also been shown to be challenging for individuals lacking plantar sensation due to peripheral neuropathy.[36] Measuring gait variability, specifically trunk acceleration RMS, while walking on uneven surfaces has been shown to increase in all directions for individuals with amputation.[112] The nature of these tasks require additional sensory input from the plantar feet and therefore are likely to be able to discern changes with sensory feedback.

Not only do these tasks test reliance on somatosensory feedback, they also mimic challenges faced in daily life for individuals with an amputation, providing further insight into situations that increase risk of falls. Using these more robust measures of balance and gait, we aim to evaluate the functional effects of sensory neuroprostheses, using SCS as a commercially available alternative to peripheral nerve stimulation.

4.2 Methods

One subject with a transtibial amputation was recruited for this study, a 69-year-old female with a dysvascular amputation. She was classified as a limited community ambulator, meaning she used an assistive device to walk in the community but ambulated independently

without a device for small distances within the home (K2 classification from Amputee Mobility Predictor [AMP Pro]).

4.2.1 Implant Procedure

In a minimally invasive procedure, three leads with 8 contacts each (Octrode, Abbott Medical) were implanted into the thoracolumbar epidural space to target the lumbosacral spinal cord. Subject was in the prone position while the leads were inserted percutaneously. Leads were steered under fluoroscopic guidance and spanned the T12-L2 vertebral levels. Lead migration was monitored closely by evaluating weekly radiographs for the first four weeks and then bi-monthly for the following weeks. Leads were sutured into the subcutaneous fascia to minimize migration for the duration of the 90-day study. At the end of the 90 days, the leads were explanted in a similar outpatient procedure.

4.2.2 Characterizing Evoked Sensations

First, we stimulated each electrode in all three leads at varying amplitudes (Nomad; Ripple, Inc.). Stimulation was delivered the custom-built circuit board and three 32-channel stimulators (Nano 2+Stim; Ripple, Inc.) used in our previous studies.[37] During initial testing, the subject was asked to report the location and quality of evoked sensations on a customized user interface.[101] The electrodes that most reliably evoked sensations on the plantar aspect of the foot were selected for sensory feedback (Figure 4.1). In this subject, we selected the electrode that evoked sensation extending into the toes and metatarsals. Prior to any functional testing,

extensive psychophysical experiments were conducted to determine how to modulate sensations to use as sensory feedback.

Experiments were performed to determine how stimulation amplitude modulates intensity of the perceived sensations. For these experiments, we delivered 1 s-long pulse trains at linearly spaced amplitudes (ranging from detection threshold to highest tolerable amplitude up to a maximum of 6 mA) and asked the subject to score their perceived intensity of the stimulus on an open-ended linear scale. The subjects were instructed to scale their responses linearly, meaning if the perceived intensity for a trial that is double the previous trial, they should report double the score from the previous trial. Amplitudes were repeated at least 5 times. Ratings were normalized to the mean rating on each set and linear regression was fit to the observed data. These results determined the usable range of amplitudes (amplitudes that were reliably perceptible without being uncomfortable or distracting) and ensured a linear relationship between intensity and amplitude of stimulation for closed-loop experiments (Figure 4.1B).

4.2.3 Stimulation as Real-Time Sensory Feedback of Plantar Pressure

To use these percepts as real-time sensory feedback, plantar pressure was used to trigger stimulation on the electrode mapped to the corresponding region of the plantar foot (Figure 4.1C-D). Above threshold, plantar pressure on the metatarsals sensed by wireless pressure-sensing insoles (Moticon Insole 3, Munich, Germany) was used to modulate stimulation amplitude. Stimulation amplitude varied linearly with plantar pressure within the pre-determined amplitude range from magnitude estimation results on that electrode (Figure 4.1D). A series of gait and balance tests were performed using this real-time stimulation. All assessments were performed with a trained physical therapist to ensure safety.

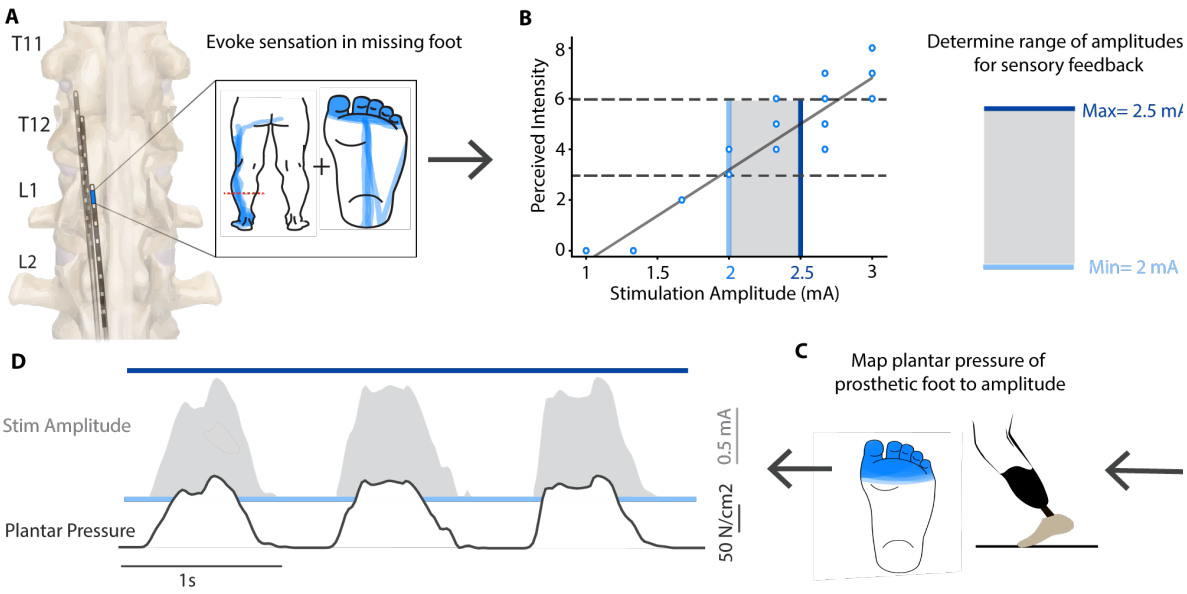


Figure 4.1. Closed-loop stimulation parameter selection and amplitude modulation. (A) We mapped and characterized sensations evoked with lumbosacral SCS. (B) On an electrode that reliably evoked useful sensations in the missing foot, we performed psychophysical tests to determine the usable range of amplitudes. (C) Plantar pressure on the corresponding region of the prosthetic foot was mapped to stimulation amplitude. (D) In a functional task, like gait or balance, stimulation amplitude was mapped linearly to plantar pressure within the pre-determined range of amplitudes found in (B).

4.2.4 Balance Tasks

4.2.4.1 Balance on Prosthetic Limb

With many balance analyses, participants compensate with their intact limb. To adapt for this, we assessed standing balance on the prosthetic side without the use of assistive devices (i.e. a cane or a walker), which restricts compensation from the intact contralateral limb. At baseline, pre-implant, SLS was timed without stimulation as part of the Amputee Mobility Predictor. During the 90-day study, SLS was tested both with and without stimulation for 10 trials each on

two separate sessions, one at one month through the study, the other at two months. Twenty trials were performed: ten with stimulation and ten without stimulation in randomized order.

4.2.4.2 Visual Feedback Test

We implemented a novel task to measure how well the participant can manipulate their balance with sensory feedback. The test was performed on the Neurocom Equitest system (Natus Inc.), which allows for real-time visual feedback of the center of pressure on a computer monitor fixed at eye level. Each condition contained four targets, set to 50% of the subject's limits of stability in each direction (anteroposterior, left and right). The subject was asked to shift her weight to the target and maintain her balance there for the remainder of the cue. The task includes 5 blocks of 3 trials each, with 2 repetitions per trial (8 targets/trial). Two blocks were performed without stimulation, first with visual feedback (in which a cursor indicates the subject's center of pressure) and then without visual feedback (no cursor). Two blocks without stimulation were performed, first with visual feedback and then without visual feedback. Lastly, one block without stimulation and without visual feedback was performed to assess effects of fatigue over time.

4.2.4.3 Balance Confidence

To assess balance confidence, the Activities-specific Balance Confidence Scale (ABC) was used.[113] This test is validated in individuals with lower-limb amputation. The questionnaire was completed at baseline and again at the end of the study. To determine the difference in balance confidence with stimulation, the participant was asked to answer the questionnaire with respect to how they felt performing the respective tasks with stimulation.

4.2.5 Gait Tasks

For all gait tasks, kinematics were recorded with a 16-camera OptiTrack motion analysis system (Natural Point, OR, USA). Sixteen reflective markers were placed on anatomical landmarks according to the OptiTrack "Conventional Lower Body" model.[71] For uneven walking, one additional marker was placed on the L5 vertebral level to analyze trunk kinematics as a measure of gait smoothness. Data was collected at 100 Hz and filtered using a 4th order low-pass Butterworth filter at 30 Hz.

4.2.5.1 The Narrowing Beam Walking Test

The NBWT is a new clinical assessment used to evaluate individuals with amputation across all ambulation levels (Appendix C).[34,35] The narrowing beam is a set of four, 1.83 m (6 ft.) beams of narrowing widths starting at 18.6 cm (7.3 in.) wide for the widest beam down to 2 cm (0.8 in.) wide at the narrowest beam. Each beam sits 3.8 cm off the ground. Participants are asked to walk as far as possible along the beam with their arms across their chest. The trial was ended when the participant uncrossed their arms or stepped off the beam, and the distance walked was recorded to the nearest 15.24 cm (0.5 ft) increment. Five trials were performed with and without stimulation (10 trials total) in a randomly assigned order. The distance for each trial was normalized to the 6.71 m (22 ft) length beam. The trials with stimulation and without stimulation were separated and the average of the last three trials for each condition was recorded.

4.2.5.2 Walking on Uneven Surfaces

Walking on uneven surfaces is a biomechanical assessment of step to step gait symmetry while walking on more challenging terrain. For this, we custom-built a 10-m walkway with wooden prisms attached in a randomly distributed manner, as described in previous publications (Appendix C).[9,36] An industrial carpet was placed over the walkway to mimic an irregular surface walking outdoors. The participant completed twelve trials with stimulation and twelve trials without stimulation at her self-selected speed. Stimulation and no stimulation trials were split into blocks of six trials each and order were pseudo-randomized to account for any fatigue or learning effects with time.

For measures of step-to-step symmetry, the harmonic ratios (HR) of trunk accelerations were utilized as a measurement that combines gait variability of kinematics, kinetics, and motor control.[114] Use of HRs for both level and irregular surfaces was found to be predictive of falls in older populations and populations with sensory impairments.[115–118] HR is an analysis of step symmetry in the frequency domain. The analysis of the harmonics of the signal assumes periodicity within each stride, with a normal acceleration pattern occurring in multiples of two, once for each limb.[119] Thus, if trunk acceleration occurs in a frequency that does not follow this pattern, that is considered “out-of-phase”. Perfect symmetry would allow for only “in-phase” harmonics. In the anteroposterior or craniocaudal axes, “in-phase” harmonics consist of even harmonics, while in the mediolateral axis, “in-phase” harmonics are odd harmonics. Through a Fourier analysis, “in-phase” harmonics are compared with “out-of-phase” harmonics, yielding a HR.[120] For more in-depth description of the HR, see Appendix D. Step width variability (coefficient of variation) and step length asymmetry (normalized to stride length) were also evaluated as measures of gait variability.

4.2.6 Statistical Analysis

For the NBWT, published standards for minimum clinically important difference (MCID) was used to compare baseline and stimulation trials.[34] For all other measures, statistical comparisons between outcomes were performed using permutation testing. Permutation testing is used for small, non-parametric samples. Permutations of both baseline and with stimulation groups were completed (n=10,000) and the difference in means was determined. A p-value is the number of permutations in which the observed difference in means is above the true difference in means out of the total number of permutations. An alpha of 0.05 was used for all statistical analyses.

4.3 Results

4.3.1 Sensory Feedback Improved Balance on the Prosthetic Limb

When combined across sessions, the subject was able to balance on her prosthetic side for 1.045s more ($p=0.045$), which is a 40% increase in time (Figure 4.2). For the intact limb, there was no significant difference in time spent in SLS with stim and SLS without stim ($p>0.05$).

Sensory feedback also improved the subject's manipulation of balance on the VFT. With stimulation, there were significant improvements in target accuracy for the posterior target only (+1.465 au, $p=0.023$) with respect to the last condition without vision or sensory feedback (Figure 4.3). There were no significant differences between conditions with visual feedback ($p>0.05$). Target error across trials increased with time.

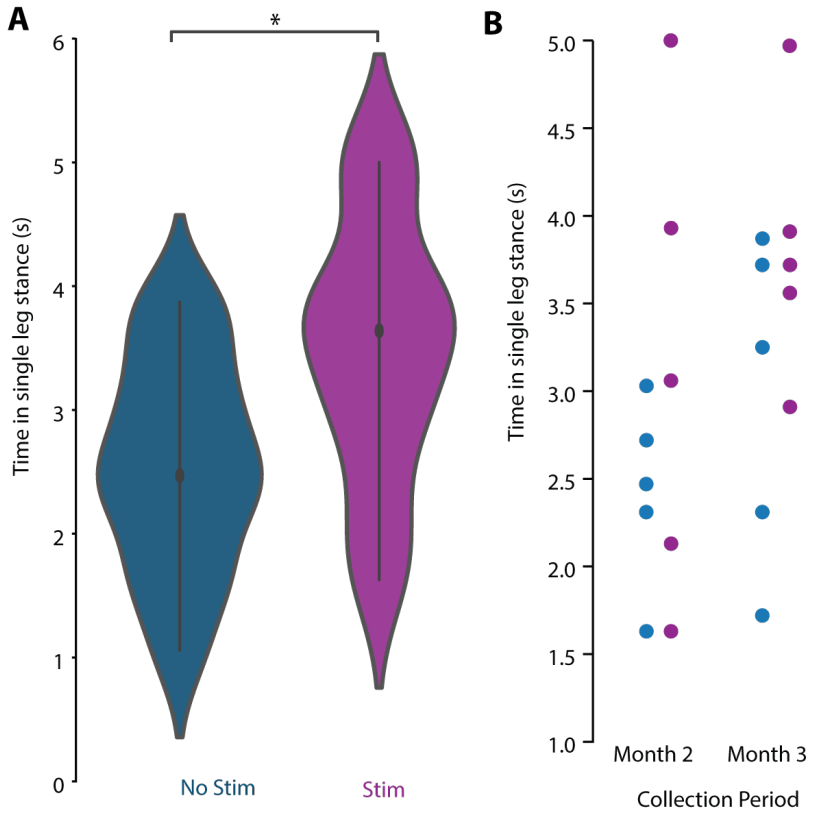


Figure 4.2. Single Leg Stance (SLS) time on the prosthetic leg. (A) Combined across sessions, there was a significant increase in time spent standing on the prosthetic leg (p=0.045) with stimulation (magenta) compared to without stimulation (teal). (B) Per session SLS time increased with stimulation.

With stimulation, the subject had a 15.65% improvement in confidence, meeting the threshold for a minimum clinically important difference in balance confidence (60.6% confidence at baseline, 76.25% confidence with stimulation, MCID=14.36% improvement).

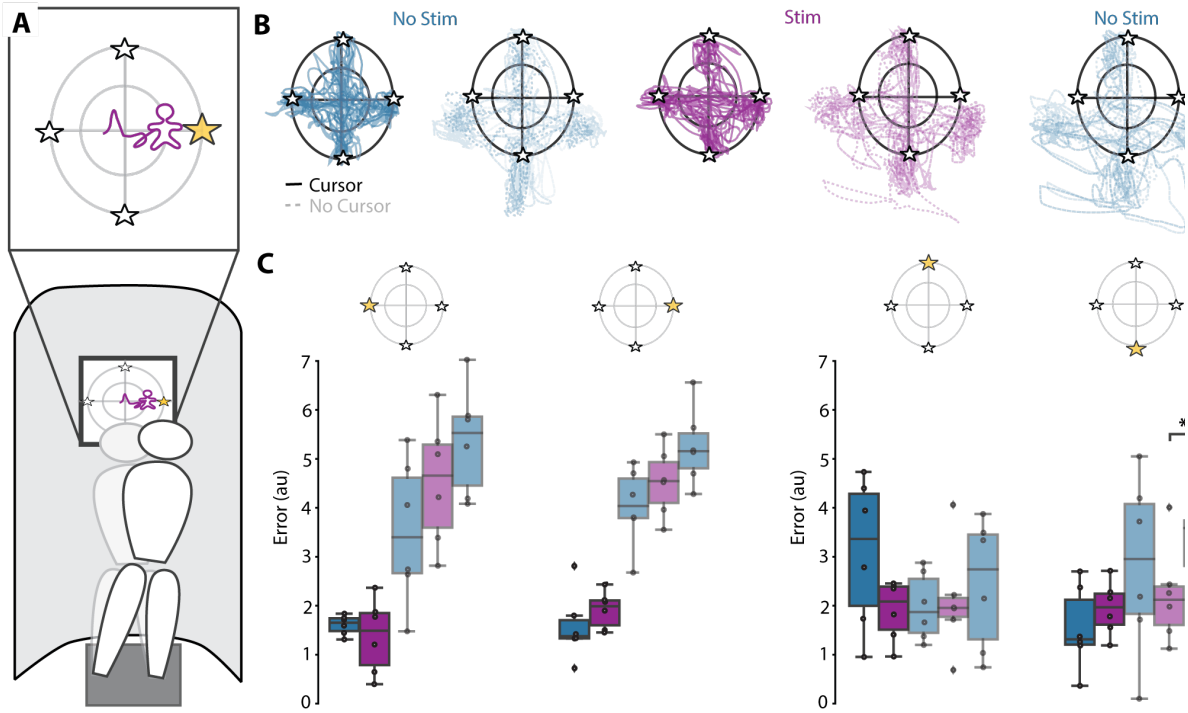


Figure 4.3. Visual feedback test (VFT) without stimulation (teal) and with stimulation (magenta). (A) The subject stands in the Equitest and shifts their center of pressure (COP) to the desired target (star). For conditions with visual feedback, the subject had a cursor indicating their COP throughout the trial. (B) Individual COP traces for each condition. Targets were presented right, back, left, front. These were repeated twice for a total of eight targets per condition. Conditions were performed both with visual feedback of the subject's COP (cursor, darker shades) and without visual feedback (no cursor, light shade). (C) Error (distance between actual COP and the target) by target location. There was a significant decrease in error with respect to the posterior target (far right) between the stim without cursor condition and the last no stim without cursor condition ($p=0.023$).

4.3.2 Gait in Challenging Conditions Improves with Stimulation

There was a clinically meaningful difference (CMD) in the distance walked in the NBWT with stimulation (4.013 m [0.60 au] with stimulation, 2.895 m [0.43 au] without stim, CMD=0.96 m [.14 normalized] improvement, Figure 4.4A-B). Additionally, pelvis sway without sensory feedback displayed a more irregular gait pattern versus pelvis sway with sensory feedback.

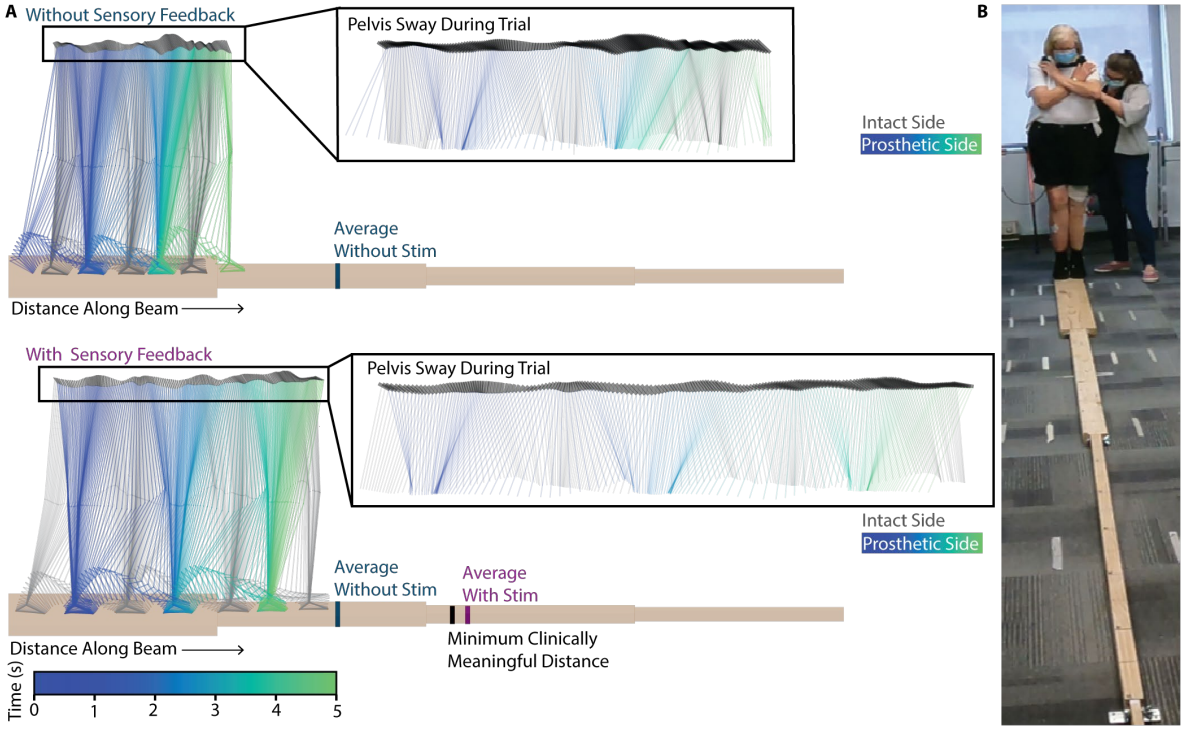


Figure 4.4. Stimulation improves walking with the Narrowing Beam Walking Task (NBWT). (A) Trial without sensory feeback (top) and with sensory feedback (bottom). The teal bar indicates the average of the trials without stimulation and the magenta represents the average of trials with stimulation. With stimulation, the subject exceeded the threshold for the clinically meaningful difference (CMD, black bar). Additionally, pelvis sway without stimulation was more irregular, whereas maintained a smoother trajectory throughout stimulation trials. (B) Subject on the narrowing beam. Assessments were performed with supervision of a physical therapist for safety.

For walking on an uneven surface, the HR of trunk acceleration in the mediolateral direction improved significantly (0.564 au, $p<0.01$, Figure 4.5A-C). HRs in the anteroposterior or longitudinal directions improved, but not significantly ($p>0.05$). There were no significant differences in step width variability or step length asymmetry ($p>0.05$).

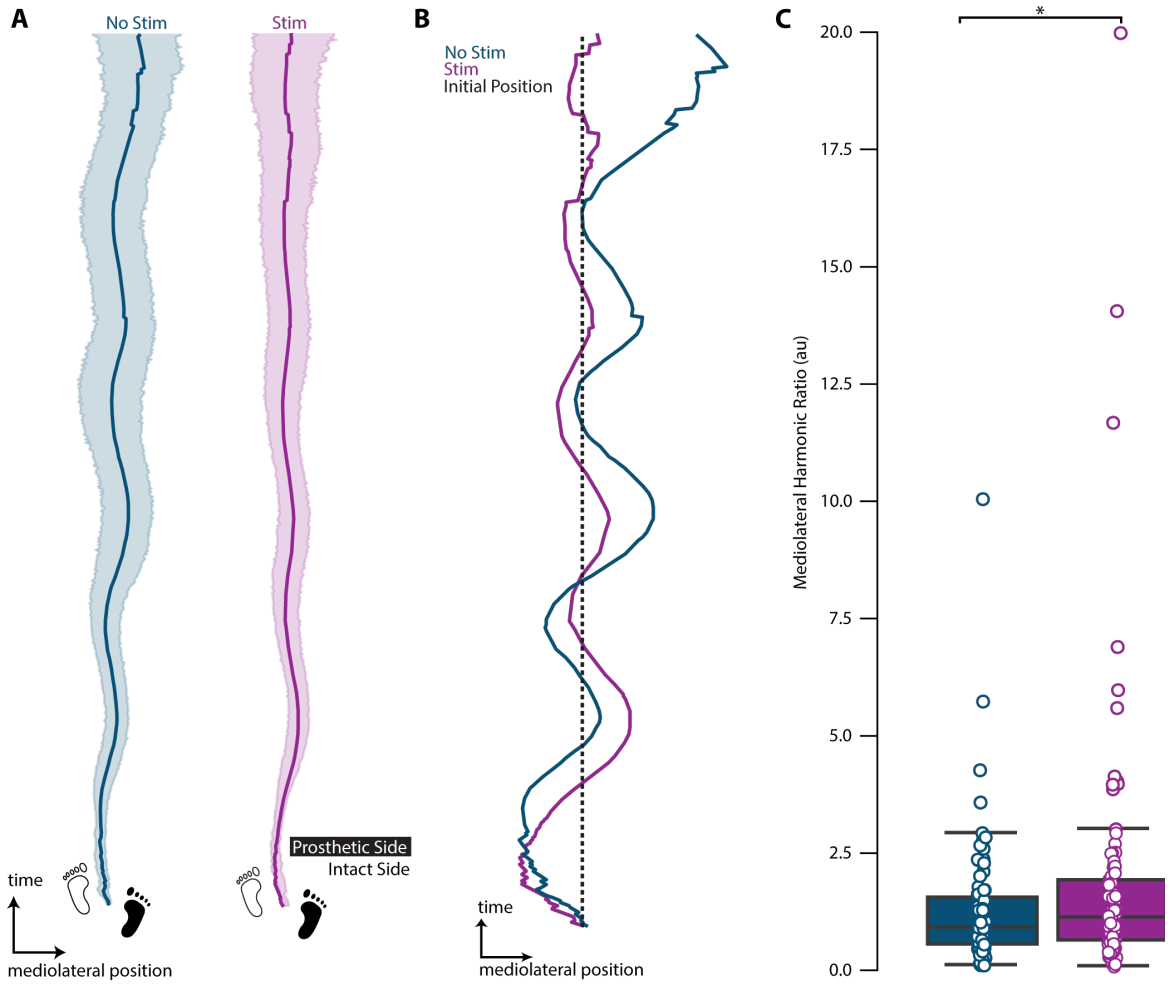


Figure 4.5. Stimulation effects walking on uneven ground. (A) Trunk position in the mediolateral direction while walking on the uneven surface. (B) Ennlarged mean trunk position centered around the initial trunk position (dotted line).While the trunk oscillates evenly around th initial position with stim (magenta), trunk position is not as symmetrical without stim (teal). (C) Harmonic ratio in the mediolateral direction improved significantly (0.564 au, $p<0.01$) with stimulation.

4.4 Discussion

This is the first study to demonstrate significant and clinically meaningful improvements in balance and gait with SCS-evoked sensory feedback in more robust functional measures. These findings corroborate work from groups using peripheral nerve stimulation for sensory neuroprostheses, which found improvements with sensory feedback in balance and gait on challenging conditions. Notably, the measures used here showed improvements in one individual with limited mobility, whereas most participants in previous studies had advanced mobility skills (K3 or K4 level ambulators, indicating abilities beyond usual community ambulation). As nearly half of individuals with an amputation use an assistive device for community ambulation, including our participant, they are likely not able to complete the tasks used in previous studies. Here we demonstrate that more robust functional tasks capture the extent of improved stability with sensory feedback, even in individuals with limited mobility. During this study, we found improvements with sensory feedback in both balance and walking.

Throughout the study, we found that SCS sensory feedback consistently improved SLS time on the prosthetic limb. These observed balance improvements on this assessment isolate the effect of restoring sensation to the prosthetic limb, as SLS restricts any potential compensation from the contralateral intact limb to maintain balance. Furthermore, sensory feedback improved the subject's ability to shift their weight toward specific targets on the VFT. Accuracy improved on the posterior target as expected, given the location of the evoked sensation was on the toes and metatarsals. Because sensory feedback provided information regarding the subjects' anteroposterior pressure on that limb, we expected improved accuracy in the corresponding anteroposterior targets more than mediolateral targets. The lack of significant changes in accuracy for the anterior target, however, is likely due to fatigue, as the anterior target was

presented last in each sequence. Not surprisingly, performance was worse for the anterior target both with and without sensory feedback. Unfortunately, the system we use to complete the VFT does not allow for randomization of targets within each trial. Future changes to allow for randomization of targets would reduce the effects of fatigue on the accuracy for any one target. Additionally, fatigue played a clear role in the accuracy of targets across conditions, with accuracy decreasing substantially over time. Adding rest breaks and selecting only the relevant target directions may improve the robustness of the measure going forward.

SCS-evoked sensory feedback also improved balance confidence. Balance confidence is associated with reduced fall risk and is therefore an important factor in improving function in individuals with lower-limb amputation. These improvements occurred over the course of two months, suggesting these improvements have the potential to continue with longer interventions with SCS-evoked sensory feedback.

For walking, we observed significant improvements in both walking with a narrow base of support (on the NBWT) and walking over uneven surfaces. Improvements on these tasks, but not on more simple gait tasks, such as walking on level surfaces, are expected given the larger role of sensation in challenging walking tasks in animal models.[96,99] Walking with a narrow base of support requires both cutaneous and proprioceptive input from the lower extremity to maintain mediolateral stability.[96,99,121] Furthermore, this subject's improvement in NBWT distance (0.43 to 0.60) moved her from the fall risk category ($\leq$0.43) to a low-risk category for individuals with a unilateral lower-limb amputation.[122] On uneven surfaces, we saw significant improvements for the gait step to step symmetry in the mediolateral direction, but not the anteroposterior or longitudinal directions. These findings are in accordance with previous studies that demonstrated

reductions in mediolateral stability only for individuals with impaired plantar sensation when walking on an uneven surface.[123]

This study was a case study and only included one subject; additional subjects are needed to analyze the full extent of these functional effects. Furthermore, with SCS, we were unable to obtain focal sensations localized to the missing foot only. Ideally, sensory feedback would induce sensations that the subject would usually experience during walking from a small area on the plantar foot. Instead, evoked sensations extended from the residual limb into the missing foot in a dermatomal pattern (see Chapter 3). Despite the lack of focality, our results indicate that SCS-evoked sensory feedback is beneficial for balance and gait.

In one subject, we have demonstrated that using a commercially available approach for a sensory neuroprosthesis provides functional improvements on outcome measures that both challenge sensory feedback and can be completed by individuals with a wide range of mobility. Studies with peripheral nerve stimulation may also benefit from utilizing these more robust gait and balance measures to assess sensory neuroprostheses. Additionally, using these measures to may provide a clearer and more clinically meaningful picture of potential functional improvements with stimulation for the field of lower-limb neuroprosthetics.

5.0 Cross-Modal Congruency Task to Quantify Integration of Sensory Feedback

5.1 Introduction

After an amputation, the lack of sensory feedback from the prosthesis can lead to substantial functional impairments during balance and gait. Providing this missing sensory feedback may be a critical aspect to improving these functional deficits. To this end, a variety of techniques can provide or augment sensory feedback, including sensory substitution (using a separate modality to compensate for the lack of sensory feedback) or sensory restoration using peripheral nerve stimulation or SCS.[17–20,23–27] With sensory substitution, using visual or auditory cues as sensory feedback is generally not inherently intuitive to the user and requires a learning period to be able to interpret the feedback reliably.[22] This need for interpretation of unintuitive feedback can increase the cognitive load required for balance or gait tasks, hindering performance in attention-demanding motor tasks, in which falls are more likely.[21] Similarly, stimulation of the remaining afferent fibers in sensory restoration studies evokes unintuitive sensations in the missing limb. Subjects most commonly report experiencing buzzing or tingling sensations, instead of more "natural" sensations, such as touch or pressure.[37,53,124] Feedback that is "natural" and intuitive will be more easily integrated with one's neural schema, via multi-sensory integration, and has the potential to provide greater functional benefits to the user.

Unlike constant frequency stimulation that evokes unintuitive sensations, recent studies have investigated the use of biomimetic stimulus patterns that more closely match typical afferent firing patterns.[40,125] Seminal work in individuals with upper-limb amputation has shown enhanced improvements in task performance when using biomimetic patterns.[126] However, we

do not yet know to what extent intuitive sensations benefit functional tasks, largely due to a lack of reliable metrics that can quantify multi-sensory integration of sensory feedback. In lieu of such measures, research has relied heavily on subjective "naturalness" ratings. These ratings vary in definition, are often erroneous and can be highly variable both within and across participants. Additionally, "naturalness" ratings do not assess how intuitive sensation is to the user. The intuitiveness of sensory feedback lies in its integration with the neural schema and with other senses, namely vision. Thus, a measure of multi-sensory integration is necessary to evaluate the intuitiveness of stimulation in sensory neuroprostheses.

The cross-modal congruency task was introduced to assess the interaction of different sensory modalities in the body, and thus can serve as an assessment of multi-sensory integration.[41] The task involves delivery of two sensory modalities simultaneously where the participant is instructed to respond to one target stimuli while avoiding the other distractor stimuli (often visual distractors), appearing at similar or different locations. Subjects are slower in attending to target sensations when the distractor is at a different location (incongruent trials) compared to when the target and distractor are in the same location (congruent trials), as one might expect. This difference in response times on incongruent trials compared to congruent trials is termed the cross- modal congruency effect (CCE). This CCE score serves as the measure of multisensory integration. Similarly, a higher CCE score suggests that the target stimuli is better integrated with the neural schema (vision) and therefore harder attend to that by ignoring the distractor and therefore less integrated to the neural schema. A lower CCE score suggests the target stimulus is less integrated and therefore easier to detect and attend to the stimulus, while ignoring the distractor. Most recently, Blustein et al. proposed the use of CCE scores to evaluate effectiveness of different types of artificial sensory feedback for use in neuroprosthetic

studies.[46] They found that it was more challenging for users to ignore a visual distractor and attend to a more integrated mechano-tactile stimulus instead of less integrated electrical stimulus. Their work suggested the use of CCE scores as a proxy for intuitiveness of sensory feedback in neuroprosthetics studies for individuals with upper-limb amputation. Because the field of sensory restoration has recently expanded to individuals with a lower-limb amputation, an adapted version of this task must be validated for the lower extremities. The purpose of this study is to develop a cross-modal congruency task catered towards individuals with lower-limb amputation and to validate that task in able-bodied participants, with the goal of using CCE scores as a measure sensorimotor integration of sensory percepts in neuroprosthetic studies.

5.2 Methods

Of the 20 able-bodied individuals recruited, 15 individuals completed the study. Inclusion criteria were between the ages of 18 and 65 who had normal or corrected to normal vision. Participants were excluded if they: (1) had a history of neurological disease, motor impairment or chronic pain, (2) had an implanted electrical device, port, or pump, (3) were being treated for cancer or were in acute remission, (4) were pregnant, (5) had any implanted metal hardware anywhere in their body. All experiments were performed with the approval of the University of Pittsburgh's Institutional Review Board (STUDY 19040086).

Two light-emitting diodes (LEDs), electrodes and tactors were placed on the dorsal foot (5 mm distal to the midpoint of the two malleoli) and mid-thigh (10 mm proximal to the superior patella). Additionally, there was a fixation LED placed midway between the foot and knee LED on the tibia (Figure 5.1).

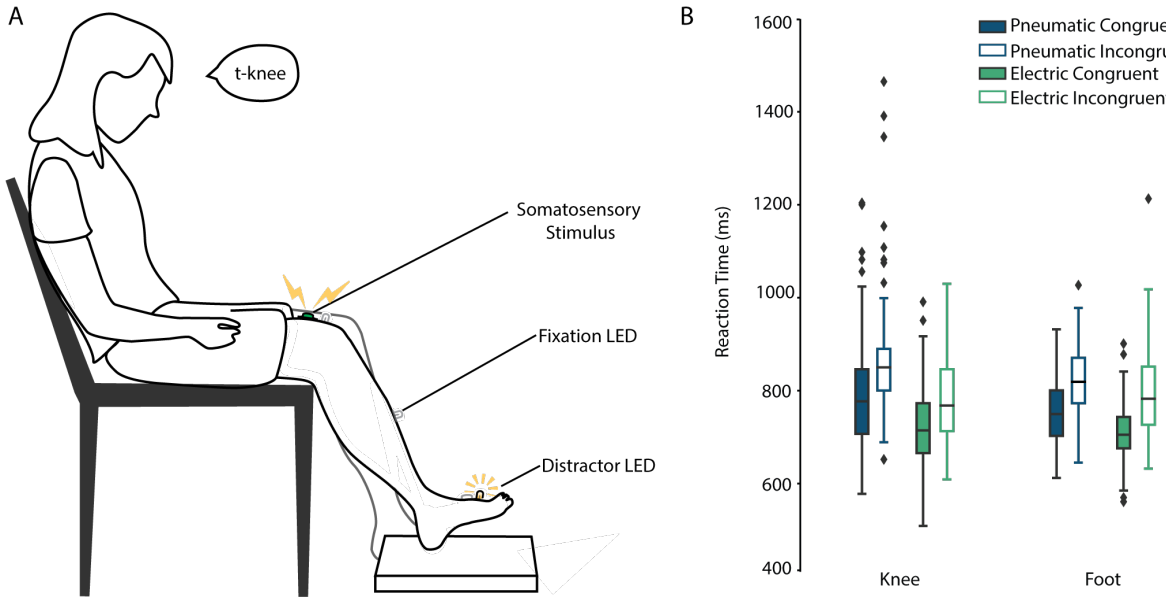


Figure 5.1 Cross-modal congruency task design for the lower-limb. (A) The participant has LEDs and stimulators (both pneumatic and electric) at both the knee and foot locations. When the trial starts, while fixating on the center LED, the participant must respond "t-knee" or "t-foot" for where they feel the sensation. The distractor LED will glow either in the same location (congruent trials) or the opposite location (incongruent trials). (B) Reaction times are recorded for congruent and incongruent trials. The difference between incongruent and congruent reaction times is the cross-modal congruency effect (CCE).

5.2.1 Visual Reaction Times

To ensure the longer visual distance to the foot LED was not affecting reaction times, we recorded reaction times in response to visual feedback only. Participants were asked to fixate on the center LED and then respond with "t-knee" or "t-foot," based on the location of the visual stimulus. Verbal reaction times were recorded by the Cedrus Voice Reaction device (SV-1 Voice Key). Verbal responses were also recorded to determine incorrect responses, which were removed prior to analysis.

5.2.2 Intensity Matching Task

A two-alternative forced choice task was completed to match the intensity of the electrical and pneumatic stimuli. Pneumo-tactile stimulation was delivered by a pneumatic tactor stimulation system (Galileo™). Transcutaneous electrical stimulation was delivered with a single channel, current-controlled stimulator (DS8R, Digitimer) and multiplexer (D188, Digitimer). Stimulation was delivered and controlled using custom software in MATLAB. Both stimuli were presented in randomized order with a 1 s interval between. The participants were asked to report which stimulus felt more intense. As amplitude of the pneumatic tactor stimulation was fixed, the electrical amplitude was then increased or decreased by 0.5mA based on their response. This was repeated until the subject responded in three consecutive trials that the two stimuli were perceived with equal intensity. Intensity matching was completed for the foot and knee electrodes separately and the matched stimulus amplitudes were then held constant throughout the cross-modal congruency task.

5.2.3 Cross-modal Congruency Task

In the cross-modal congruency task, somatosensory (target) and visual (distractor) stimuli were delivered simultaneously. The visual stimulus (LED) appeared either in the same location as the somatosensory stimulus (congruent trials) or in the opposite location (incongruent trials, Figure 5.1A). The participants responded as quickly as possible where they felt the somatosensory stimulus (“t-knee” or “t-foot”) while avoiding the distractor visual stimulus. The letter “t” was added preceding the location in verbal responses to avoid the variations in delays based on the audio signatures of the words “knee” and “foot”. Participants were instructed to fixate on the

center LED throughout the trial. The fixation LED turned on for 2 s indicating the start of a trial followed by the target and distractor stimuli.

Subjects completed a total of six sessions with 12 blocks each and 5-minute rest breaks between sessions. Each block consisted of randomly assigned incongruent and congruent trials for each stimulus modality (pneumatic or electrical) at each location (knee and foot), with a total of 8 trials per block. Additionally, six catch trials per block were added, in which both LED the participant was instructed to respond "invalid." These trials ensured the participant was attending to the fixation light. Prior to the first session, practice trials were performed until the participants were familiarized with the task. Upon the end of each session, participants reported the perceived "naturalness" of the pneumatic and electrical sensations on a scale of 1-10 (10 being completely natural). Additionally, participants reported their fatigue level on a 1-10 scale (10 being most fatigued) between each session. Incorrect trials and trials with early (<200 ms) or delayed responses (>1.5 ms) were discarded prior to analysis (the range was determined from preliminary pilot runs).

5.2.4 Statistical Analysis

Permutation tests were conducted to determine statistically significant cross-modal congruency effects (differences in means between congruent and incongruent trials) for each subject. Permutation testing is used to detect significant differences between groups with a small number of samples. Only subjects with a significant CCE scores ($p<0.05$) were included in comparisons across modalities, for both the knee and foot. A linear regression analysis was performed to determine any associations between subjective naturalness ratings and cross-modal congruency effects of each modality. For group-level analysis, Wilcoxon signed rank test was

performed to determine any differences in CCE scores or in number of incorrect responses. For visual reaction times, a one-way analysis of variance (ANOVA) was conducted to determine significant differences between reaction times for visual stimulus at the knee or the foot. An alpha of 0.05 was used for all statistical analyses.

5.3 Results

5.3.1 Subject-level Analysis

Out of 15 subjects, the congruency effect was present (indicating a significantly higher reaction time for incongruent than congruent trials) for the pneumatic and electrical sensation in 10 subjects for the knee and for 10 subjects at the foot. At the knee, 8 of 10 subjects with significant CCE scores had a higher CCE score for pneumatic (Figure 5.2). At the foot, for five subjects the pneumatic CCE score was higher and for five subjects the electric CCE score was higher (Figure 5.2).

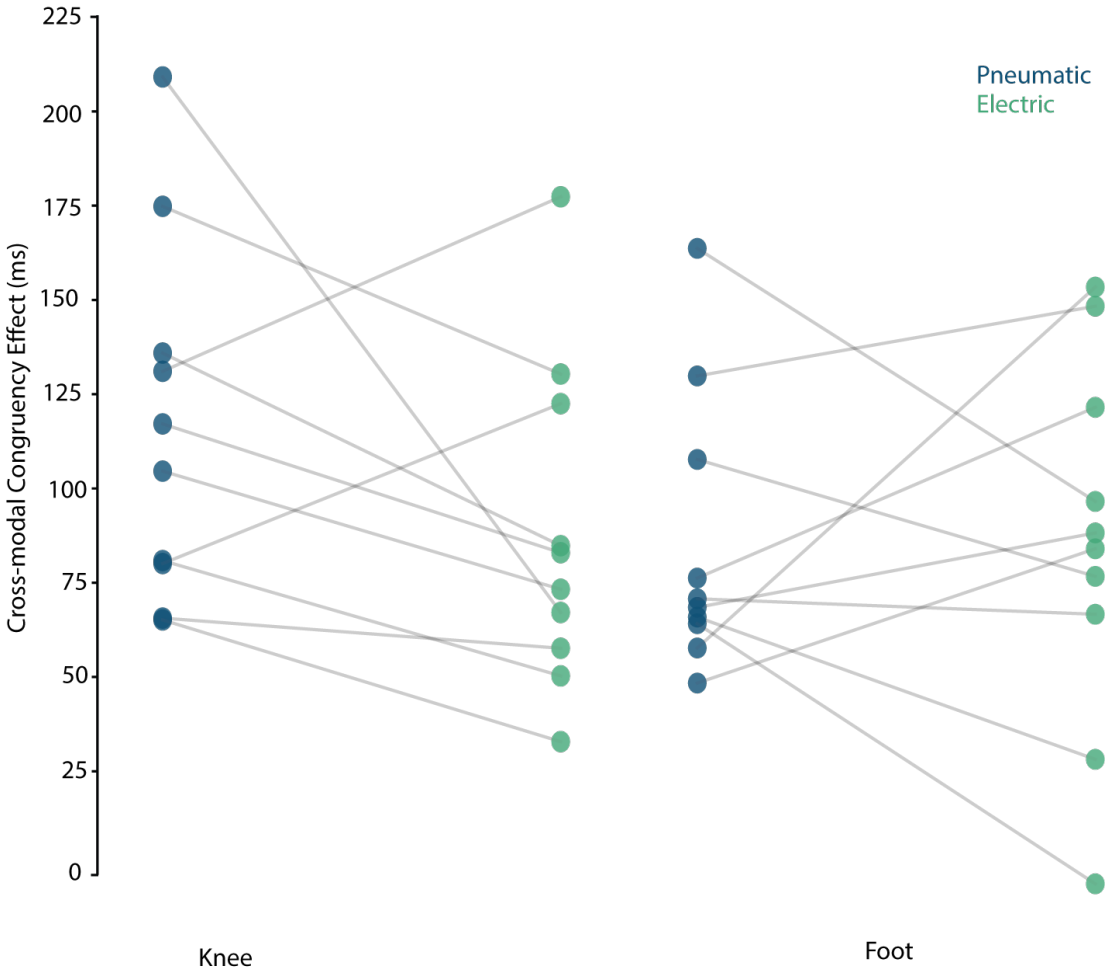


Figure 5.2. Cross-modal congruency effect (CCE) for each modality at the knee and foot. Ten subjects had significant CCE scores at the knee and ten had signficant CCE scores at the foot. For the knee, most participants had a higher CCE score for pneumatic than electric. However, at the foot, half of the participants had a higher electric CCE score than pneumatic.

5.3.2 Comparison with Naturalness Ratings

Overall, subjects reported a similar subjective rating of naturalness for both pneumatic and electrical stimuli, five subjects reported the exact same score for both sensations. There was no correlation between reported naturalness ratings and CCE score ($p>0.05$).

5.3.3 Variations with Stimulation Amplitude

Overall, thresholds were higher at the foot and therefore, the selected electrical stimulation amplitude was higher in foot compared to the knee. No significant effect of the difference in stimulation amplitude was observed in the difference in the CCE scores ($p>0.05$, Figure 5.3**)**. Though not significant, the relationship between amplitude and CCE score was opposite for electrical and pneumatic stimuli. For pneumatic, as the difference in amplitude between the foot and knee increased, the difference in CCE score decreased. For electric, there was a direct relationship between amplitude and CCE score.

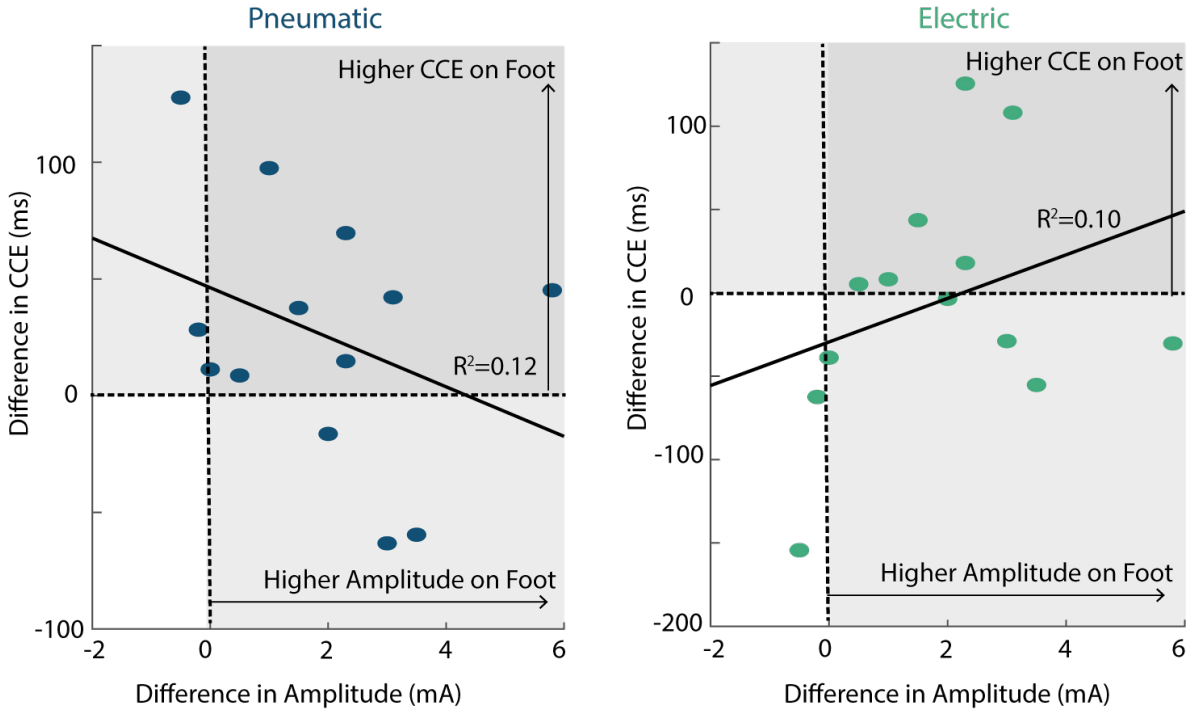


Figure 5.3. Difference in CCE score by difference in amplitude of electrical stimulation at the foot and the knee. Pneumatic and electrical thresholds are matched per location, thus for the pneumatic stimuli, electrical amplitude serves as a proxy for pneumatic intensity. There is an inverse (but not statistically significant, $p>0.05$) association between higher electrical amplitudes at the foot on the foot.

5.3.4 Reaction Time Variability

Anecdotally, some subjects would respond more slowly to answer correctly. Four subjects had a reaction time for the congruent trials (baseline reaction time) higher than 1 sec. Two of these four subjects had no significant CCE scores. There was no significant relation with baseline reaction times and the corresponding CCE score (Figure 5.4).

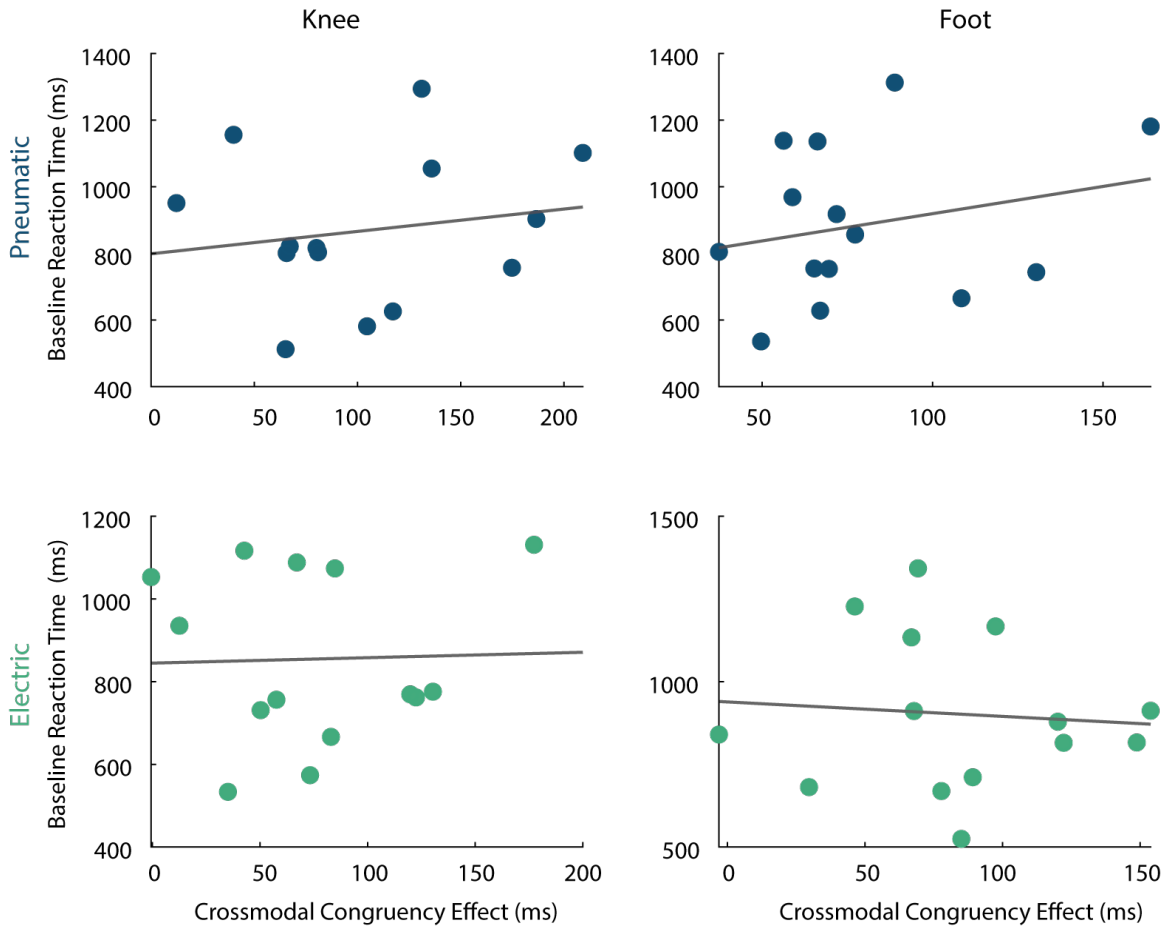


Figure 5.4. Baseline reaction times relation to CCE scores. There were no significant relationships between baseline reaction time and CCE scores at either location or for either stimulus modality ($p<0.05$). The range of baseline reaction times were similar across modalities for both locations.

5.3.5 Incorrect Responses

Subjects with >5% incorrect trials were included for analysis (n=10). At the knee, 8 out of 10 subjects had more incorrect responses for pneumatic than electric sensations (Figure 5.5). Similar to the CCE scores, we observed mixed results at the foot, with no clear patterns within or across participants with regards to stimulus type. At the foot, 7 subjects responded incorrectly more for the electric stimuli and 3 subjects responded incorrectly more for the pneumatic stimuli.

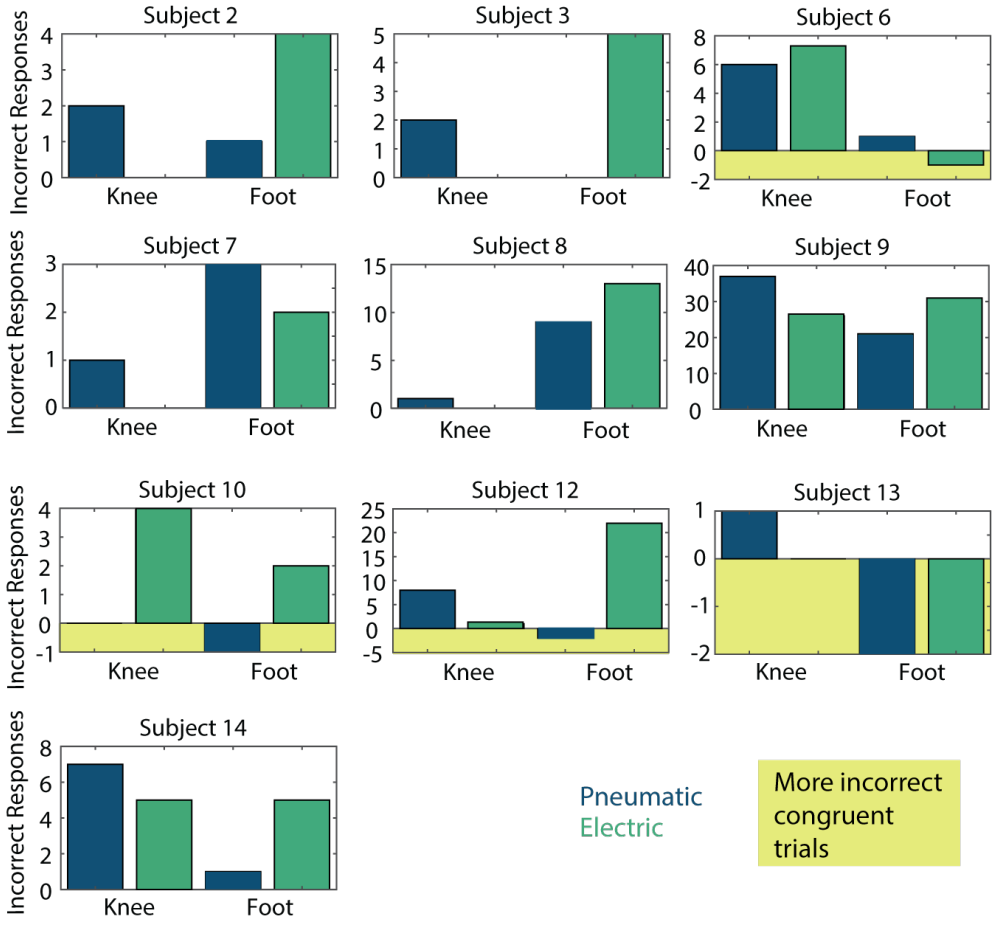


Figure 5.5. Incorrect responses by area and stimulus type. Responses grouped by location for pneumatic (blue) or electric (green) stimuli. Positive incorrect responses indicate more incorrect incongruent trials, while negative responses (green shading) indicates more incorrect congruent trials than incongruent trials. Only four subjects responded incorrectly more for congruent trials. There were no clear patterns based on stimulus type.

5.3.6 Group Analysis

Though this task is intended to use on individual subjects, we performed group-level analysis to be able to compare findings to previous studies. There were no significant differences between pneumatic and electric CCE scores at the knee or foot. (Figure 5.6A). However, there was significant difference in the number of incorrect responses for both knee and foot locations (Figure 5.6B). In the knee, the incorrect responses were significantly higher for pneumatic sensation (+1.714, p=0.035), whereas in the foot, incorrect responses were significantly higher for electric sensation (+3.57, p=0.026).

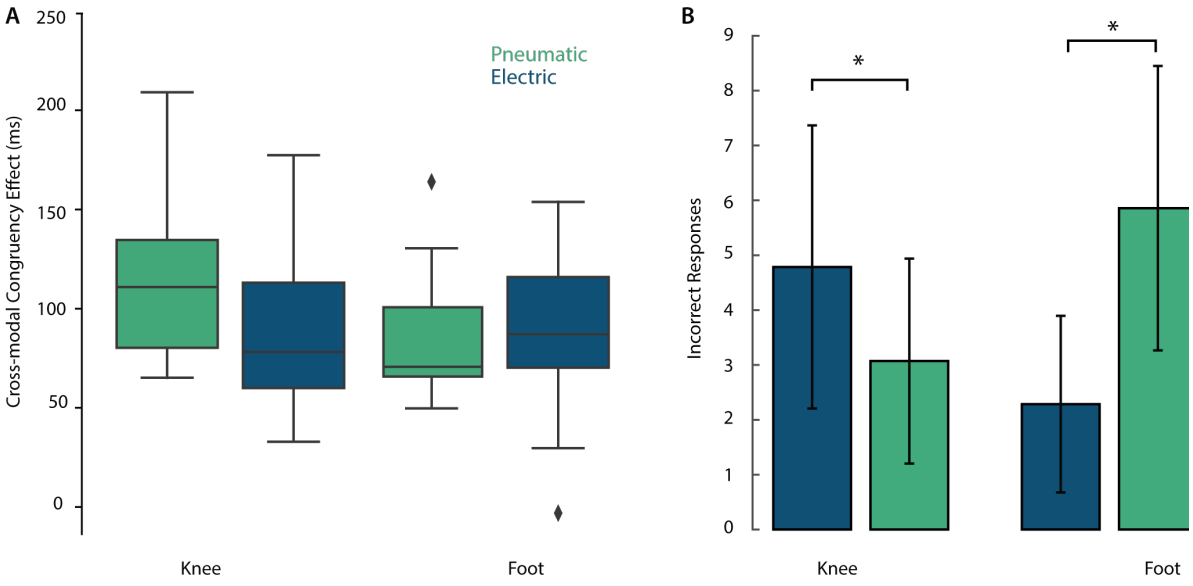


Figure 5.6. Group-level analysis of CCE scores and incorrect responses. (A) There were no significant differences ($p>0.05$) in CCE score for pneumatic or electric stimuli at either the knee or foot locations, however the pneumatic had a higher CCE score at the knee, but not the foot. Similarly, participants had significantly more incorrect responses for pneumatic stimuli at the knee and more incorrect responses for electric stimuli at the foot.

5.4 Discussion

From the results, the cross-modal congruency task detects differences in integration of the sensation based on modality type at the knee, but not at the foot. As it stands, the cross-modal congruency task would not be a suitable proxy for intuitiveness of a stimulus at all lower-limb locations and is therefore unlikely to be a successful measure in lower-limb neuroprosthetics studies. However, future studies with a modified task design could shed more light on the differences in location we see in the current task.

We found no quantitative justification (fatigue, thresholds, etc.) for the discrepancy in the relationship between CCE score at these two locations. Anecdotally, however, there are potential explanations for this effect. In half of the subjects, the perception of the electrical sensation in the foot changed throughout the duration of the experiment. Most reported that the intensity at the foot decreased with time, while some subjects reported radiating sensations to the toes. For subjects reporting differences in intensity, the amplitude was increased to correct for habituation of the electrical sensation. In subjects reporting radiating sensations, the location of the electrode was adjusted to provide a focal sensation. Despite these adjustments, these factors could have influenced the CCE score of the electrical sensations at the foot. Because the cross-modal congruency task is intended for neuroprosthetic studies using peripheral nerve stimulation or SCS, changes in intensity with stimulation throughout the experiment could pose a similar problem with evoked sensations in the missing foot. Adaptation or habituation of electrical stimuli must be taken into consideration prior to making further modifications to this task for evaluating peripheral nerve or SCS studies.

Our findings could also be affected by other factors that impact CCE scores instead of multi-sensory integration. In the field of cognitive neuroscience, there is ongoing debate

regarding the relative contributions of multi-sensory integration, attentional demands, and response conflict to the CCE scores. A study evaluating these relative contributions determined that response conflict and, to a lesser extent multi-sensory integration, were the primary mediators of the standard cross-modal congruency task.[45] Response conflict is an incorrect mental representation of the sensation locations that involuntarily occurs when a distractor stimulus appears at the same time.[45] In the traditional cross-modal congruency task, a block is held with the index finger and the thumb in each hand with LEDs and tactile stimulators at each location.[41] Subjects are asked to discriminate between location (index finger or thumb) while visual distractors appear on either hand (same or opposite hand of the stimulus) on the same finger (congruent) or the other finger (incongruent). The slowing of response times in incongruent trials represents the amount of time it takes to inhibit these incorrect representations of the stimulus, regardless of the location of the distractor (same or opposite hand incongruent trials). However, multi-sensory integration is location dependent. Thus, multi-sensory integration explains the increase in CCE score on only the ipsilateral hand. If only response conflict were at play, the CCE score would be similar on both hands, as the location of the distractor should not matter, which is not the case. Thus, both response conflict and multi- sensory integration have a role in the cross-modal congruency task.

Response conflict in this task can be considered a better indicator of the interference or dominance of visual stimuli on somatosensory stimuli, while multi-sensory integration is a better indicator of how well the visual and somatosensory stimuli merge in the neural schema. Both aspects of cross-modal perception are important, however for the purposes of evaluating varying types of somatosensory stimuli, evaluating multi-sensory integration in lieu of response conflict may be more appropriate. To eliminate the contribution of response conflict, a shift to a “Go-No

Go" task would be necessary. In this task, the subject is asked simply to report if they felt a sensation, not discriminate the location of the sensation.

A limitation of this study was that we were unable to match the perceived intensity of the pneumatic stimulus at the knee and the foot. Because higher intensity stimuli are more salient and one would respond faster to it, the CCE score for higher intensity stimuli would decrease, as the distractor is less effective at overcoming the target stimulus effect. Additionally, some subjects reported numbness in the distal foot with their leg extended resting on a step for 2-3 hours. This numbness may influence CCE scores and reducing the experiment time could ameliorate this effect. Going forward, as the analysis using just the first three sessions did not change the results of the study, three sessions should be sufficient in future studies. This is similar to the findings of Blustein et al. who found learning effects with a larger number of trials per experiment.[47] Additionally, the similarity in subjective naturalness ratings between the two modalities was surprising, and despite a lack of relationship between naturalness ratings and CCE scores, using a sensation that is more clearly distinct from electrical stimulation, such as vibrotactors, may be useful in future studies.

Though we focused on relationships of CCE scores across modalities per subject, we also analyzed group effects to compare with the Blustein study. Our results differ from those in the Blustein study, which demonstrated a clear group-level effect of modality on CCE score.[46,47] Notably, that study was conducted in the hand, with a substantially smaller distance between the two locations of stimuli and distractors. The sensory acuity in the hand is higher than in the foot and lower limb, which could account for these differences.[93] Additionally, the large differences in distance between these two locations could possibly influence CCE scores.

To explore this potential relationship, we collected pilot data (n=5) using the same protocol, using two stimulus locations on the foot (Appendix G). Due to our findings that three sessions are sufficient, we also utilized only three sessions for this preliminary data collection. Evoking sensations in two locations on the foot would likely not be possible for SCS studies, as this stimulation evokes sensations that cover large areas of the missing foot and residual limb (Chapter 2 and Chapter 3). However, two locations on the foot would be feasible for peripheral nerve stimulation studies that are able to achieve more focal sensations with stimulation. Though this was only preliminary data, we saw no significant findings across CCE scores for pneumatic or electric modalities at either the toe or heel location. These results suggest that distance between the stimuli was not the primary factor in these conflicting results across studies, however more data would be necessary to fully evaluate the effects of distance between distractor and target stimuli.

In summary, while the CCE score was an indicator of multi-sensory integration of stimuli in the knee, this finding did not generalize to the foot. Given these results, the discussed additional steps need to be taken to further develop a more useful method of measuring multi-sensory integration of stimuli and evaluate the functional effects of somatosensory neuroprostheses. These findings also call into question the role of response conflict and multi-sensory integration in the cross-modal congruency task. When using this CCE score as a proxy for intuitiveness, the importance of sensory interference or multi-sensory integration must be established to design the task appropriately.

6.0 Summary of Results and Future Directions

This dissertation highlights the importance of utilizing appropriate measures for evaluation of sensory neuroprosthetics. Evaluation of standard clinical outcome measures revealed a lack of sensitivity to even large differences in sensation across individuals with amputation. Despite these shortcomings, these measures were still able to demonstrate improvements in balance and gait with a sensory neuroprosthesis, but only on the most challenging conditions. With more sensitive clinical research measures of balance and gait, the functional improvements of a sensory neuroprosthesis were more apparent. Additionally, to advance neuroprosthetic techniques to optimize functional benefits, appropriate measures are vital to the success of these projects. This work reports the adaptation of the cross-modal congruency task as a potential measure of multi- sensory integration for assessing different types of stimulation. Despite its promise, this measure is subject to differences in CCE score based on location and is not a reliable proxy for intuitiveness of SCS-evoked sensations.

These findings establish the functional effects of a sensory neuroprosthesis using SCS and allow for further evaluation of the effects of variations of SCS on function. Using more robust outcome measures will be a crucial aspect to neuroprosthetic advancements and optimizing stimulation parameters. Namely, biomimetic stimulation, or the use of stimulus patterns that more closely match the natural firing patterns of the nervous system, has the potential to provide additional functional improvements and the groundwork in this dissertation has been set for these future projects.

6.1 Clinical Measures are Not Sensitive to Measures of Sensation

The current clinical assessments often used for assessing reliance on sensation for static, reactive and dynamic balance are the SOT, MCT, and FGA respectively.[69,102] However, in a cross- sectional study of twenty individuals with lower-limb amputation, these measures were unable to detect even very large differences in sensation. Most clinical measures merge individual assessments of multiple systems into one composite score, such as in the SOT. While the SOT determines reliance on visual and somatosensory systems, it also tests reliance on vestibular systems for balance, which is not relevant to assessing sensory feedback. Though this is beneficial for clinical use, many of the components of these measures are not designed to determine the role of sensation in balance and gait. As these assessments are not sensitive to differences in somatosensory integrity, they are likely not the best assessments for evaluating more fine changes in sensation seen with neuroprosthetics to restore sensory feedback. Despite the lack of sensitivity to somatosensory integrity in these measures, the SOT is the clinical standard for evaluating reliance on visual, somatosensory or vestibular systems for balance and has been used in early stage studies evaluating neuroprostheses across multiple stimulation strategies (peripheral nerve stimulation, SCS, transcutaneous electrical stimulation).[25,66]

6.1.1 Limitations

Both the SOT and the FGA assess multiple components of mobility and function. The SOT tests reliance of visual, somatosensory and vestibular systems for balance, while the FGA contains ten items, some of which are designed to evaluate vestibular disorders.69 Because these measures include components that are intended to evaluate aspects of balance and gait that are

irrelevant to these studies, these measures inherently do not focus on the effects of sensory feedback. Furthermore, participants have likely learned to compensate for lacking sensory impairments in daily balance and gait tests, such as level walking, relying more on their intact limb for balance. Thus, these outcome measures, while valuable clinical assessments, are not suited to gauge the effectiveness of a sensory neuroprosthetic.

In addition to the functional measures, the measures to quantify sensation in this study were also designed for clinical use and they may not have been able to appropriately characterize the mild to moderate range of somatosensory impairments. Additionally, this study included twenty individuals with transtibial and transfemoral amputations, but only four individuals had transfemoral amputations. Because of this sample size, we were underpowered to be able to determine with any statistical significance how other factors, such as level of amputation or time since amputation, affected functional outcomes.

6.1.2 Broader Impact

While the SOT, MCT, FGA and gait kinematics on level surfaces were able to adequately detect differences between able-bodied individuals and individuals with an amputation, these assessments were not able to distinguish differences in somatosensory impairments within the amputation group. The findings from this study encourage the use of more challenging and robust metrics to evaluate the role of sensation on functional impairments in people with lower-limb amputation, such as the NBWT. Though the outcome measures in this study have been used in sensory restoration studies for individuals with amputations, measures that are more sensitive to sensory integrity may be better suited to detect finer changes in function with the addition of sensory feedback.

6.2 Addition of Sensory Feedback via Sensory Neuroprosthesis Improves Function

In this study, we found that a sensory neuroprosthesis that restores somatosensation in the missing limb using SCS improves balance and gait and reduces PLP. In three subjects, we used SCS to activate afferent neural pathways in the lumbosacral spinal cord to evoke sensations in the missing foot and use these sensations as real-time sensory feedback. SCS-evoked sensations were consistent with dermatomal distribution for the lower-limb, however, we were never able to obtain a sensation localized exclusively to the missing foot. Thus, all sensations used in functional testing for sensory feedback spanned the missing limb and the residual limb. By modulating stimulation intensity in real-time based on plantar pressure signals from a wireless insole, we demonstrated improvements in balance and gait stability in two subjects. Due to time constraints with Subject 1, we were unable to complete functional testing with this participant.

With stimulation, both subjects had a reduced number of falls in the SOT. Furthermore, Subject 3 had a clinically meaningful improvement in composite score for the SOT, while Subject 2 had an improvement above the threshold for a minimum detectable change. We saw results similar to previous studies using more standard measures of balance and gait, seeing the clinically meaningful improvements on the most challenging conditions.[25] Additionally, over the duration of the study, subjects experienced a clinically meaningful decrease in PLP. These combined results demonstrate that, with a clinically viable implantable electrode technology, our approach has potential as an intervention to improve function and pain after lower-limb amputation.

6.2.1 Limitations

Like other neuroprosthetics projects, only a small number of subjects (n=3) completed this study. Furthermore, in Subject 1, migration of the electrodes caused changes in the location of evoked sensations. The changing locations of stimulation and the time limitations with this subject restricted our ability to complete closed-loop testing. Thus, only two of these individuals, with vastly different clinical presentations, completed the functional testing. One subject had a dysvascular amputation and limited community mobility, while the other subject had a traumatic amputation with a very active lifestyle. Larger randomized controlled trials will be an important step in applying SCS to a broader range of individuals for improvements in function and PLP.

6.2.2 Broader Impact

Despite using the standard clinical measures (SOT, FGA and gait analysis) used in this study, both individuals experienced improvements in challenging conditions of balance and gait with SCS-evoked sensory feedback. These changes suggest SCS is a clinically viable stimulation method for providing sensory feedback in a lower-limb sensory neuroprosthesis. While peripheral nerve stimulation requires training surgeons for a new implantation procedure, SCS is already a device used regularly in clinics. Thus, SCS can be repurposed for sensory neuroprosthetics instead of requiring additional steps that slow wide-scale translation. These findings are a significant advancement in clinical translation of neuroprosthetics for individuals with lower-limb amputation.

6.3 New Outcome Measures Shed Light on Improvements with Sensory Feedback

Even with the limitations of standard outcome measures, we found that restoring sensation can improve function on more difficult conditions of standard clinical tasks. Critically, we demonstrated that the effects of a SCS sensory neuroprosthesis on function are more evident using measures that are designed to challenge and enhance the need for sensory feedback. In one individual with a transtibial amputation, we saw clinically meaningful and statistically significant improvements in these measures of balance and gait using SCS as real-time sensory feedback. Stimulation amplitude was linearly modulated by plantar pressure in a wireless pressure-sensing insole, meaning that if the subject shifted her weight more onto her prosthetic toes, she experienced a more intense sensation extending into her missing toes. The functional tasks used in this study are particularly relevant to individuals with a lower-limb amputation: balance on the prosthetic leg, manipulating balance with and without visual feedback, walking on uneven surfaces and walking with a narrow base of support.

Balance is a critical component to regaining function following an amputation and reducing fall risk. Because individuals with an amputation can often compensate using their intact limb, situations where they need to rely on their prosthetic limb can put them at an unnecessary risk of falls. Therefore, evaluating the ability to remain standing on the prosthetic limb is an important component of balance for this population. Additionally, the VFT, though not a clinical outcome measure, evaluates how the participant learns a new task with this stimulation and, by doing so, sheds light on several aspects of motor learning using a sensory neuroprosthesis.

Walking on uneven surfaces is a challenging task for individuals with amputation and individuals lacking sensation.[36,111] In a recent focus group study on falls in individuals with a

lower-limb amputation, a primary theme of fall-related events was surface conditions, with uneven surfaces creating more instability and a greater risk to the user.[127] In fact, trips and slips account for most falls in ambulatory individuals with a lower-limb amputation.[109,128,129] Walking with a confined, narrow base of support also occurs regularly in daily life in environments (both indoor and outdoor) with space constraints, which poses additional fall risk due to greater instability with a narrowing base of support. For this reason, the NBWT has been used to assess fall risk after lower-limb amputation.[122]

Notably, these tasks are challenging, but still able to be performed by individuals with lower mobility levels (e.g. limited community ambulators). Using these measures, improvements in balance and gait with a sensory neuroprosthesis were more evident.

6.3.1 Limitations

The primary limitation of this study is the lack of additional subjects. Currently, only two subjects have undergone closed-loop functional testing and only one has completed these more robust measures of balance and gait. Additionally, the VFT is not a clinical task, but instead mimics upper-limb tasks used commonly in studies involving neural interfaces and motor learning. Future studies using the VFT should reduce fatigue by selecting only the relevant targets (anteroposterior or mediolateral directions) based on the area of the sensory stimulation. Additionally, separating the eight target sessions into two sets of four targets and providing a rest break in between would reduce the effects of fatigue within each trial and across conditions.

6.3.2 Broader Impact

Using these more robust measures going forward in sensory neuroprosthetics studies may clarify the importance of sensory feedback in daily tasks and therefore highlight any functional improvements of restoring sensory feedback through a neuroprosthesis. Additionally, having standardized outcome measures across labs and clinics that can serve as a reliable measure of effectiveness for these devices, which will be necessary to make critical advancements in stimulation patterns or parameters to optimize functional benefits.

6.4 Evaluating the Role of Intuitiveness in Sensory Feedback

Part of advancing neuroprosthetics lies in optimizing stimulation patterns and parameters to provide the best possible feedback to the user. To maximize potential functional benefits of stimulation, many hypothesize that more intuitive sensations, that integrate more into the neural schema, will provide greater functional improvements. Along these lines, it is likely that biomimetic stimulation, which has the potential to provide more intuitive sensory percepts, will provide further functional benefits, as well. However, a metric of intuitiveness or multi-sensory integration of the evoked sensation is a key development needed for these studies. We adapted the cross-modal congruency task for the lower-limb to be able to assess multi-sensory integration of different stimulus types in able-bodied individuals.

While this assessment shows promise, there were different patterns across stimulus types depending on the location of the stimulus, which was contrary to our hypothesis. At the knee, the pneumatic stimulation had greater multi-sensory integration (indicated by a greater CCE score),

however at the foot, the electrical sensation had a greater CCE score. These findings also differ from previous studies using the cross-modal congruency task in the hand. These discrepancies are likely due to the adaptations we made to use the cross-modal congruency task for the lower-limb. In previous studies, the two locations were on two different fingers in one hand, however here we have one stimulus on the foot and one on the knee. The foot and the knee have differences sensory acuity, which could lead to these differences based on location. Additionally, in comparison to previous studies, the foot has less sensory acuity than the hand, which could account for these differences, as well. Regardless of the explanations behind these findings, with the differences in location we observed, this assessment is not a feasible method of quantifying multi-sensory integration or functioning as a proxy for intuitiveness of stimulation in neuroprosthetic studies at this time.

6.4.1 Limitations

There were several limitations to this study. First, as the pneumatic tactor amplitude is fixed, we could not match the perceived intensity of the pneumatic stimuli at the knee and the foot. Additionally, the considerable distance between these two locations could likely influence CCE scores in comparison to previous studies, which had two locations on one hand.46,47 However, the preliminary results (n=5) from repeating this experimental paradigm using two locations on the foot suggest that distance between locations is not a primary factor confounding these results. More data would be necessary to explore these findings. Additionally, the similarity in subjective naturalness ratings between the two modalities was surprising. For future iterations, using a sensation that is more clearly distinct from electrical stimulation, such as vibrotactile sensation, may shed light on the relationship between stimulus type and CCE score.

Lastly, response conflict (taking time to inhibit an incorrect response to instead say the correct response) likely played a larger role in the cross-modal congruency task than multi-sensory integration. Thus, in the future, using a "Go-No Go" task (in which the participant simply states whether they felt a stimulus, not the location of the stimulus) would shed light on the role of multi-sensory integration exclusively, without response conflict.

6.4.2 Broader Impact

The difference in CCE score patterns at the two locations does not allow for the cross-modal congruency task to be used as a measure of multi-sensory integration or intuitiveness in lower-limb neuroprostheses. Additional modifications would need to be taken to develop a more useful method of measuring these aspects of stimulation. Furthermore, due to the large area of sensation evoked in SCS studies, the CCE score is likely not a viable solution for these specific neuroprosthetic studies. With current electrode limitations (large size for the small nerve roots), it is unlikely that SCS would evoke two distinct locations that were isolated to either the foot or the knee, or two places on the foot. Thus, the potential overlap in the two locations of stimulation is a clear confounding factor and does not allow for the use of CCE score as a proxy for intuitiveness of sensation in these studies.

6.5 Biomimetic Stimulation Patterns

All SCS used in these studies was constant frequency stimulation, which often evoked unintuitive percepts (Appendix B). Previous upper-limb neuroprosthetics studies reported a

change in percept quality with different stimulation patterns, with biomimetic stimulation evoking more natural percepts (from subjective ratings).131 From early work with biomimetic stimulation in our SCS subjects, there was a significant difference in quality, though not in naturalness as seen in previous studies (Appendix G). Using constant frequency stimulation, Subject 3 had reported a temporal component to the evoked sensations in her missing foot. Anecdotally, she described them as a "wave passing from my leg down into my toes." To provide useful sensory feedback, real- time stimulation should evoke the missing foot sensation as quickly as possible. Thus, the slow time course of these sensations was not ideal. We trialed two sessions with ten trials of biomimetic stimulation and ten trials of constant frequency stimulation (matched to the peak frequency of the biomimetic stimulation) in random order. Biomimetic stimulation was modeled as a rapid-firing 1A afferent fiber response to a pressure indentation, or a sequence of press-hold-release over the course of 1s, as used in previous studies.40,126,131 With biomimetic stimulation, the speed at which the sensation traveled to her foot was rated significantly faster than with constant frequency stimulation at the same peak frequency (+2.31, ratings out of 10, $p=0.003$). Though a difference in timing is not what we expected with biomimetic stimulation, this is inherently a change in sensation quality. Furthermore, these results suggest a potential difference in the integration of the sensation into the subject's neural schema.

As each subject experiences slightly different types of sensations, the changes with biomimetic stimulation may not be as straightforward as a change from buzzing to pressure sensation. Instead, future studies should focus on if these changes in subjective ratings correlate with improvements in function with stimulation or, more simply, if biomimetic stimulation can provide greater functional improvements than constant frequency stimulation. Though we used

closed-loop biomimetic stimulation (co-modulation of stimulation frequency and amplitude mapped linearly to plantar pressure during gait) on the corresponding region of the prosthetic foot in one subject, several modifications were made to test this stimulation in another subject. Anecdotally, Subject 3 reported different qualities of biomimetic stimulation in a functional task, as anticipated based on the changes in sensation with open-loop biomimetic stimulation. She reported a quicker, sharper sensation with standing and walking. As the sharpness of this sensation was occasionally too distracting for her, we adjusted range of amplitudes and frequencies to ensure we used appropriate, safe parameters for stimulation. The need for constant adjustments did not allow for a systematic assessment of functional improvements with varying stimulus patterns (biomimetic vs constant frequency stimulation), however, the framework has been established to perform these experiments in future subjects.

6.6 Evoking and Assessing Proprioceptive Percepts

The work in this dissertation has focused on restoring tactile sensation. However, individuals with an amputation lack both tactile sensation from the missing foot and proprioception from the missing ankle (or ankle and knee in transfemoral amputations). When navigating environments that pose a high risk for falls, such as low light conditions and on unsteady surfaces, proprioceptive signals are crucial for maintaining stability. Proprioception is an integral part of the somatosensory system that combines static joint position sense and dynamic changes in joint position. Though we have been able to evoke proprioception a handful of times in our participants with SCS, we only have minimal anecdotal evidence. We were unable to reliably evoke proprioceptive percepts in open-loop SCS testing, as they often changed

in nature with posture or were context dependent. Furthermore, when we were able to evoke them, these sensations often accompanied a reflexive electromyographic (EMG) response. It was unclear whether the subjects felt a proprioceptive percept without actual prosthetic movement or if they felt their limb move because the prosthetic was moving as a result of the reflexive activity. Unfortunately, because we could not evoke proprioception reliably in our study, we were unable to use those percepts in closed-loop functional testing.

In future studies, it is likely that we will not be able to obtain proprioception in absence of reflexive EMG activity or that proprioception will only occur in certain contexts, such as different functional tasks. However, if the reflexive activity coincides with the natural EMG activity during gait, these evoked percepts have the potential to provide even greater functional benefits. Future studies characterizing the ability to evoke these percepts in the context of a functional task and the corresponding reflexive activity are necessary to providing useful proprioceptive percepts for sensory feedback.

6.7 Evaluating the Use of a Sensory Neuroprosthetic in a Home Setting

The goal of sensory restoration is a fully implantable system that individuals would use daily. Much of the work developing and testing these neuroprosthetic devices has taken place in the laboratory and little is known about use of lower-limb neuroprostheses in participants' daily lives. Recently, upper-limb studies have implemented take-home trials, in which the participant can use a sensory neuroprosthesis unsupervised in their home or community setting after being properly trained in the lab.[130–132] These studies have found that home use of the neuroprosthesis has improved efficiency, sensory acuity, wear time, psychosocial factors, and perceived

naturalness and quality of sensations.[130–132] Though most of the take-home studies were conducted on upper-limb neuroprostheses, similar effects are likely possible with lower-limb neuroprostheses. We do not yet know what extent extended use or motor learning may impact with functional improvements seen with lower-limb neuroprostheses.

Subjects in our studies had variable levels of training and time to familiarize themselves with the device due to differences in study duration (29 days for subject 2 and 90 days for Subject 3). Due to time constraints, Subject 2 had only two and a half days of functional testing out of the 29-day study. Most of that time was spent determining the appropriate parameters for stimulation, leaving very little time for training with the stimulation. As development of lower-limb neuroprostheses is still in its early stages, little is known about the necessary amount or type of training that should be used to optimize the functional effects of stimulation. Just as individuals with a lower-limb amputation receive additional training after getting a new prosthetic leg, physical therapy or prosthetic training could improve the user's efficiency with the device. Furthermore, this training could improve their ability to incorporate the sensory feedback in a meaningful way during complex motor tasks.[22] Future studies evaluating what training is necessary or beneficial with the addition of a sensory neuroprosthesis will be an integral part of translating neuroprosthetic devices into widespread clinical use.

6.8 Advancements in SCS Sensory Neuroprostheses

With the goal of a long-term, take-home system in mind, ideally stimulation would evoke more focal sensation in the missing limb without an accompanying sensation in the residual limb. This is possible with peripheral nerve stimulation, where electrodes are placed around

(epineural electrodes) or through (intrafasicular electrodes) the peripheral nerve, allowing for very small areas of sensation evoked by stimulation. Interestingly, our labs prior work using cervical SCS in individuals with upper-limb amputation allowed for greater focality of sensations localized to the missing limb, as well. In two subjects stimulation evoked sensations in individual fingers or spots on the fingers. These differences with lumbosacral and cervical SCS are likely due to the differences in anatomical arrangement of lumbar and cervical rootlets we are targeting. The dorsal rootlets in the cervical spinal cord are more confined and evenly spaced, thus the SCS electrodes can still provide sensations in a small region of the missing hand. However, in the lumbar spinal cord and cauda equina, the large number of closely packed and overlapping nerve roots does not permit focal sensations in the missing limb with the SCS electrodes. Smaller electrodes may be able to provide more focal sensations with lumbosacral SCS.

Additionally, we are using percutaneous SCS leads for these clinical trials, which are designed to be implanted on a temporary basis. The fully implantable permanent SCS systems utilize larger paddle electrodes. These paddle electrodes minimize lead migration but need development prior to implantation to allow for the more focal sensations needed in sensory neuroprostheses. To ease the translation of sensory neuroprostheses to clinics, future studies should aim to develop smaller paddle electrodes to target more specific locations in the missing foot with lumbosacral SCS.

No modifications were made to the lower-limb prostheses used in this study, sensors were added to the subjects' original prosthesis. Additionally, none of the participants with the sensory neuroprosthesis had active components to their prosthetic devices. In future studies, the functional improvements seen using myoelectric control and active prostheses would likely add

to improvements seen with sensory neuroprostheses. Furthermore, myoelectric control would allow further testing of proprioceptive feedback, which is currently limited as many proprioceptive tests require active control of the prosthetic ankle.

Lastly, our studies have been limited to individuals with a transtibial amputation, however a benefit of using SCS sensory neuroprostheses is that they allow for any level of lower-limb amputation, including transfemoral amputations and hip disarticulations. As SCS does not require implantation in the residual limb, the length of the residual limb or the quality or accessibility of nerves in the residual limb are of no consequence to the techniques used in this study. Additionally, SCS sensory neuroprostheses could ultimately be expanded to restore sensation in individuals with peripheral neuropathy, who suffer from similar functional impairments as individuals with a lower-limb amputation. These individuals would likely benefit from restored sensation with SCS evoked feedback, as well.

6.9 Overall Conclusions

The work presented in this dissertation discusses in detail the importance of using appropriate outcome measures for evaluating the effectiveness of sensory neuroprostheses at improving function. Appropriate functional outcome measures are both sensitive to differences in somatosensory integrity and can be performed by the wide range of mobility levels seen in this population. We provide potential alternative measures that fit these criteria. Using both standard and more robust outcome measures, we see functional improvements with a somatosensory neuroprosthesis using lumbosacral SCS in individuals with a lower-limb amputation. Additionally, we provided a framework for further evaluating the functional effects of improving

stimulation parameters or patterns, which will be a significant step to make necessary advancements in SCS sensory neuroprostheses. These studies set a precedent for functional evaluation of sensory neuroprosthetics across a wide range of stimulation approaches and thus allow for optimizing functional benefits of these devices.

Appendix A Measures of Balance and Gait Supplementary Material

Light touch and protective (pin prick) sensation were assessed proximally to distally and subjects were instructed to report if they detected a stimulus for light touch or whether a Neurotip examination pin was "sharp" or "dull" for protective sensation. For light touch, a stimulus (finger tip) was applied at target locations for standard neurological assessment of the lower-limb.[133] Stimuli were applied three times per location and sensation was scored out of 2. A score of 0 (indicating no sensation) was recorded if participants were unable to detect a stimulus or correctly identify the stimulus at all, a score of 1 (impaired sensation) if they were able to correctly identify for some trials, and a score of 2 (intact sensation) if they were able to correctly identify the stimulus for all trials. The quality of lower extremity reflexes of the Achilles tendon and the patellar tendon on the intact limb and the patellar tendon (for individuals with transtibial amputation) on the residual limb were assessed using a Taylor percussion reflex hammer (hyperreflexive response=3, normal response=2, hyporeflexive response=1, no response=0). Vibration sense was tested using a 128 Hz tuning fork applied perpendicular to the medial malleolus and distal interphalangeal joint of the hallux of the intact limb.[134] For able-bodied controls, both limbs were assessed and the better score was used as a comparison with the AMP group. The tuning fork was struck maximally and participants were asked to report when the sensation started and when they could no longer detect the sensation. The average time between these two points was taken across three trials. Proprioceptive sensation was measured as in standard clinical neurological assessments. The hallux and ankle were moved into a flexed or extended position, using only the sides of the toe or foot to avoid any anteroposterior tactile

feedback, and the participant was asked to report either “up” or “down” from the original position. Percent correct out of ten trials was recorded.

The sensory loss patterns observed in the AMP group are diverse, yet consistent with neuropathy patterns. Typically, the distalmost limbs are most impaired, while the more proximal residual limb is less affected, which may explain why half of our subjects with sensory impairment exhibited impairments only on the intact limb. These findings are also consistent with previous literature reporting sensory loss in this population.[16]

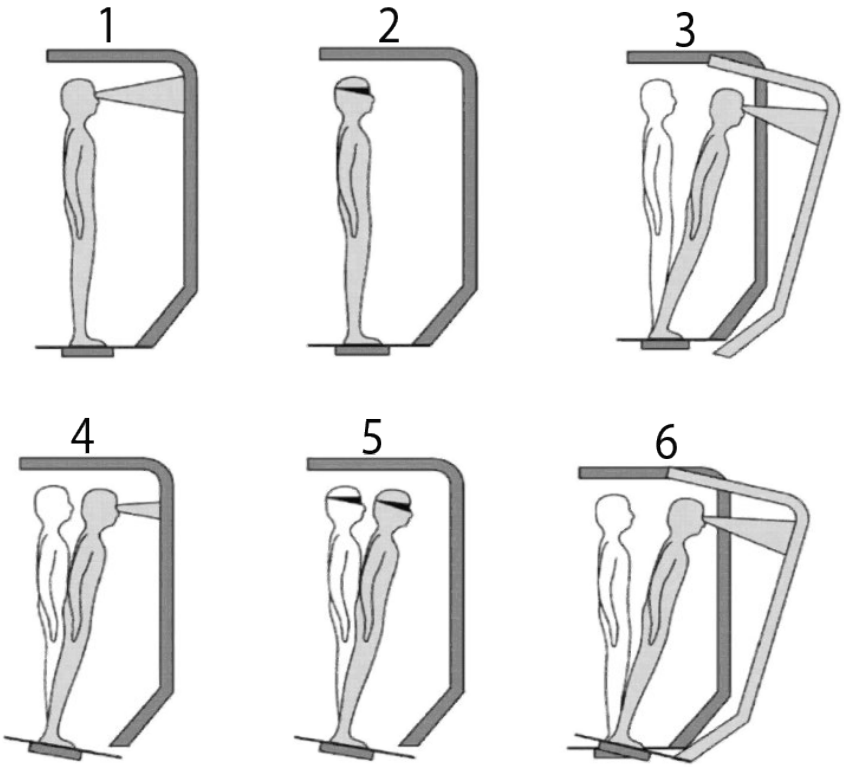
The Sensory Organization Test was implemented using a Neurocom Equitest System, which includes a visual surround that can rotate around the frontal axis and two force plates on a platform that can impart anteroposterior translations and rotate around the frontal axis at the ankles and. During the Sensory Organization Test, the subject is instructed to maintain balance during standing in one of six conditions (Supplementary Figure 1), including (1) stable support surface, eyes open, (2) stable support surface, eyes closed, (3) stable support surface, sway-referenced visual surround, (4) sway-referenced rotating support surface, eyes open, (5) sway-referenced rotating support surface, eyes closed, and (6) sway-referenced visual surround and rotating support surface. Three 20-second trials were completed per condition. Center of pressure (COP) traces were recorded from the force plates (100 Hz), filtered with a low-pass fourth-order Butterworth filter, and analyzed for standard measures of posturography, in addition to clinical measures. Equilibrium scores indicate a participant’s ability to stay within a normative 12.5° anteroposterior sway envelope (Equation A-1).

$$\text{Equilibrium Score} = \frac{12.5° - (\theta_{max} - \theta_{min})}{12.5°} \quad \text{(A-1)}$$

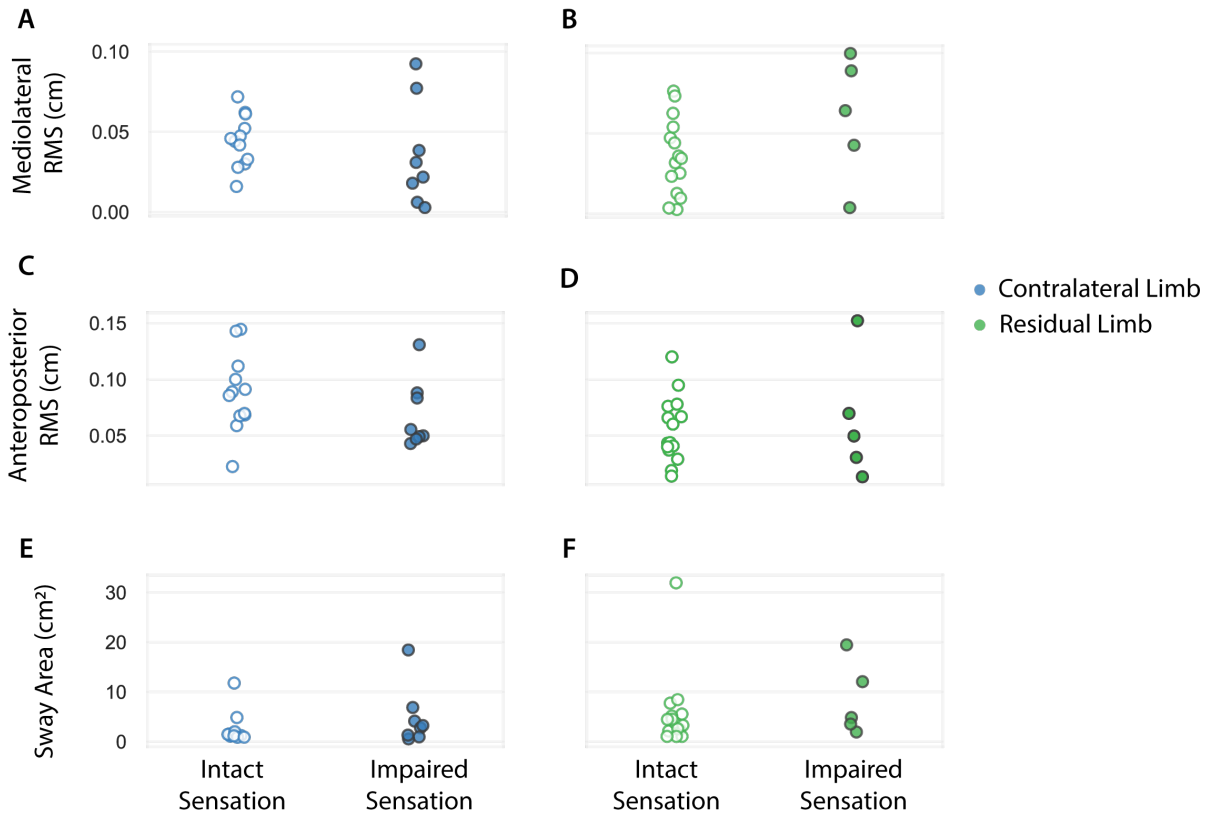
If a fall was recorded or a full trial was not completed, in accordance with NeuroCom and standard clinical protocol, a zero was recorded for the equilibrium score and the trial was not analyzed for posturography measures. Somatosensory ability (ratio of equilibrium scores in static conditions without vision, condition 2, to equilibrium scores with normal vision, condition 1) indicates a participant's ability to utilize somatosensation for balance when vision is impaired (Equation A-2).

$$\text{Somatosensory Ability} = \frac{\text{Equilibrium Score}_{\text{condition 2}}}{\text{Equilibrium Score}_{\text{condition 1}}} \quad \text{(A-2)}$$

In addition to measures of total body COP, posturography analyses were completed separately for data from the force plate under each of the limbs. Standard posturography measures include excursion (maximum displacement), sway velocity, 95% confidence interval ellipse of sway area, root-mean-square (RMS) distance, sample, and approximate entropy in both anteroposterior and mediolateral directions, as described elsewhere.[135] Approximate and sample entropy were calculated with a subseries length (m=4), similarity tolerance (r= 0.3), and a time delay (τ=5) according to entropy analyses with posturography data.[136] Additionally, posturography analyses were performed on left and right force plates, separately, to determine potential influence of sensation on each limb's stability (Supplementary Figure 2). There were no significant differences due to sensory impairment across limbs.



Supplementary Figure 1. Conditions of the Sensory Organization Test (SOT). The SOT is used to measure reliance on visual, vestibular and somatosensory systems for balance. Either visual (eyes closed, surround movement) or somatosensory feedback (platform movement) are altered while measures of sway are assessed across six conditions.



Supplementary Figure 2. Posturography measures for contralateral (blue) and residual limb (green) separated by sensory impairment in that limb. Empty circles indicate intact sensation and filled circles indicate impaired sensation on that limb. (A,B) RMS in mediolateral direction, (C,D) RMS in anteroposterior direction, (E,F) sway area did not significantly differ ($p>0.01$) when separated by sensory impairment on that limb.

The Motor Control Test is a test of reactive balance in which participants' responses to a translational perturbation are evaluated. The participants were not made aware of the expected motion of this platform, they were instructed only to maintain their balance to the best of their ability. Latency of an active force response after the onset of the perturbation was measured, as well as weight symmetry in stance prior to the perturbation. These are the standard clinical measures of the Equitest system. In individuals with an amputation, the "active response" to the

perturbation is too small to detect reliably. Because of this, only the intact limb was used for analysis for latency and the limb with the lower latency was used for able-bodied controls.

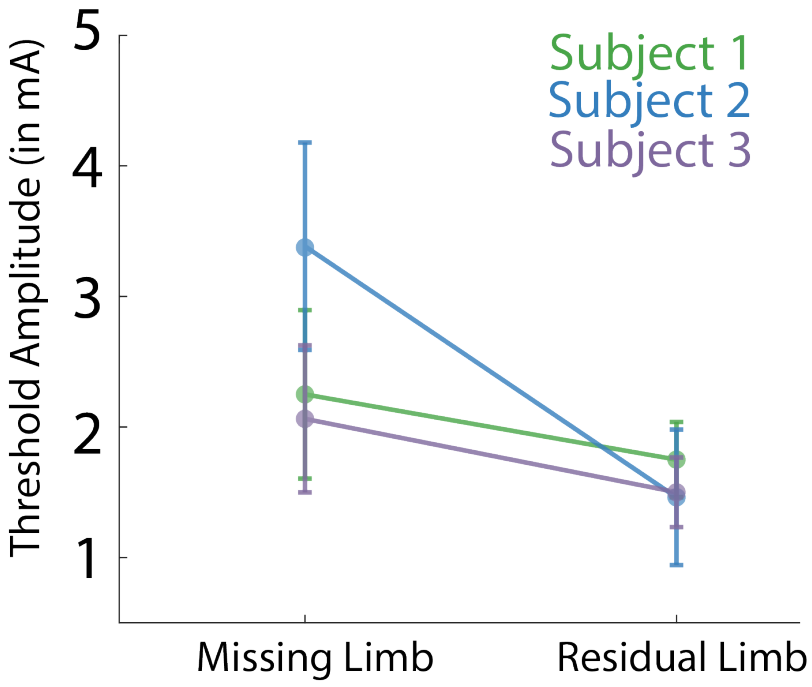
Gait kinematics during walking on a level surface were recorded using a 16-camera OptiTrack motion analysis system (Flex3 cameras, Natural Point, OR, USA). Six trials were analyzed across a 6-m walkway. Kinematic marker data was collected at 100 Hz and filtered using a 4th order low-pass Butterworth filter at 12 Hz. Step length asymmetry (normalized to stride length, Equation A-3), step length variability, and step width variability (standard deviation of step width and coefficient of variation of step width, Equation A-4) were calculated as measures of gait stability. Step length was calculated as anteroposterior distance between two consecutive heel markers at heel strike. Step width was calculated as the mediolateral distance between lateral malleolus markers of two consecutive steps. Gait assessments were only collected from 12 of the 20 AMP participants and matched to the corresponding 12 CON participants.

$$\text{Step Length Asymmetry (SLA)} = \frac{SL_{intact} - SL_{residual}}{SL_{intact} + SL_{residual}} \tag{A-3}$$

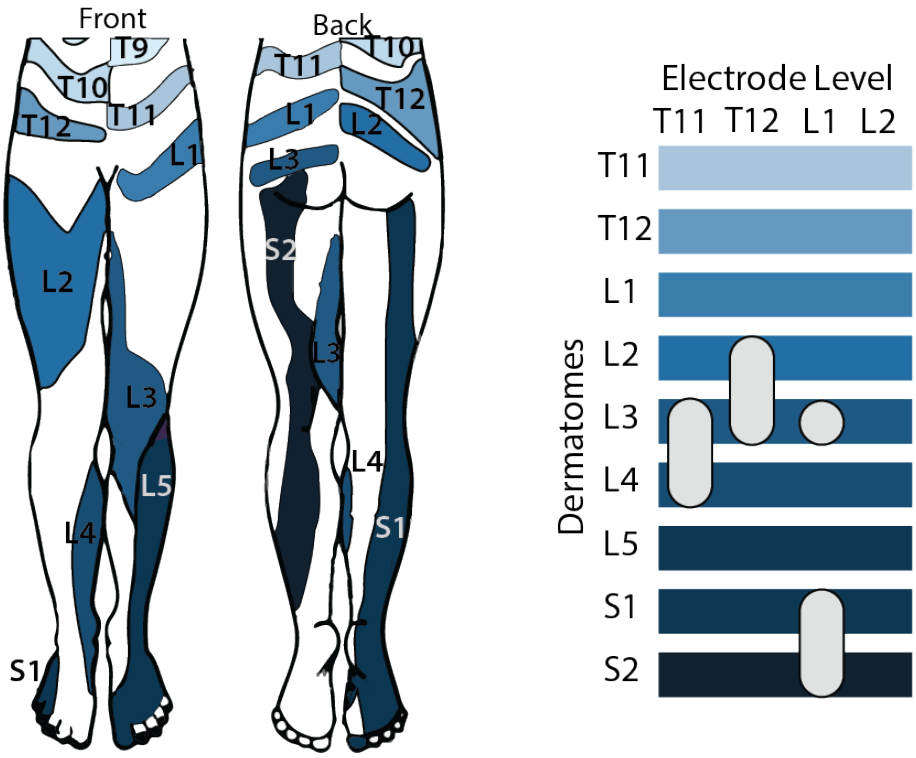
$$\text{Step Width Coefficient of Variation (CV)} = \frac{\text{Standard Devation (SD) Step Width}}{\text{Mean Step Width}} * 100 \tag{A-4}$$

Though there are only four individuals with a transfemoral amputation, these individuals were not outliers in our data. Furthermore, when used as a co-variate in a linear regression model, the relationship between level of amputation and functional measures was not significant. Thus, these individuals were included in our analysis with the rest of the AMP group.

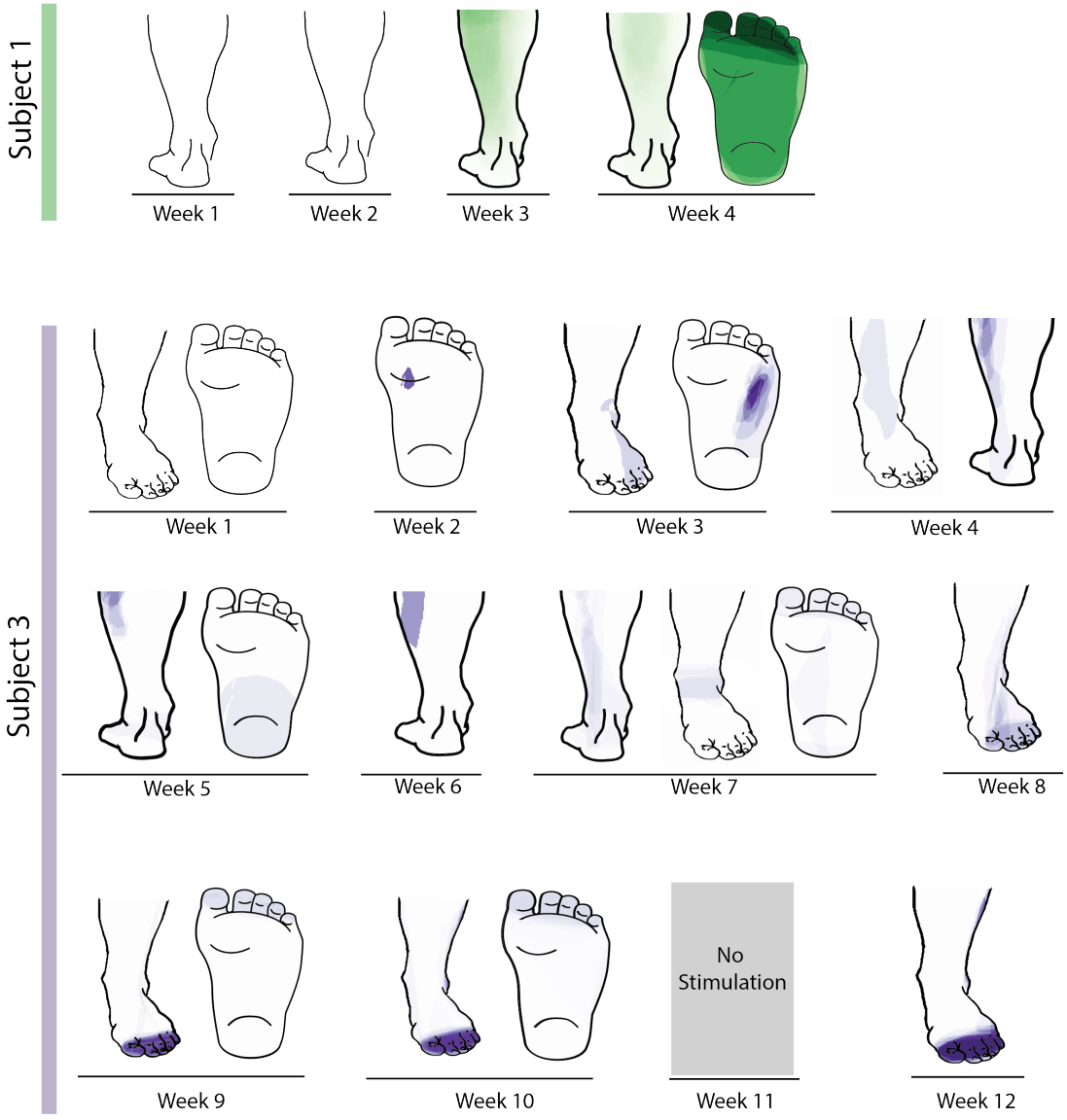
Appendix B SCS Sensory Restoration Supplementary Material



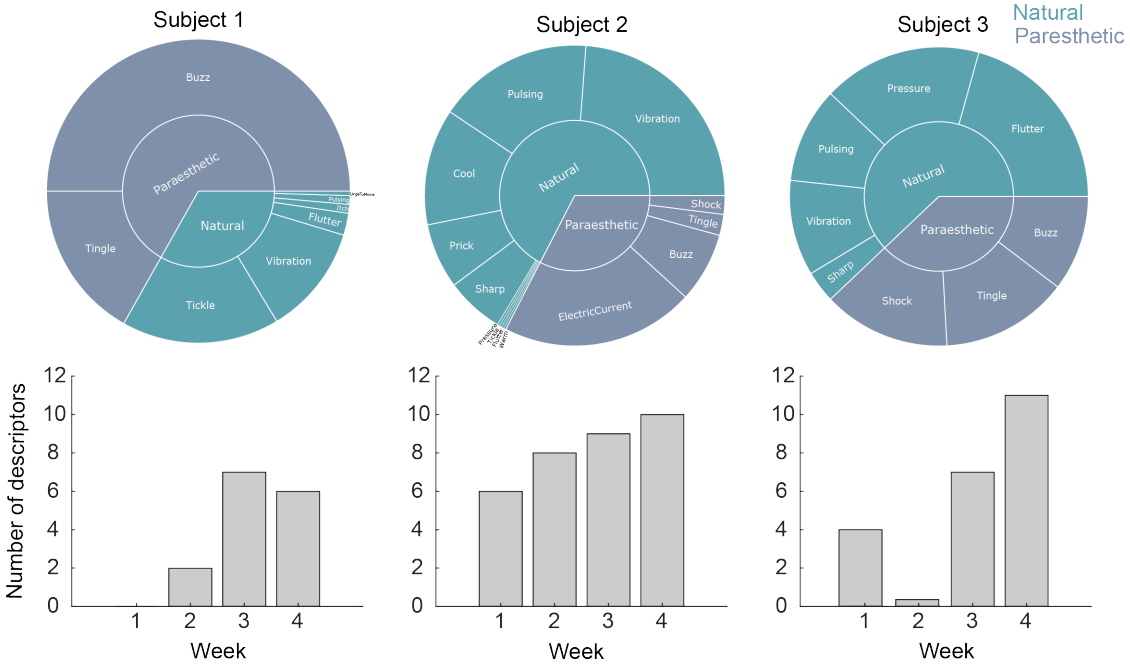
Supplementary Figure 3. Comparison of the threshold amplitude that evokes sensation in missing limb (with co-activation in the residual limb) and to the threshold amplitude that evoked sensation only in the residual limb. The threshold amplitude was determined by increasing the stimulation amplitude in 0.5 or 1 mA steps and asking the subjects to report the location where they perceive the evoked sensation. The error bars show the standard deviation across multiple days.



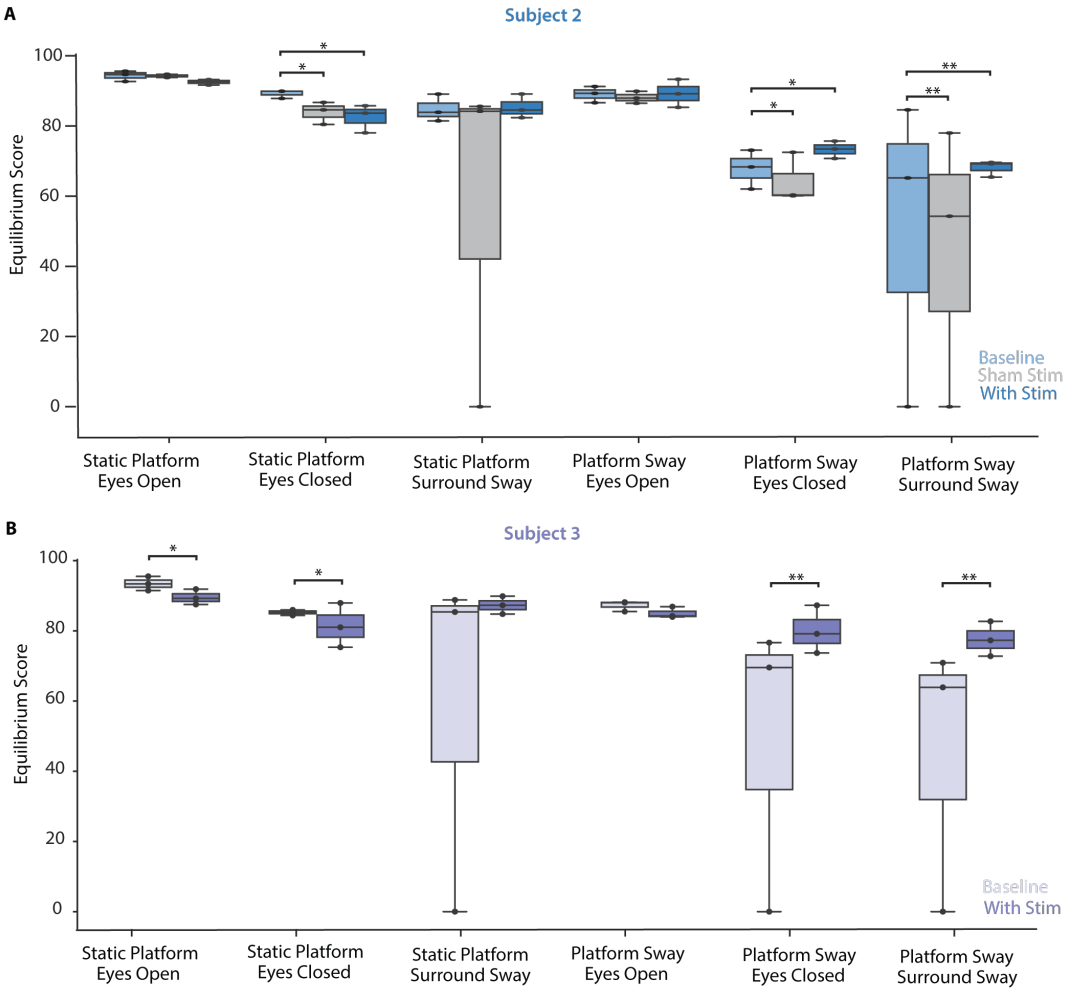
Supplementary Figure 4. Dermatome activation by electrodes located at different vertebrae levels for Subject 2. The left image shows the expected dermatomal innervation in the leg. In the right, the horizontal bars indicate different dermatomes and the vertical columns indicate the electrode position with respect to the vertebrae level. Subject 1 had significant lead migration across the weeks and therefore predicting the location of the electrodes with respect to the vertebrae level is less reliable. Hence, we have not shown these results for Subject 1.



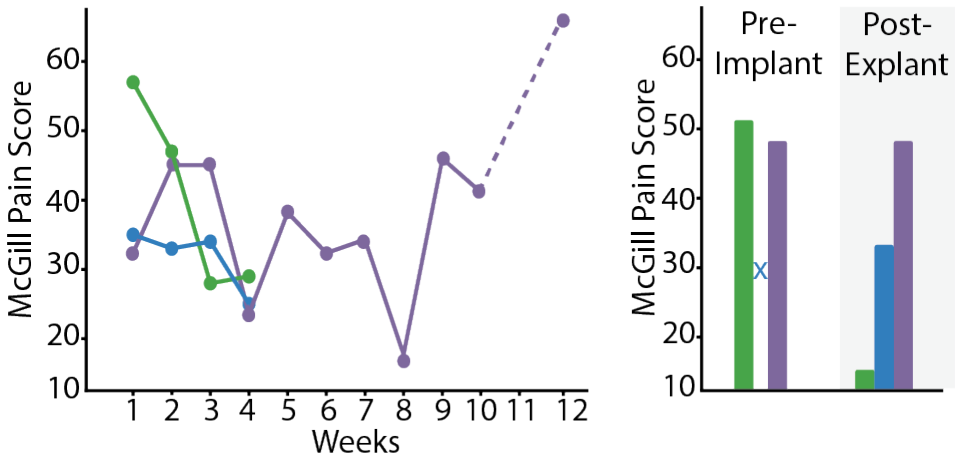
Supplementary Figure 5. Heatmap showing the rate of occurrence of sensations in the missing limb across weeks in Subject 1 and Subject 3. The darker shade indicate higher rate of occurrence of the sensations in that location. No testing was performed on week 11.



Supplementary Figure 6. Percept quality of the evoked sensations in the missing limb. The subjects were given a list of 13 natural descriptors and 5 paresthetic descriptors using which they had to describe the quality of the sensation. The top panel shows the frequency of each descriptor used to describe the evoked sensations. The bottom panel shows the total number of descriptors each week to describe the sensations.

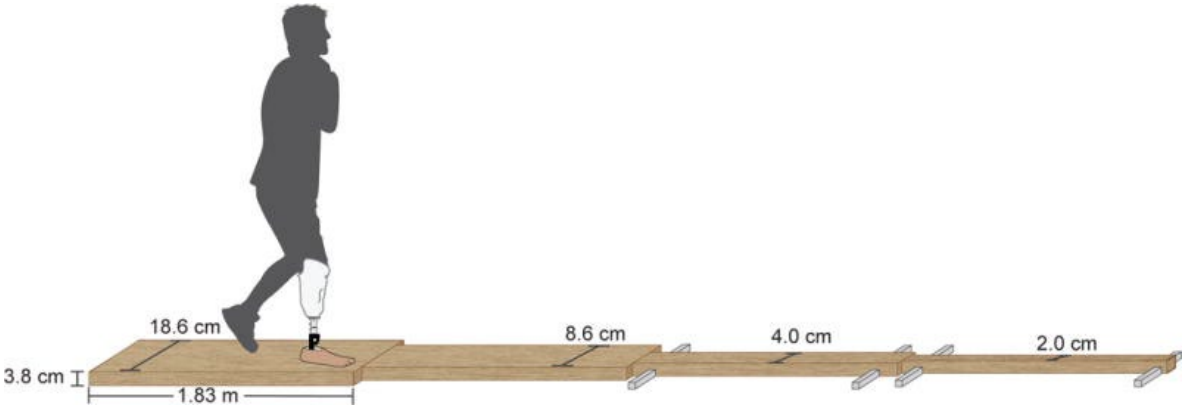


Supplementary Figure 7. Full results of Sensory Organization Test (SOT). (A) Subject 2 performed the SOT without stimulation (light blue), with sham stimulation (stimulation in the residual limb only, green) and with stimulation (stimulation in the prosthetic foot, dark blue). Sham stimulation substantially decreased performance in all trials (with greater than minimum detectable change [MDC, 3.98] for three of six conditions, suggesting that stimulation alone was not the motivator for improved performance with useful stimulation. Subject 3 performed the SOT without stimulation (light purple) and with useful stimulation (dark purple) only. Both Subject 2 and Subject 3 improved performance on conditions with platform sway and eyes closed (+5.12 Subject 2, +9.60 Subject 3) and with surround sway (+4.04 Subject 2, +13.39 Subject 3), however decreased performance on static standing with eyes closed (-6.25 Subject 2, -4.32 Subject 3). Additionally, for Subject 3 had worse performance on static standing with eyes open with stimulation (-4.13). Change in median values reported. * represents a MDC, ** represents a clinically meaningful difference (>8.0).

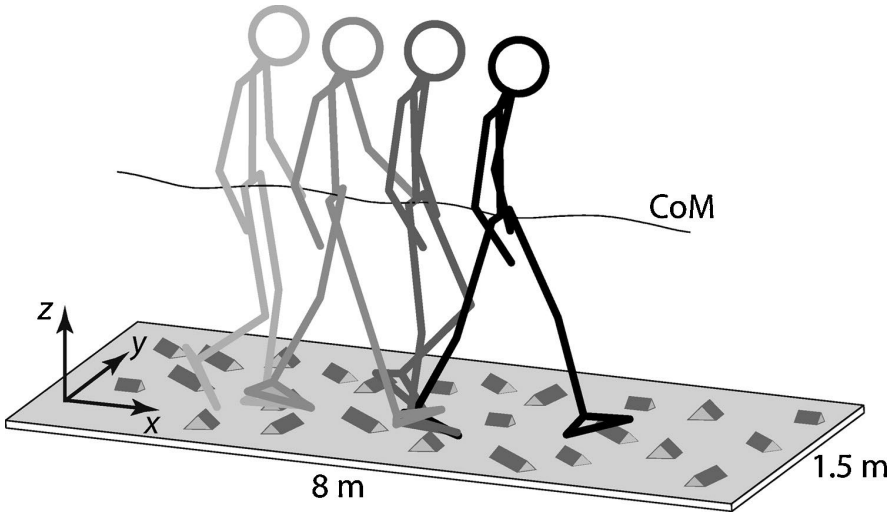


Supplementary Figure 8. Full form McGill Pain Questionnaire results. On the right, the bar plots show the McGill pain score before the implant and 1-month post-explant. The pre-implant score for Subject 2 was not recorded and we did not do any testing on week 11 for Subject 11.

Appendix C Narrowing Walking Beam Test and Uneven Walking



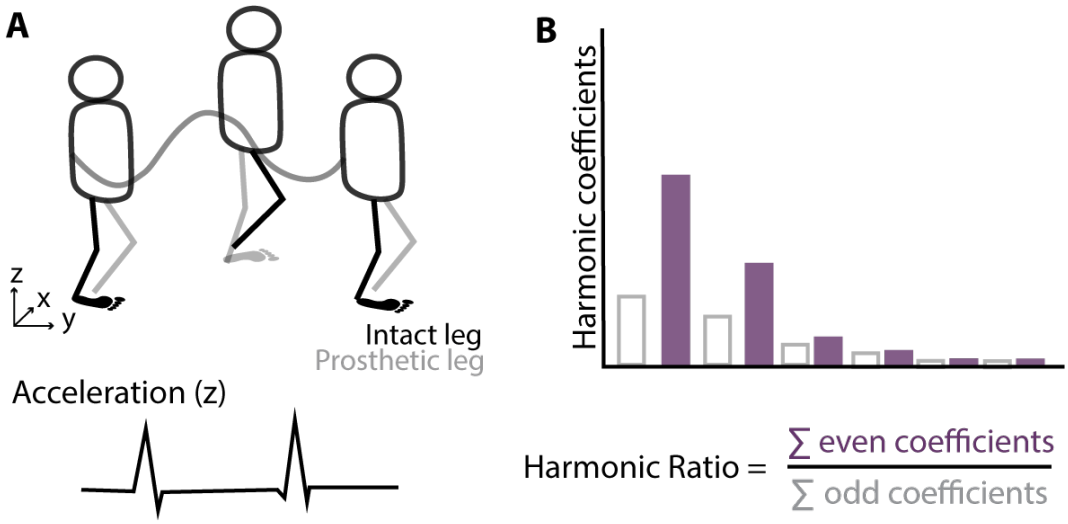
Supplementary Figure 9. Narrowing Beam for the Narrowing Beam Walking Test (NBWT). Distance traveled along the beam is recorded (to the nearest 6 in. mark) and the last 3 scores of 5 trials are averaged and normalized to the length fo the beam (6.71 m). Figure reprinted from [35].



Supplementary Figure 10. Irregular surface walking. Prisms of varying lengths are randomly placed on a 8 x1.5 m board with an industrial carpet on top. The harmonic ratio of the center of mass (CoM) is assessed by the trunk L5/S1 marker position and acceleration. Figure reprinted from [111].

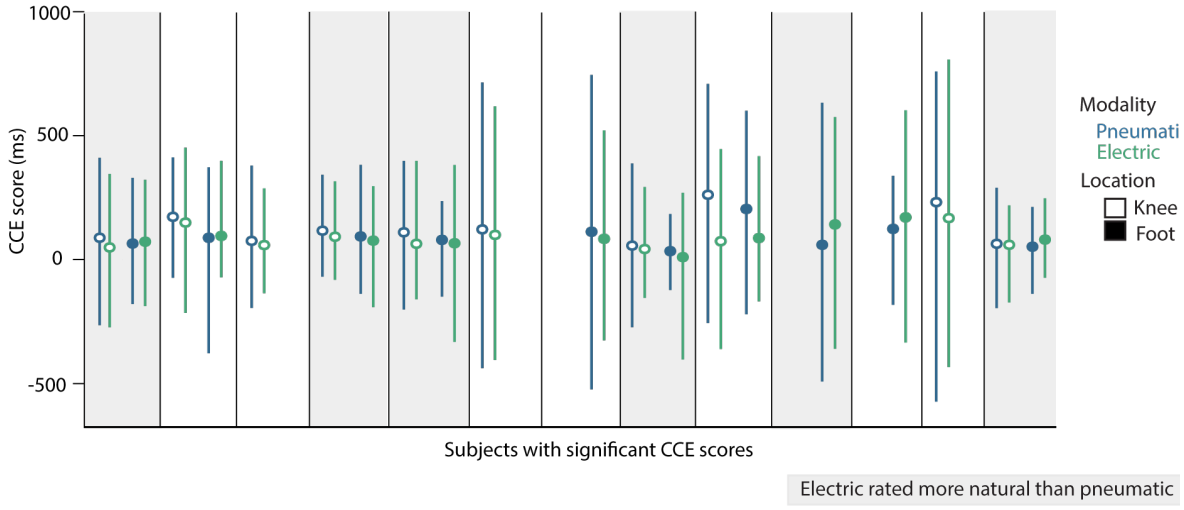
Appendix D Calculation of Harmonic Ratios

Smoothness of gait, as indicated by the harmonic ratio (HR) of trunk accelerations, is a measurement that combines gait variability of kinematics, kinetics, and motor control. In a stride, a normal acceleration pattern occurs in multiples of two, once for each limb. Thus, if trunk acceleration does not follow this pattern, that frequency is considered "out-of-phase".[115,119] Through a Fourier analysis, "in-phase" harmonics are compared with "out-of-phase" harmonics, yielding a HR. A higher HR is indicative of greater smoothness or symmetry, suggesting a higher quality of gait. To calculate this, strides were segmented according to heel and toe marker trajectories. For each stride, a Fourier transform of the trunk marker, in each direction separately, decomposed the time series to reveal the harmonics of the signal.



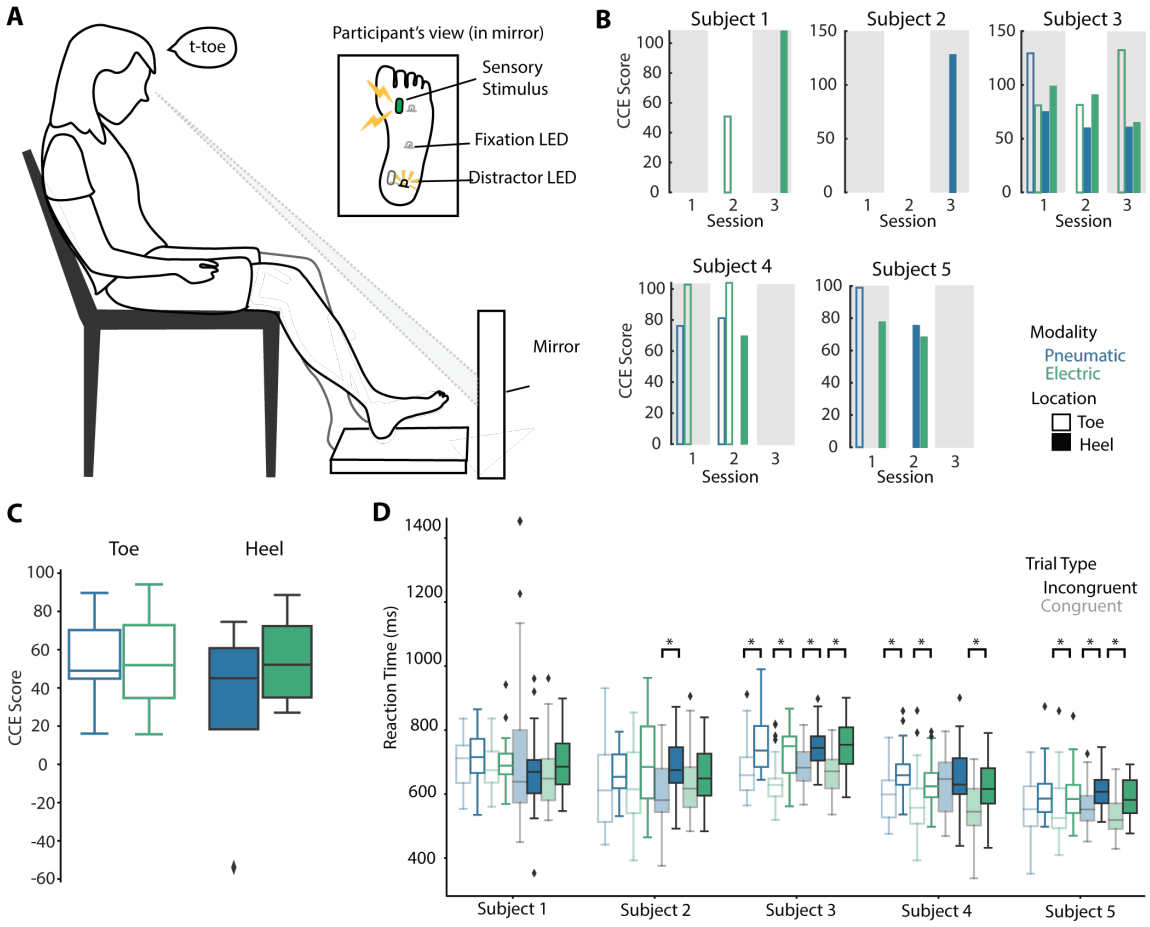
Supplementary Figure 11. Harmonic ratio calculations. Trunk acceleration for each stride is analyzed to compare the ratio of "in-phase" harmonics (even coefficients) to "out-of-phase" harmonics (odd coefficients). A higher HR indicates greater gait symmetry. For HR in the mediolateral (x) direction, "in-phase" harmonics are odd coefficients and "out-of-phase" harmonics are even coefficients.

Appendix E CCE Scores by Subject



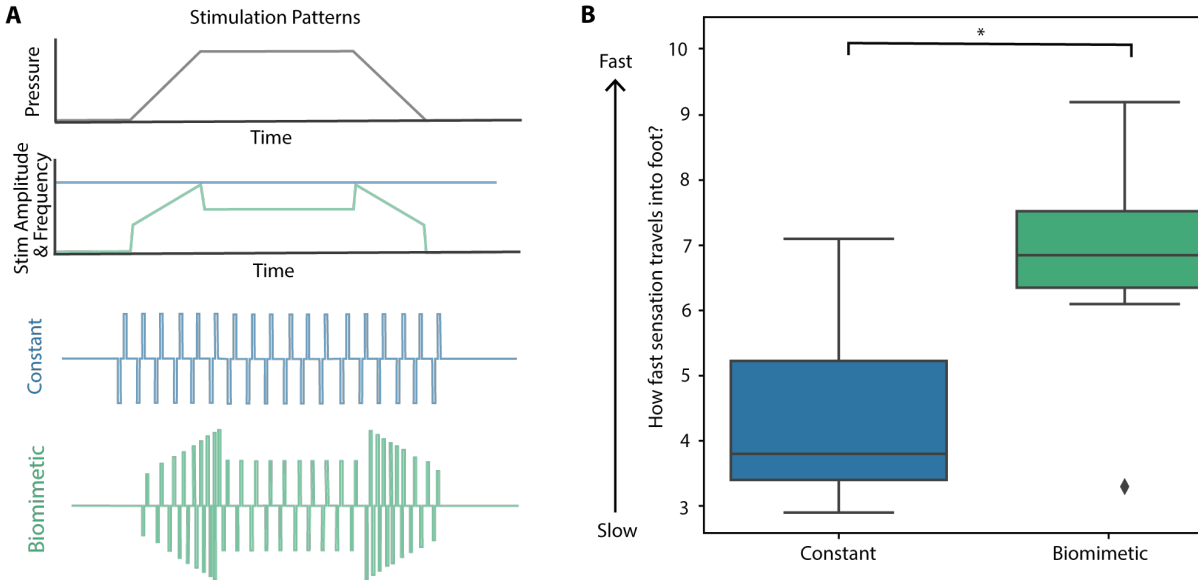
Supplementary Figure 12. CCE score for individual subjects. CCE scores for pneumatic (blue) and electric (green) stimuli at the knee (filled) and at the foot (empty). Only significant CCEs (significant differences between incongruent and congruent trials, $p<0.05$) per session and per experiment were compared across modalities. No significant patterns emerged for subjects with a higher CCE score for electric stimuli. Individuals that subjectively rated the electric sensation as more natural than the pneumatic sensation are shaded in grey. No clear patterns are seen among these individuals.

Appendix F CCE Results from Pilot Study on Foot



Supplementary Figure 13. Cross-modal congruency task using locations on the plantar foot. (A) Locations of stimuli and distractors were placed on the metatarsals ("t-toe") or on the heel ("t-heel"). The subject was able to see the distractors and fixation LED through a mirror placed at their feet. (B) Individual subjects CCE for pneumatic (blue) and electric (green) at each location by session. Only sessions with significant CCEs ($p<0.05$) were included in these plots. There were no clear findings across participants. (C) Group-level analysis of CCE scores at both locations, with both stimulus modalities. There were no significant differences ($p>0.05$) across modalities. (D) Individual plots of reaction times for congruent (lighter shades) and incongruent (darker shade)s when combining sessions. * indicates significant CCE scores. Reaction times were highly variable for all participants.

Appendix G Changes in Quality of Evoked Sensations with Biomimetic Stimulation



Supplementary Figure 14. Temporal quality of biomimetic vs constant frequency stimulation. (A) Constant frequency and amplitude stimulation (blue) versus biomimetic stimulation (green) which co-modulates amplitude and frequency according to the modelled pressure indentation of the skin. (B) Subjective ratings of how fast the sensation traveled into the foot. The subject was asked to report on a scale of 1-10 how quickly the sensation traveled down into her foot. Biomimetic stimulation (green) had a significantly quicker time course (+2.31 au, p=0.003). * indicates a statistically significant difference.

Bibliography

1. Miller WC, Speechley M, Deathe B. The prevalence and risk factors of falling and fear of falling among lower extremity amputees. *Arch Phys Med Rehabil*. 2001;82(8):1031-1037. doi:https://doi.org/10.1053/apmr.2001.24295

2. Wong CK, Chihuri ST, Li G. Risk of fall-related injury in people with lower limb amputations: A prospective cohort study. *J Rehabil Med*. 2016;48(1):80-85. doi:10.2340/16501977-2042

3. Prochazka A, Gorassini M. Models of ensemble firing of muscle spindle afferents recorded during normal locomotion in cats. *J Physiol*. 1998;507(1):277-291. doi:10.1111/j.1469-7793.1998.277bu.x

4. Pearson KG. Generating the walking gait: role of sensory feedback. *Prog Brain Res*. 2004;143:123-129.

5. Schubert M, Curt A, Jensen L, Dietz V. Corticospinal input in human gait: modulation of magnetically evoked motor responses. *Exp Brain Res*. 1997;115(2):234-246. doi:10.1007/PL00005693

6. Viseux FJF. The sensory role of the sole of the foot: Review and update on clinical perspectives. *Neurophysiol Clin*. 2020;50(1):55-68. doi:https://doi.org/10.1016/j.neucli.2019.12.003

7. Varrecchia T, Serrao M, Rinaldi M, et al. Common and specific gait patterns in people with varying anatomical levels of lower limb amputation and different prosthetic components. *Hum Mov Sci*. 2019;66:9-21. doi:10.1016/j.humov.2019.03.008

8. Timar B, Timar R, Gaiță L, Oancea C, Levai C, Lungeanu D. The Impact of Diabetic Neuropathy on Balance and on the Risk of Falls in Patients with Type 2 Diabetes Mellitus: A Cross-Sectional Study. *PLoS One*. 2016;11(4):e0154654. https://doi.org/10.1371/journal.pone.0154654

9. DeMott TK, Richardson JK, Thies SB, Ashton-Miller JA. Falls and Gait Characteristics Among Older Persons with Peripheral Neuropathy. *Am J Phys Med Rehabil*. 2007;86(2):125-132. doi:10.1097/PHM.0b013e31802ee1d1

10. Brown SJ, Handsaker JC, Bowling FL, Boulton AJM, Reeves ND. Diabetic Peripheral Neuropathy Compromises Balance During Daily Activities. *Diabetes Care*. 2015;38(6):1116 LP - 1122. doi:10.2337/dc14-1982

11. Ducic I, Short KW, Dellon AL. Relationship Between Loss of Pedal Sensibility, Balance, and Falls in Patients With Peripheral Neuropathy. *Ann Plast Surg*. 2004;52(6).

https://journals.lww.com/annalsplasticsurgery/Fulltext/2004/06000/Relationship_Between_Loss_of_Pedal_Sensibility,.1.aspx

12. Wang T-Y, Lin S-I. Sensitivity of plantar cutaneous sensation and postural stability. *Clin Biomech*. 2008;23(4):493-499. doi:https://doi.org/10.1016/j.clinbiomech.2007.11.014

13. McKeon PO, Hertel JAY. Plantar hypoesthesia alters time-to-boundary measures of postural control. *Somatosens Mot Res*. 2007;24(6):171-177. doi:10.1080/08990220701637224

14. Sood P, Chatterjee SA, Skinner JW, et al. Somatosensory impairment of the feet is associated with higher activation of prefrontal cortex during walking in older adults. *Exp Gerontol*. 2022;165:111845. doi:https://doi.org/10.1016/j.exger.2022.111845

15. Fasano A, Plotnik M, Bove F, Berardelli A. The neurobiology of falls. *Neurol Sci*. 2012;33(6):1215-1223. doi:10.1007/s10072-012-1126-6

16. Templeton CA, Strzalkowski NDJ, Galvin P, Bent LR. Cutaneous sensitivity in unilateral trans-tibial amputees. *PLoS One*. 2018;13(6):e0197557. https://doi.org/10.1371/journal.pone.0197557

17. Chen L, Feng Y, Chen B, Wang Q, Wei K. Improving postural stability among people with lower-limb amputations by tactile sensory substitution. *J Neuroeng Rehabil*. 2021;18(1):159. doi:10.1186/s12984-021-00952-x

18. Crea S, Cipriani C, Donati M, Carrozza MC, Vitiello N. Providing Time-Discrete Gait Information by Wearable Feedback Apparatus for Lower-Limb Amputees: Usability and Functional Validation. *IEEE Trans Neural Syst Rehabil Eng*. 2015;23(2):250-257. doi:10.1109/TNSRE.2014.2365548

19. Zambarbieri D, Schmid M, Verni G. Sensory feedback for lower limb prostheses. *Intell Syst Technol Rehabil Eng*. 2001;(January). doi:10.1201/9781420042122.ch4

20. Davis JBDBL, Frazier DM. Use of an instrumented treadmill for real-time gait symmetry evaluation and feedback in normal and trans-tibia1 amputee subjects. 1996;(1989):101-110.

21. Pagel A, Arieta AH, Riener R, Vallery H. Effects of sensory augmentation on postural control and gait symmetry of transfemoral amputees: a case description. *Med Biol Eng Comput*. 2016;54(10):1579-1589. doi:10.1007/s11517-015-1432-2

22. Escamilla-Nunez R, Michelini A, Andrysek J. Biofeedback Systems for Gait Rehabilitation of Individuals with Lower-Limb Amputation: A Systematic Review. *Sensors* . 2020;20(6). doi:10.3390/s20061628

23. Petrini FM, Bumbasirevic M, Valle G, et al. Sensory feedback restoration in leg amputees improves walking speed, metabolic cost and phantom pain. *Nat Med*. 2019;25(9):1356-1363. doi:10.1038/s41591-019-0567-3

24. Petrini FM, Valle G, Bumbasirevic M, et al. Enhancing functional abilities and cognitive integration of the lower limb prosthesis. *Sci Transl Med*. 2019;11(512):eaav8939. doi:10.1126/scitranslmed.aav8939

25. Charkhkar H, Christie BP, Triolo RJ. Sensory neuroprosthesis improves postural stability during Sensory Organization Test in lower-limb amputees. *Sci Rep*. 2020;10(1):6984. doi:10.1038/s41598-020-63936-2

26. Christie BP, Charkhkar H, Shell CE, Burant CJ, Tyler DJ, Triolo RJ. Ambulatory searching task reveals importance of somatosensation for lower-limb amputees. *Sci Rep*. 2020;10(1):10216. doi:10.1038/s41598-020-67032-3

27. Shell CE, Christie BP, Marasco PD, Charkhkar H, Triolo RJ. Lower-Limb Amputees Adjust Quiet Stance in Response to Manipulations of Plantar Sensation . *Front Neurosci* . 2021;15. https://www.frontiersin.org/article/10.3389/fnins.2021.611926

28. Charkhkar H, Shell CE, Marasco PD, Pinault GJ, Tyler DJ, Triolo RJ. High-density peripheral nerve cuffs restore natural sensation to individuals with lower-limb amputations. *J Neural Eng*. 2018;15(5):056002. doi:10.1088/1741-2552/aac964

29. Valle G, Saliji A, Fogle E, Cimolato A, Petrini FM, Raspopovic S. Mechanisms of neuro-robotic prosthesis operation in leg amputees. *Sci Adv*. 2021;7(17):eabd8354.

30. Jayakaran P, Johnson GM, Sullivan SJ. Postural Control in Response to Altered Sensory Conditions in Persons With Dysvascular and Traumatic Transtibial Amputation. *Arch Phys Med Rehabil*. 2015;96(2):331-339. doi:https://doi.org/10.1016/j.apmr.2014.09.037

31. Jayakaran P, Johnson GM, Sullivan SJ. Test-Retest Reliability of the Sensory Organization Test in Older Persons With a Transtibial Amputation. *PM&R*. 2011;3(8):723-729. doi:https://doi.org/10.1016/j.pmrj.2011.01.005

32. Jayakaran P, Johnson GM, Sullivan SJ, Nitz JC. Instrumented measurement of balance and postural control in individuals with lower limb amputation: a critical review. *Int J Rehabil Res*. 2012;35(3). https://journals.lww.com/intjrehabilres/Fulltext/2012/09000/Instrumented_measurement_of_balance_and_postural.1.aspx

33. Sinha R, van den Heuvel WJA, Arokiasamy P. Factors affecting quality of life in lower limb amputees. *Prosthet Orthot Int*. 2011;35(1):90-96. doi:10.1177/0309364610397087

34. Sawers A, Kim J, Balkman G, Hafner BJ. Interrater and Test-Retest Reliability of Performance-Based Clinical Tests Administered to Established Users of Lower Limb Prostheses. *Phys Ther*. 2020;100(7):1206-1216. doi:10.1093/ptj/pzaa063

35. Sawers A, Hafner BJ. Narrowing beam-walking is a clinically feasible approach for assessing balance ability in lower-limb prosthesis users. *J Rehabil Med*. 2018;50(5):457-464. doi:10.2340/16501977-2329

36. Thies SB, Richardson JK, Ashton-Miller JA. Effects of surface irregularity and lighting on step variability during gait: A study in healthy young and older women. *Gait Posture*. 2005;22(1):26-31. doi:10.1016/j.gaitpost.2004.06.004

37. Chandrasekaran S, Nanivadekar AC, McKernan G, et al. Sensory restoration by epidural stimulation of the lateral spinal cord in upper-limb amputees. Makin TR, Ivry RB, Makin TR, Perich MG, eds. *Elife*. 2020;9:e54349. doi:10.7554/eLife.54349

38. Cameron T. Safety and efficacy of spinal cord stimulation for the treatment of chronic pain: a 20-year literature review. *J Neurosurg*. 2004;100(3 Suppl Spine):254-267. doi:10.3171/spi.2004.100.3.0254

39. Nanivadekar AC, Chandrasekaran S, Helm ER, et al. Closed-loop stimulation of lateral cervical spinal cord in upper-limb amputees to enable sensory discrimination. *medRxiv*. Published online January 1, 2022:2022.03.25.22272820. doi:10.1101/2022.03.25.22272820

40. Saal HP, Bensmaia SJ. Biomimetic approaches to bionic touch through a peripheral nerve interface. *Neuropsychologia*. 2015;79:344-353. doi:10.1016/j.neuropsychologia.2015.06.010

41. Spence C, Nicholls ME, Gillespie N, Driver J. Cross-modal links in exogenous covert spatial orienting between touch, audition, and vision. *Percept Psychophys*. 1998;60(4):544-557.

42. Spence C. Multisensory attention and tactile information-processing. *Behav Brain Res*. 2002;135(1-2):57-64.

43. Spence C, Pavani F, Maravita A, Holmes N. Multisensory contributions to the 3-D representation of visuotactile peripersonal space in humans: evidence from the crossmodal congruency task. *J Physiol Paris*. 2004;98(1-3):171-189. doi:10.1016/j.jphysparis.2004.03.008

44. Zopf R, Savage G, Williams MA. The crossmodal congruency task as a means to obtain an objective behavioral measure in the rubber hand illusion paradigm. *J Vis Exp*. 2013;(77). doi:10.3791/50530

45. Marini F, Romano D, Maravita A. The contribution of response conflict, multisensory integration, and body-mediated attention to the crossmodal congruency effect. *Exp Brain Res*. 2017;235(3):873-887. doi:10.1007/s00221-016-4849-4

46. Blustein D, Wilson A, Sensinger J. Assessing the quality of supplementary sensory feedback using a crossmodal congruency task. *Sci Rep*. 2017;(October 2017):1-19. doi:10.1038/s41598-018-24560-3

47. Blustein D, Gill S, Wilson A, Sensinger J. Crossmodal congruency effect scores decrease with repeat test exposure. *PeerJ*. 2019;7:e6976. doi:10.7717/peerj.6976

48. Detrembleur C, Vanmarsenille JM, De Cuyper F, Dierick F. Relationship between energy cost, gait speed, vertical displacement of centre of body mass and efficiency of pendulum-like mechanism in unilateral amputee gait. *Gait Posture*. 2005;21(3):333-340. doi:10.1016/j.gaitpost.2004.04.005

49. Weinert-Aplin RA, Twiste M, Jarvis HL, et al. Medial-lateral centre of mass displacement and base of support are equally good predictors of metabolic cost in amputee walking. *Gait Posture*. 2017;51:41-46. doi:10.1016/j.gaitpost.2016.09.024

50. Barnett CT, Vanicek N, Polman RCJ. Postural responses during volitional and perturbed dynamic balance tasks in new lower limb amputees: A longitudinal study. *Gait Posture*. 2013;37(3):319-325. doi:https://doi.org/10.1016/j.gaitpost.2012.07.023

51. Dillingham TR, Pezzin LE, MacKenzie EJBT-SMJ. Limb amputation and limb deficiency: epidemiology and recent trends in the United States. 2002;95(8):875+. https://link.gale.com/apps/doc/A90569925/AONE?u=anon~9bbf278b&sid=googleScholar&xid=31d7d7d6

52. Dhillon GS, Horch KW. Direct neural sensory feedback and control of a prosthetic arm. *IEEE Trans Neural Syst Rehabil Eng*. 2005;13(4):468-472. doi:10.1109/TNSRE.2005.856072

53. Rossini PM, Micera S, Benvenuto A, et al. Double nerve intraneural interface implant on a human amputee for robotic hand control. *Clin Neurophysiol*. 2010;121(5):777-783. doi:10.1016/j.clinph.2010.01.001

54. Horch K, Meek S, Taylor TG, Hutchinson DT. Object discrimination with an artificial hand using electrical stimulation of peripheral tactile and proprioceptive pathways with intrafascicular electrodes. *IEEE Trans Neural Syst Rehabil Eng*. 2011;19(5):483-489. doi:10.1109/TNSRE.2011.2162635

55. Valle G, Petrini FM, Strauss I, et al. Comparison of linear frequency and amplitude modulation for intraneural sensory feedback in bidirectional hand prostheses. *Sci Rep*. 2018;8(1):16666. doi:10.1038/s41598-018-34910-w

56. Ziegler-Graham K, MacKenzie EEJEEJ, Ephraim PPLPPL, Travison TTG, Brookmeyer R. Estimating the prevalence of limb loss in the United States: 2005 to 2050. *Arch Phys Med Rehabil*. 2008;89(3):422-429. doi:10.1016/j.apmr.2007.11.005

57. Miller WC, Speechley M, Deathe B, Wc AM, Speechley M, The DB. The prevalence and risk factors of falling and fear of falling among lower extremity amputees. *Arch Phys Med Rehabil*. 2001;82(8):1031-1037. doi:10.1053/apmr.2001.24295

58. Moreland B, Kakara R, Henry A. Trends in Nonfatal Falls and Fall-Related Injuries Among Adults Aged ≥65 Years - United States, 2012-2018. *MMWR Morb Mortal Wkly Rep*. 2020;69(27):875-881. doi:10.15585/mmwr.mm6927a5

59. Vanicek N, Strike S, McNaughton L, Polman R. Postural Responses to Dynamic

Perturbations in Amputee Fallers Versus Nonfallers: A Comparative Study With Able-Bodied Subjects. *Arch Phys Med Rehabil*. 2009;90(6):1018-1025. doi:https://doi.org/10.1016/j.apmr.2008.12.024

60. Claret CR, Herget GW, Kouba L, et al. Neuromuscular adaptations and sensorimotor integration following a unilateral transfemoral amputation. *J Neuroeng Rehabil*. 2019;16(1):115. doi:10.1186/s12984-019-0586-9

61. Ku PX, Abu Osman NA, Wan Abas WAB. Balance control in lower extremity amputees during quiet standing: a systematic review. *Gait Posture*. 2014;39(2):672-682. doi:10.1016/j.gaitpost.2013.07.006

62. Mancini M, Horak FB. The relevance of clinical balance assessment tools to differentiate balance deficits. *Eur J Phys Rehabil Med*. 2010;46(2):239-248. https://pubmed.ncbi.nlm.nih.gov/20485226

63. Honeycutt CF, Nichols TR. Disruption of cutaneous feedback alters magnitude but not direction of muscle responses to postural perturbations in the decerebrate cat. *Exp brain Res*. 2010;203(4):765-771. doi:10.1007/s00221-010-2281-8

64. Nakajima H, Yamamoto S, Katsuhira J. Effects of diabetic peripheral neuropathy on gait in vascular trans-tibial amputees. *Clin Biomech*. 2018;56:84-89. doi:10.1016/j.clinbiomech.2018.05.005

65. Ephraim PL, Wegener ST, MacKenzie EJ, Dillingham TR, Pezzin LE. Phantom pain, residual limb pain, and back pain in amputees: Results of a national survey. *Arch Phys Med Rehabil*. 2005;86(10):1910-1919. doi:10.1016/j.apmr.2005.03.031

66. Koehler-McNicholas SR, Danzl L, Cataldo AY, Oddsson LIE. Neuromodulation to improve gait and balance function using a sensory neuroprosthesis in people who report insensate feet – A randomized control cross-over study. *PLoS One*. 2019;14(4):e0216212. https://doi.org/10.1371/journal.pone.0216212

67. Molina-Rueda F, Molero-Sánchez A, Alguacil-Diego IM, Carratalá-Tejada M, Cuesta-Gómez A, Miangolarra-Page JC. Weight Symmetry and Latency Scores for Unexpected Surface Perturbations in Subjects With Traumatic and Vascular Unilateral Transtibial Amputation. *PM&R*. 2016;8(3):235-240. doi:https://doi.org/10.1016/j.pmrj.2015.05.024

68. Wrisley DM, Marchetti GF, Kuharsky DK, Whitney SL. Reliability, Internal Consistency, and Validity of Data Obtained With the Functional Gait Assessment. *Phys Ther*. 2004;84(10):906-918. http://dx.doi.org/10.1093/ptj/84.10.906

69. Wrisley DM, Kumar NA. Functional Gait Assessment: Concurrent, Discriminative, and Predictive Validity in Community-Dwelling Older Adults. *Phys Ther*. 2010;90(5):761-773. doi:10.2522/ptj.20090069

70. Leddy AL, Crowner BE, Earhart GM. Functional Gait Assessment and Balance Evaluation System Test: Reliability, Validity, Sensitivity, and Specificity for Identifying

Individuals With Parkinson Disease Who Fall. *Phys Ther*. 2011;91(1):102-113. http://dx.doi.org/10.2522/ptj.20100113

71. Skeleton Tracking—NaturalPoint Product Documentation Ver 2.2. https://v22.wiki.optitrack.com/index.php

72. Feng Y, Schlösser FJ, Sumpio BE. The Semmes Weinstein monofilament examination as a screening tool for diabetic peripheral neuropathy. *J Vasc Surg*. 2009;50(3):675-682.e1. doi:https://doi.org/10.1016/j.jvs.2009.05.017

73. Miller S, Ruit JB, Van der Meche FG. Reversal of sign of long spinal reflexes dependent on the phase of the step cycle in the high decerebrate cat. *Brain Res*. 1977;128(3):447-459.

74. Keklicek H, Kirdi E, Yalcin A, et al. Comparison of gait variability and symmetry in trained individuals with transtibial and transfemoral limb loss. *J Orthop Surg (Hong Kong)*. 2019;27(1):2309499019832665. doi:10.1177/2309499019832665

75. Pröbsting E, Bellmann M, Schmalz T, Hahn A. Gait characteristics of transtibial amputees on level ground in a cohort of 53 amputees-Comparison of kinetics and kinematics with non-amputees. *Can PROSTHETICS Orthot J*. 2019;2(2).

76. Stenum J, Choi JT. Disentangling the energetic costs of step time asymmetry and step length asymmetry in human walking. *J Exp Biol*. 2021;224(12):jeb242258. doi:10.1242/jeb.242258

77. Charkhkar H, Shell CE, Marasco PD, Pinault GJ, Tyler DJ, Triolo RJ. High-density peripheral nerve cuffs restore natural sensation to individuals with lower-limb amputations. *J Neural Eng*. 2018;15(5):056002. doi:10.1088/1741-2552/aac964

78. Raspopovic S, Capogrosso M, Petrini FM, et al. Restoring natural sensory feedback in real-time bidirectional hand prostheses. *Sci Transl Med*. 2014;6(222):222ra19. doi:10.1126/scitranslmed.3006820

79. Ortiz-Catalan M, Hakansson B, Branemark R. An osseointegrated human-machine gateway for long-term sensory feedback and motor control of artificial limbs. *Sci Transl Med*. 2014;6(257):257re6-257re6. doi:10.1126/scitranslmed.3008933

80. Tan DW, Schiefer MA, Keith MW, Anderson JR, Tyler J, Tyler DJ. A neural interface provides long-term stable natural touch perception. *Sci Transl Med*. 2014;6(257):257ra138-257ra138. doi:10.1126/scitranslmed.3008669

81. Davis TS, Wark HAC, Hutchinson DT, et al. Restoring motor control and sensory feedback in people with upper extremity amputations using arrays of 96 microelectrodes implanted in the median and ulnar nerves. *J Neural Eng*. 2016;13(3):036001. doi:10.1088/1741-2560/13/3/036001

82. Kumar K, Rizvi S. Historical and Present State of Neuromodulation in Chronic Pain. *Curr*

Pain Headache Rep. 2014;18(1):387. doi:10.1007/s11916-013-0387-y

83. Fanciullo GJ, Rose RJ, Lunt PG, Whalen PK, Ross E. The State of Implantable Pain Therapies in the United States: A Nationwide Survey of Academic Teaching Programs. *Anesth Analg*. 1999;88(6). https://journals.lww.com/anesthesia-analgesia/Fulltext/1999/06000/The_State_of_Implantable_Pain_Therapies_in_the.21.aspx

84. Lee MWL, McPhee RW, Stringer MD. An evidence-based approach to human dermatomes. *Clin Anat Off J Am Assoc Clin Anat Br Assoc Clin Anat*. 2008;21(5):363-373.

85. Kim LH, McLeod RS, Kiss ZHT. A new psychometric questionnaire for reporting of somatosensory percepts. *J Neural Eng*. 2018;15(1):13002.

86. Raspopovic S, Capogrosso M, Petrini FMM, et al. Restoring Natural Sensory Feedback in Real-Time Bidirectional Hand Prostheses. *Sci Transl Med*. 2014;6(222):222ra19-222ra19. doi:10.1126/scitranslmed.3006820

87. Graczyk EL, Schiefer MA, Saal HP, Delhaye BP, Bensmaia SJ, Tyler DJ. The neural basis of perceived intensity in natural and artificial touch. *Sci Transl Med*. 2016;8(362):1-11. doi:10.1126/scitranslmed.aaf5187

88. Page DM, George JA, Wendelken SM, et al. Discriminability of multiple cutaneous and proprioceptive hand percepts evoked by intraneural stimulation with Utah slanted electrode arrays in human amputees. *J Neuroeng Rehabil*. 2021;18(1):12. doi:10.1186/s12984-021-00808-4

89. Mastinu E, Clemente F, Sassu P, et al. Grip control and motor coordination with implanted and surface electrodes while grasping with an osseointegrated prosthetic hand. *J Neuroeng Rehabil*. 2019;16(1):49. doi:10.1186/s12984-019-0511-2

90. Flesher SN, Collinger JL, Foldes ST, et al. Intracortical microstimulation of human somatosensory cortex. *Sci Transl Med*. 2016;8(361):361ra141-361ra141. doi:10.1126/scitranslmed.aaf8083

91. Melzack R. The McGill Pain Questionnaire: major properties and scoring methods. *Pain*. 1975;1(3):277-299.

92. Cho TA. Spinal cord functional anatomy. *Contin Lifelong Learn Neurol*. 2015;21(1):13-35.

93. Corniani G, Saal HP. Tactile innervation densities across the whole body. *J Neurophysiol*. 2020;124(4):1229-1240.

94. Kim S, Callier T, Tabot GA, Gaunt RA, Tenore F V, Bensmaia SJ. Behavioral assessment of sensitivity to intracortical microstimulation of primate somatosensory cortex. *Proc Natl Acad Sci U S A*. 2015;112(49):15202-15207. doi:10.1073/pnas.1509265112

95. Petersen B, Sparto PJ, Fisher LE. Clinical measures of balance and gait cannot differentiate somatosensory impairments in people with lower-limb amputation. *medRxiv*. Published online 2022.

96. Bouyer LJG, Rossignol S. Contribution of cutaneous inputs from the hindpaw to the control of locomotion. I. Intact cats. *J Neurophysiol*. 2003;90(6):3625-3639.

97. Steele AG, Manson GA, Horner PJ, Sayenko DG, Contreras-Vidal JL. Effects of transcutaneous spinal stimulation on spatiotemporal cortical activation patterns: a proof-of-concept EEG study. *J Neural Eng*. 2022;19(4):46001. doi:10.1088/1741-2552/ac7b4b

98. Manson G, Atkinson DA, Shi Z, et al. Transcutaneous spinal stimulation alters cortical and subcortical activation patterns during mimicked-standing: A proof-of-concept fMRI study. *Neuroimage Reports*. 2022;2(2):100090. doi:10.1016/j.ynirp.2022.100090

99. Bouyer LJG, Rossignol S. Contribution of cutaneous inputs from the hindpaw to the control of locomotion. II. Spinal cats. *J Neurophysiol*. 2003;90(6):3640-3653.

100. Jones MG, Rogers ER, Harris JP, et al. Neuromodulation using ultra low frequency current waveform reversibly blocks axonal conduction and chronic pain. *Sci Transl Med*. 2021;13(608):eabg9890. doi:10.1126/scitranslmed.abg9890

101. Nanivadekar A, Chandrasekaran S, Gaunt R, Fisher L. RNEL PerceptMapper. Published online May 2, 2020. doi:10.5281/ZENODO.3939658

102. Cripps A, Livingston SC, DeSantis BM. The Test- Retest Reliability and Minimal Detectable Change of the Sensory Organization Test and Head- Shake Sensory Organization Test. In: ; 2016.

103. Beninato M, Fernandes A, Plummer LS. Minimal Clinically Important Difference of the Functional Gait Assessment in Older Adults. *Phys Ther*. 2014;94(11):1594-1603. doi:10.2522/ptj.20130596

104. Crea S, Edin BB, Knaepen K, Meeusen R, Vitiello N. Time-Discrete Vibrotactile Feedback Contributes to Improved Gait Symmetry in Patients With Lower Limb Amputations: Case Series. *Phys Ther*. 2017;97(2):198-207. http://dx.doi.org/10.2522/ptj.20150441

105. Valgeirsdóttir VV, Sigurðardóttir JS, Lechler K, et al. How Do We Measure Success? A Review of Performance Evaluations for Lower-Limb Neuroprosthetics. *JPO J Prosthetics Orthot*. 2022;34(1). https://journals.lww.com/jpojournal/Fulltext/2022/01000/How_Do_We_Measure_Success__A_Review_of_Performance.12.aspx

106. Dean JC. Proprioceptive feedback and preferred patterns of human movement. *Exerc Sport Sci Rev*. 2013;41(1):36-43. doi:10.1097/JES.0b013e3182724bb0

107. Hurteau M, Thibaudier Y, Dambreville C, Danner SM, Rybak IA, Frigon A. Intralimb and

Interlimb Cutaneous Reflexes during Locomotion in the Intact Cat. *J Neurosci*. 2018;38(17):4104-4122. doi:10.1523/JNEUROSCI.3288-17.2018

108. Schillings AM, van Wezel BM, Mulder T, Duysens J. Muscular responses and movement strategies during stumbling over obstacles. *J Neurophysiol*. 2000;83(4):2093-2102. doi:10.1152/jn.2000.83.4.2093

109. Anderson CB, Miller MJ, Murray AM, Fields TT, So NF, Christiansen CL. Falls After Dysvascular Transtibial Amputation: A Secondary Analysis of Falling Characteristics and Reduced Physical Performance. *PM&R*. 2021;13(1):19-29. doi:https://doi.org/10.1002/pmrj.12376

110. Hunter SW, Batchelor F, Hill KD, Hill A-M, Mackintosh S, Payne M. Risk Factors for Falls in People With a Lower Limb Amputation: A Systematic Review. *PM R*. 2017;9(2):170-180.e1. doi:10.1016/j.pmrj.2016.07.531

111. Curtze C, Hof AL, Postema K, Otten B. Over rough and smooth: Amputee gait on an irregular surface. *Gait Posture*. 2011;33(2):292-296. doi:10.1016/j.gaitpost.2010.11.023

112. Sinitski EH, Lemaire ED, Baddour N, Besemann M, Dudek N, Hebert JS. Maintaining stable transtibial amputee gait on level and simulated uneven conditions in a virtual environment. *Disabil Rehabil Assist Technol*. 2021;16(1):40-48. doi:10.1080/17483107.2019.1629186

113. Powell LE, Myers AM. The activities-specific balance confidence (ABC) scale. *Journals Gerontol Ser A Biol Sci Med Sci*. 1995;50(1):M28-M34.

114. Menz H, Lord S, Fitzpatrick R. Acceleration patterns of the head and pelvis when walking on level and irregular surfaces. *Gait Posture*. 2003;18:35-46. doi:10.1016/S0966-6362(02)00159-5

115. Doi T, Hirata S, Ono R, Tsutsumimoto K, Misu S, Ando H. The harmonic ratio of trunk acceleration predicts falling among older people: results of a 1-year prospective study. *J Neuroeng Rehabil*. 2013;10(1):7. doi:10.1186/1743-0003-10-7

116. Dingwell JB, Ulbrecht JS, Boch J, Becker MB, O'Gorman JT, Cavanagh PR. Neuropathic gait shows only trends towards increased variability of sagittal plane kinematics during treadmill locomotion. *Gait Posture*. 1999;10(1):21-29. doi:10.1016/S0966-6362(99)00016-8

117. Moe-Nilssen R. A new method for evaluating motor control in gait under real-life environmental conditions. Part 1: The instrument. *Clin Biomech*. 1998;13(4-5):320-327. doi:10.1016/S0268-0033(98)00089-8

118. Kojima M, Obuchi S, Henmi O, Ikeda N. Comparison of Smoothness during Gait between Community Dwelling Elderly Fallers and Non-Fallers Using Power Spectrum Entropy of Acceleration Time-Series. *J Phys Ther Sci*. 2008;20:243-248. doi:10.1589/jpts.20.243

119. Brach JS, McGurl D, Wert D, et al. Validation of a Measure of Smoothness of Walking. *Journals Gerontol Ser A*. 2011;66A(1):136-141. http://dx.doi.org/10.1093/gerona/glq170

120. Sejdić E, Lowry KA, Bellanca J, Redfern MS, Brach JS. A comprehensive assessment of gait accelerometry signals in time, frequency and time-frequency domains. *IEEE Trans neural Syst Rehabil Eng a Publ IEEE Eng Med Biol Soc*. 2014;22(3):603-612. doi:10.1109/TNSRE.2013.2265887

121. Bolton DAE, Misiaszek JE. Contribution of Hindpaw Cutaneous Inputs to the Control of Lateral Stability During Walking in the Cat. *J Neurophysiol*. 2009;102(3):1711-1724. doi:10.1152/jn.00445.2009

122. Sawers A, Hafner BJ. Using Clinical Balance Tests to Assess Fall Risk among Established Unilateral Lower Limb Prosthesis Users: Cutoff Scores and Associated Validity Indices. *PM&R*. 2020;12(1):16-25. doi:https://doi.org/10.1002/pmrj.12160

123. Richardson JK, Thies SB, DeMott TK, Ashton-Miller JA. Interventions Improve Gait Regularity in Patients with Peripheral Neuropathy While Walking on an Irregular Surface Under Low Light. *J Am Geriatr Soc*. 2004;52(4):510-515. doi:10.1111/j.1532-5415.2004.52155.x

124. Dhillon GS, Lawrence SM, Hutchinson DT, Horch KW. Residual function in peripheral nerve stumps of amputees: implications for neural control of artificial limbs. *J Hand Surg Am*. 2004;29(4):605-615; discussion 616-8. doi:10.1016/j.jhsa.2004.02.006

125. Okorokova E V, He Q, Bensmaia SJ. Biomimetic encoding model for restoring touch in bionic hands through a nerve interface. *J Neural Eng*. 2018;15(6):66033. doi:10.1088/1741-2552/aae398

126. A. GJ, T. KD, S. DT, et al. Biomimetic sensory feedback through peripheral nerve stimulation improves dexterous use of a bionic hand. *Sci Robot*. 2019;4(32):eaax2352. doi:10.1126/scirobotics.aax2352

127. Kim J, McDonald CL, Hafner BJ, Sawers A. Fall-related events in people who are lower limb prosthesis users: the lived experience. *Disabil Rehabil*. Published online March 10, 2021:1-12. doi:10.1080/09638288.2021.1891467

128. Kim J, Major MJ, Hafner B, Sawers A. Frequency and Circumstances of Falls Reported by Ambulatory Unilateral Lower Limb Prosthesis Users: A Secondary Analysis. *PM&R*. 2019;11(4):344-353. doi:https://doi.org/10.1016/j.pmrj.2018.08.385

129. Clemens S, Gaunaurd I, Raya M, Kirk-Sanchez N, Klute G, Gailey R. Using theoretical frameworks to examine fall history and associated prosthetic mobility in people with nondysvascular lower limb amputation. *Prosthet Orthot Int*. Published online 2022. https://journals.lww.com/poijournal/Fulltext/9900/Using_theoretical_frameworks_to_examine_fall.39.aspx

130. Cuberovic I, Gill A, Resnik LJ, Tyler DJ, Graczyk EL. Learning of Artificial Sensation

Through Long-Term Home Use of a Sensory-Enabled Prosthesis . *Front Neurosci* . 2019;13. https://www.frontiersin.org/articles/10.3389/fnins.2019.00853

131. Graczyk EL, Resnik L, Schiefer MA, Schmitt MS, Tyler DJ. Home Use of a Neural-connected Sensory Prosthesis Provides the Functional and Psychosocial Experience of Having a Hand Again. *Sci Rep*. 2018;8(1):9866. doi:10.1038/s41598-018-26952-x

132. Ortiz-Catalan M, Mastinu E, Sassu P, Aszmann O, Brånemark R. Self-Contained Neuromusculoskeletal Arm Prostheses. *N Engl J Med*. 2020;382(18):1732-1738. doi:10.1056/NEJMoa1917537

133. Maynard FM, Bracken MB, Creasey G, et al. International standards for neurological and functional classification of spinal cord injury. *Spinal Cord*. 1997;35(5):266-274.

134. Prabhakar AT, Suresh T, Kurian DS, et al. Timed Vibration Sense and Joint Position Sense Testing in the Diagnosis of Distal Sensory Polyneuropathy. *J Neurosci Rural Pract*. 2019;10(2):273-277. doi:10.4103/jnrp.jnrp_241_18

135. Prieto TE, Myklebust JB, Hoffmann RG, Lovett EG, Myklebust BM. Measures of postural steadiness: differences between healthy young and elderly adults. *IEEE Trans Biomed Eng*. 1996;43(9):956-966. doi:10.1109/10.532130

136. Montesinos L, Castaldo R, Pecchia L. On the use of approximate entropy and sample entropy with centre of pressure time-series. *J Neuroeng Rehabil*. 2018;15(1):116. doi:10.1186/s12984-018-0465-9