Title: The firing characteristics of foot sole cutaneous mechanoreceptor afferents in response to vibration stimuli

Authors: Nicholas D.J. Strzalkowski[1], R. Ayesha Ali[2], Leah R. Bent[3]

Affiliations: [1]University of Calgary, Department of Clinical Neuroscience, Calgary AB, Canada, [2]University of Guelph, Department of Mathematics and Statistics, Guelph ON, Canada, [3]University of Guelph, Department of Human Health and Nutritional Science, Guelph ON, Canada

Running head: Vibration response of foot sole cutaneous afferents

Corresponding author:
Dr. Leah R. Bent
Associate Professor
Department of Human Health and Nutritional Science
Guelph, Ontario, N1G 2W1
E-mail: lbent@uoguelph.ca
Phone: 519 824 4129 ext. 56442

Abstract

Single unit microneurography was used to record the firing characteristics of the four classes of foot sole cutaneous afferents (fast and slowly adapting type I and II; FAI, FAII, SAI, SAII) in response to sinusoidal vibratory stimuli. Frequency (3-250Hz) and amplitude (0.001-2mm) combinations were applied to afferent receptive fields through a 6mm diameter probe. The impulses per cycle, defined as the number of action potentials evoked per vibration sine wave, were measured over one second of vibration at each frequency-amplitude combination tested. Afferent entrainment threshold (lowest amplitude at which an afferent could entrain 1:1 to the vibration frequency) and afferent firing threshold (minimum amplitude for which impulses per cycle was greater than zero) were then obtained for each frequency. Increases in vibration frequency are generally associated with decreases in expected impulses per cycle ($p < 0.001$), but each foot sole afferent class appears uniquely tuned to vibration stimuli. FAII afferents tended to have the lowest entrainment and firing thresholds ($p < 0.001$ for both); however, these afferents seem to be sensitive across frequency. In contrast to FAII afferents, SAI and SAII afferents tended to demonstrate optimal entrainment to frequencies below 20Hz, and FAI afferents faithfully encoded frequencies between 8-60Hz. Contrary to the selective activation of distinct afferent classes in the hand, application of class specific frequencies in the foot sole is confounded due to the high sensitivity of FAII afferents. These findings may aid in the development of sensorimotor control models or the design of balance enhancement interventions.

Key words: cutaneous afferents, mechanoreceptor, foot sole, vibration, microneurography

New & Noteworthy

Our work provides a mechanistic look at the capacity of foot sole cutaneous afferents to respond to vibration of varying frequency and amplitude. We found that foot sole afferent classes are uniquely tuned to vibration stimuli; however, unlike in the hand, they cannot be independently activated by class specific frequencies. Viewing the foot sole as a sensory structure, the present findings may aid in the refinement of sensorimotor control models and design of balance enhancement interventions.

Introduction

Skin feedback from the feet and ankles plays an important role in standing balance and the control of gait. It is well established that cutaneous afferents from the foot sole and dorsum can modulate lower (Fallon et al., 2005) and upper limb (Bent and Lowrey, 2013) motor neuron excitability, evoke posturally relevant reflexes (Zehr and Stein, 1999), in addition to providing proprioceptive (Collins, 2005; Aimonetti et al., 2007; Mildren and Bent, 2016) and exteroceptive (Kavounoudias et al., 1998) feedback about body orientation and interactions with the environment. This understanding has led to a growing interest in improving postural control through the enhancement of foot sole cutaneous feedback (Priplata et al., 2005; Perry et al., 2008; Zehr et al., 2014; Lipsitz et al., 2015). Facilitatory shoe insoles, which employ suprathreshold (Novak and Novak, 2006) and subthreshold (Priplata et al., 2005; Galica et al., 2009; Lipsitz et al., 2015) vibrations, have been shown to improve balance and gait parameters in older adults and clinical populations. The benefits of these subthreshold insole vibrations are believed to manifest themselves by lowering the activation threshold of cutaneous receptors, thus making natural inputs suprathreshold and able to generate viable and appropriate balance responses. Vibration has been shown to induce sensations of whole body lean in restrained subjects (Roll et al., 2002) and evoke postural sway away from stimulated sites during quiet stance (Kavounoudias et al., 1998; 1999; Roll et al., 2002), thereby suggesting that the central nervous system (CNS) uses cutaneous feedback from the soles of the feet to deduce body spatial position. The sensitivity of foot sole cutaneous afferents and their ability to provide vibration feedback has direct consequences on standing balance.

To comprehend the contributions of foot sole cutaneous feedback in postural control, it is imperative to have an accurate understanding of the firing properties of the four primary cutaneous afferent classes in response to vibration (i.e., when and how do they respond). As an experimental tool, vibration provides a controllable stimulus that can be used to investigate these properties. Impulses per vibratory cycle (ImpCycle) represents the number of discharges evoked in response to vibration divided by the stimulus frequency, and indicates the ability of the afferent to entrain to the stimulus. An ImpCycle response of 1:1 signifies entrainment, where the afferent discharges once per probe indentation. Similarly, an ImpCycle response of 0.5:1 indicates that the afferent is firing on average once every other indentation. As such, the ImpCycle response provides a normalized afferent response, which highlights the capacity of the different afferent classes to encode vibration stimuli across frequencies.

Previous work has investigated cutaneous afferent firing characteristics in the leg and dorsum of the foot (Vedel and Roll, 1982; Ribot-Ciscar et al., 1989; Trulsson, 2001), and specific afferent class tuning curves have been developed for afferents innervating the glabrous skin on the hand (Johansson et al., 1982). Johansson et al. (1982) identified the specific frequency ranges over which each cutaneous afferent class (Fast and slowly adapting type I and II; FAI, FAII, SAI, SAII) is tuned to respond (has ability to entrain) in the hand and found that FA afferents were tuned to high frequencies. Specifically, FAI afferents were most easily activated between 8-64Hz, and FAII afferents between 64-400Hz. In contrast, SA afferents were tuned to low frequencies; SAIs between 2-32Hz, and SAIIs below 8Hz. The presence of unique cutaneous afferent class vibration tuning in the hand indicates that the CNS has access to a range of feedback that can be used to shape tactile experience and reflex responses.

The hands and feet are used for different functional roles and it is reasonable that differences exist in the response properties of the underlying mechanoreceptor afferents. Foot sole cutaneous afferents have been shown to have higher thresholds in response to light touch compared to afferents in the hand and arm (Johansson and Vallbo, 1979a; Vallbo et al., 1995; Kennedy and Inglis, 2002; Strzalkowski et al., 2015a). Further, there is a distinct proximal to distal increase in type I receptors in the hand (Johansson and Vallbo, 1979b), while a more even distribution has been proposed in the foot sole (Kennedy & Inglis 2002). Although these findings have provided valuable insights into the general firing and receptive field characteristics of cutaneous afferents, the firing properties of the specific afferents classes innervating the skin of the foot sole in response to vibration remains unknown.

The cutaneous mechanoreceptor classes that innervate the glabrous skin on foot sole are thought to be the same as those in the hand (Kennedy and Inglis, 2002). We hypothesized that foot sole afferent class vibration tuning curves would be similar to those in the hand (Johansson et al., 1982), but with higher thresholds as previously demonstrated with light touch (Strzalkowski et al., 2015a). In the hand, the availability of distinct afferent class feedback may be necessary to provide the sensory resolution associated with texture perception and fine motor control (Yau et al., 2016). The tactile stimuli associated with postural sway and gait may also require distinct afferent responses that cannot be fully represented by a single vibration stimuli. In order to evaluate the contribution of cutaneous input in postural control, it is essential to have an understanding of the capability of individual foot sole cutaneous afferents to contribute to postural control. As such, understanding the capacity of foot sole afferents to respond to select vibration is an important step in creating a model of tactile perception and motor control.

The objectives of the present study are to: (i) investigate the vibration response characteristics of cutaneous afferents in the glabrous skin of the foot; and (ii) expand upon previous studies conducted on the glabrous skin of the hand. Accordingly, single unit microneurography was used to record the firing responses of cutaneous afferents, i.e., ImpCycle. These data provide a measure of afferent vibration tuning, considered to be the frequency, or range of frequencies where individual afferents can easily entrain (at a low amplitude) and when entrainment is insensitive to amplitude changes.

Methods

Ethical Approval

Fifty-nine recording sessions were performed on 21 healthy subjects (12 males, 9 females; mean age 24 with range 20-27 years). None of the participants had any known neurological or musculoskeletal disorders. Following an explanation of the protocol, each subject gave written informed consent to participate in the experiment. The protocol was approved by the University of Guelph research ethics board and complied with the declaration of Helsinki.

Experimental setup

Subjects lay prone on an adjustable treatment table. Both legs were straight with the right, test leg supported with a Versa Form positioning pillow at the level of the ankle. All recordings were performed in the right tibial nerve. The path of the tibial nerve was located at the level of the popliteal fossa using transdermal electrical stimulation (1-ms square wave pulse, 1Hz 0-10mA, SIU-C Grass stimulus isolation unit and a S88X Grass stimulator; Grass Instruments Astro-Med, West Warwick, RI). Stimuli were applied through a handheld probe with a reference surface electrode (Ag/AgCl) attached to the patella of the right knee. The tibial nerve location was established by observable muscle twitches in the plantar flexor muscles, paired with subject reported sensations of parasthesia in the foot and leg (representative of the tibial innervation zone). The location for insertion was established as the location with the largest response at the lowest current. A low impedance reference electrode (uninsulated, tungsten, 200µm diameter; FHC Inc. Bowdoin, ME, USA) was inserted through the skin ~2cm medial to the recording site at a depth of ~5mm. The recording electrode (insulated 10MΩ, tungsten, 200µm diameter, 1-2 µm recording tip, 55mm length; FHC Inc.) was then inserted at the predetermined tibial nerve recording location. Using audio feedback of the neural activity, the tibial nerve was located and penetrated using small manipulations of the recording electrode. Mechanical activation (light tapping, stroking and stretching) of the foot sole skin was then applied to help guide fine manipulations of the electrode to isolate single afferents. Neural recordings were amplified (gain 10^4, bandwidth 300Hz-10kHz, model ISO-180; World Precision Instruments, Sarasota, FL), digitally sampled (40kHz), and stored for analysis (CED 1401 and Spike2 version 6; Cambridge Electronic Design).

Cutaneous mechanoreceptor classification

Single afferents were classified as fast adapting (FAI or FAII) or slowly adapting (SAI or SAII) based on previously described criteria (Johansson, 1978; Kennedy and Inglis, 2002). FA afferents are sensitive to dynamic events and adapt quickly to sustained indentations. In contrast, SA afferents respond throughout sustained skin indentation, and their firing rates are proportional to the magnitude of deformation. FAI and SAI afferents typically have small receptive fields with multiple hotspots (locations of highest sensitivity) and distinct borders, while FAII and SAII afferents have large receptive fields, a single hotspot and less well-defined borders. To improve classification accuracy, additional tests were performed to identify units, such as manual skin stretch of SAII afferent receptive fields, blowing across the receptive field of FAII afferents, and calculations of the instantaneous frequency (SAII regular, SAI irregular). Semmes-Weinstein monofilaments (North Coast Medical Inc, Gilroy, California) capable of applying ~0.078-2941mN of force, were applied by hand to calculate receptive field size, monofilament firing threshold, and hotspot location (site of maximum sensitivity). Receptive fields were then determined with a monofilament 4-5 times afferent firing threshold and drawn on the skin with a fine tip pen. Only units whose receptive fields fell within the plantar surface of the foot sole were included in this study.

Vibration protocol

Sinusoidal vibrations were delivered through a 6mm diameter probe driven by a vibration exciter (Mini-shaker type 4810, Power amplifier type 2718, Bruel & Kjaer, Naerum, Denmark) secured on an adjustable arm (143BKT, LinoManfrotto, Markham, Canada). The probe was positioned perpendicular to the receptive field hotspot, and 2mm of pre-indentation was applied with a manual displacement gauge. Force was measured with a force transducer (load cell model 31, Honeywell, MN, USA) placed in series with the probe. Force feedback was used to monitor the probe position and to ensure consistent contact with the foot sole at that position. Probe acceleration (ΔV/sec) was recorded with an accelerometer (sampled at 2kHz; 4507 B 002; Bruel and Kjaer, Germany), and used in a closed-loop system to control stimulus frequency and amplitude (VR8500 Vibration Controller, VibrationVIEW v. 7.1.4; Vibration Research corporation, Jenison MI).

A single frequency was tested at a time and delivered through a vibration ramp consisting of multiple 2-second vibration bursts of increasing amplitudes. To limit habituation, there was a four-second pause given between each vibration burst within each ramp. A broad range of control stimulus frequencies (3, 5, 8, 10, 20, 30, 60, 100, 150 and 250Hz) and of amplitudes (0.001, 0.0025, 0.005, 0.0075, 0.01, 0.025, 0.05, 0.075, 0.1, 0.25, 0.5, 0.75, 1, 1.25, 1.5, 1.75 and 2mm) were tested. Note that the order of frequencies tested was pseudo-randomly selected, to limit any influence of order while maximizing the range of frequencies tested for each afferent given the unpredictability of recording stability. Further, amplitudes were not randomized within each ramp to ensure consistent stimuli across recordings and to facilitate real time firing threshold identification. Not every frequency-amplitude combination was possible due to an acceleration feedback requirement of the closed loop system necessary for the accurate control of peak-to-peak probe displacement. Consequently, some low frequency-small amplitude and high frequency-large amplitude combinations were not testable. For *example,* we could not vibrate below 3Hz (acceleration signal was too weak), and only at low-subthreshold amplitudes at 400Hz. We were unable to evoke responses at 400Hz at the highest amplitude

(2.5um) and 400Hz vibration ramps were not tested. Depending on the stability/quality of the neural recording, 1 to 10 vibration ramps (representing 1 to 10 different frequencies) were delivered to each afferent and 7 to 15 amplitudes (vibration bursts) were tested within a vibration ramp at any delivered frequency.

Analysis of neural recordings

Recorded afferent signals were analyzed using Spike2 (version 6; Cambridge Electronics Design). Spike morphology was used to generate a template for the visual classification of single units. Figure 1 depicts an example of a 30Hz vibration ramp and FAII afferent firing response. The top panel presents the instantaneous afferent firing frequency response (Hz), the 30Hz acceleration profile of the relative timing and amplitude of each vibration ramp (ΔV/sec), the peak-to-peak amplitude of each vibration burst (mm), and the raw neurogram of the single unit recording (V). Recordings in which a single afferent could not be confidently identified without interference from multiple units or excessive signal noise were excluded from further analysis.

Afferent firing characteristics were calculated from a representative one-second of each two-second vibratory burst. The one-second period was selected at the end of each vibration burst just before the vibration amplitude decreased; see Figures 1A) and 1B). This period was selected to standardize the analysis period, and to limit the influence of an amplitude overshoot present at the beginning of some vibration bursts. The ImpCycle response was calculated from this one-second period.

Subsequently, afferent firing threshold and entrainment threshold were identified. For this analysis, entrainment threshold was defined as the lowest amplitude for which impulses per cycle was greater than 0.9 for a given frequency. Greater than 0.9 was chosen as entrainment threshold to account for instances where the afferent could clearly entrain but failed to discharge on an indentation due to inherent spiking variability. Tables and figures are labelled as 1:1 instead of >0.9:1 for ease of interpretation.

Statistical analyses

Afferent served as the observational unit in all statistical analyses. Descriptive statistics of the monofilament threshold, receptive field area, ImpCycle, entrainment threshold, and firing threshold were used to provide an overview of the data. At each frequency-amplitude combination, the mean impulses per cycle were plotted (Figure 3, MATLAB 6.1, The MathWorks Inc., Natick MA, and Figures 4-7, GraphPad Prism version 5.0c for Mac OS X, San Diego CA). The percentage of afferents firing (% firing) and percentage firing at or greater than 1:1 (% ≥1:1) were also tabulated to highlight afferent firing behaviour both within and across vibration ramps at select frequency-amplitude combinations.

Regression analyses of ImpCycle, entrainment threshold, and afferent firing threshold were performed. In order to accommodate the correlation of multiple observations within an afferent, the data were modelled using generalized estimating equations (GEE) (Zeger and Liang, 1986); exchangeable correlation structure and robust standard errors via the `gee` function from the gee package in R (R Core Team, 2017). There are several correlation structures one could specify, with typical ones being independent (the responses at each frequency-amplitude combination are independent of each other), exchangeable (equal correlation for all observations across the frequency-amplitude plane), and auto-regressive (correlations exist but are stronger for nearby frequency-amplitude combinations and decrease for combinations that are further away). While the exchangeable correlation structure is likely inaccurate, (Zeger and Liang, 1986) show that misspecification of correlation structure does not affect consistency of coefficient estimates. Further, the use of robust standard errors helps mitigate any potential influence on standard errors.

Both frequency and amplitude were transformed to the natural logarithm scale to improve model fit. Afferent class was treated as a factor with FAI afferents forming the reference group. Accordingly, coefficients associated with every other afferent class in the regression of ImpCycle can be interpreted as the expected difference in ImpCycle between that afferent class and FAI afferents. Since amplitude was logged, in the regressions for (log) entrainment and for (log) firing threshold, the interpretation of coefficients is no longer in terms of the mean response (i.e., entrainment or firing threshold), but in terms of the median response.

Results

Out of 111 recordings in which an afferent was confidently identified, 52 individual cutaneous afferents were recorded from 16 healthy subjects over 39 recordings sessions. Some of the identified afferents were reported in a previous study (Strzalkowski et al., 2015a). For each subject, the number of successful recording sessions ranged from 1 to 6, and at most 3 cutaneous afferents were identified in any single recording session. The total number of afferents identified on any subject ranged from 1 to 9.

The recorded afferents all had receptive fields in the plantar surface of the foot sole, and the recordings were stable enough to permit at least one complete vibration ramp to be applied. On average, five different frequency ramps, out of a possible ten, were tested on each of the fifty-two recorded afferents. Afferents were not observed to habituate to the 2-second bursts or across multiple vibration ramps. The afferents tested included 19 FAI (37%), 9 FAII (17%), 14 SAI (27%) and 10 SAII (19%) afferents.

The monofilament firing thresholds tend to be larger for slowly adapting afferents compared to fast adapting afferents, with both larger means and variation (Table 1). The receptive fields for type II afferents (FAII and SAII) tended to be larger, with larger variation, compared to the type I afferents (FAI and SAI). The location and distribution of the cutaneous afferent receptive fields are presented in Figure 2.

Descriptive Statistics

Table 2 presents descriptive statistics for the impulses per cycle, entrainment threshold, and firing threshold by afferent class. All afferent classes were tested across the respective tested frequency ranges, though SA afferents responded over a restricted range of frequencies (FAs: 3-250Hz, SAI: 3-150Hz, SAII: 3-100Hz). On average, ImpCycle responses for FAI (mean: 0.63), SAI (mean: 0.55), and SAII (mean: 0.33) were slightly lower than that of FAII afferents (mean: 1.07). Entrainment and afferent firing threshold tended to be highest for SAII afferents (means: 1.11mm and 0.79 mm, respectively) and lowest for FAII afferents (means: 0.33mm and 0.17mm, respectively).

Figure 3A) shows the mean ImpCycle for each afferent as a heat map over the frequency-amplitude plane, with higher ImpCycle responses corresponding to hotter colours. Despite the restricted frequency range for SA afferents, there is little that differentiates the SAI afferent responses from that of the FAI afferents. For reference, Figure 3B) provides the analogous plot for the hand, adapted from (Johansson et al., 1982).

Impulses per cycle

Figures 4-7 show the tuning curves for each afferent class, respectively. The largest FAI afferent ImpCycle responses (>2:1) were observed at low frequencies (3-8Hz), and responses greater than 1:1 were observed at frequencies up to 60Hz (Figure 4). Similar to the other afferent classes, the ImpCycle responses varied greatly across individual FAI afferents (Figure 4B,C). FAII afferents were found to be the most sensitive class to vibration stimuli, demonstrating the highest ImpCycle responses across frequencies compared to the other classes (Figure 5). Average ImpCycle responses >3:1 were observed up to 10Hz, and >1:1 up to 150Hz at the available vibration amplitudes. Individual FAII afferent responses varied within the population, indicating a continuum of coverage (varying levels of sensitivity) class for a particular frequency (Figure 5B, C). Two different FAII afferent responses were observed: a high amplitude (>0.5mm) response where the impulses per cycle decreased with increasing stimulus frequency; and, a low amplitude response (≤0.5mm), where the impulses per cycle were similar across frequencies. These trends support a high capacity for FAII afferents to respond across frequencies.

SAI afferents tended to respond across the range of frequencies tested and at elevated thresholds compared to FA afferents (Table 2, Figure 6). SAI afferents demonstrate their largest ImpCycle responses at low frequencies, but do not appear to be tuned to a specific range like FAI afferents, as a consistent ImpCycle response is not seen across stimulation frequencies at a given vibration frequency. SAII afferents were the most insensitive to vibration. They were associated with the lowest ImpCycle responses across most frequencies and a near absence of firing at amplitudes below 0.25mm (Figure 7). Average SAII afferent ImpCycle responses >1:1 were only achieved at 3Hz and 5Hz; however, average responses >0.5:1 were found up to 60Hz (Figure 7).

Table 3 presents the regression of the ImpCycle on frequency and amplitude. These results validate the trends seen in Figure 3. At a given frequency-amplitude combination, the ImpCycle tended to be larger for FAII afferents but smaller for SAII afferents ($p < 0.001$ for both afferents) compared to FAI afferents; however, there was no statistically significant difference between SAI and FAI afferents (Table 3, $p = 0.274$). ImpCycle response was also found to depend on the interaction of frequency and amplitude ($p < 0.001$). In particular, for a given frequency, as amplitude was ramped up, ImpCycle also increased; however, the amount of increase depended on the frequency, with the effect of amplitude dampened at higher frequencies. For a given amplitude, as frequency was increased, ImpCycle tended to decrease, but the amount of decrease depended on the amplitude.

Entrainment threshold

FAI afferent firing remained robust at higher frequencies, where 77% and 43% of the FAI afferents achieved 1:1 firing at 100Hz and 150Hz at the largest available amplitudes of 0.5mm and 0.25mm respectively (Table 4). These ImpCycle responses reflect the fast adapting nature of FAI afferents and their ability to entrain to high frequencies. FAI had the most consistent ImpCycle responses between 8-60Hz, as the ability of FAI afferents to entrain did not deviate greatly between 8Hz and 60Hz at low vibration amplitudes (Figure 4). As such, 8-60Hz may specify a frequency range over which FAI afferents are tuned.

FAII afferents had the lowest entrainment thresholds compared to the other afferent classes. Entrainment was reached in all FAII afferents at amplitudes of 0.25mm or 0.5mm (except 100Hz 75% at 0.5mm and 250Hz 20% at 0.075mm) (Table 4). Note that at 250Hz one afferent (of five tested) did not respond, and one entrained to the stimulus. Further, entrainment threshold at 10Hz could not be isolated for FAII afferents because >1:1 firing was evoked on average at the smallest applied amplitude (0.05mm). As a population, FAII afferents responded and entrained at all the frequencies tested (except at 250Hz where an average afferent entrainment threshold was not observed at the largest amplitude of 0.075mm).

SAI afferent entrainment thresholds increased with increasing frequency up until 30Hz, above which an average population response of 1:1 was not observed (Figure 6, Table 4). At 3Hz SAI entrainment threshold was 0.1mm, which increased to around 1.25mm at 30Hz. An individual afferent (1 of 5) was found to entrain up to 100Hz; however, entrainment was not observed in frequencies above 30Hz in the majority of SAI afferents (Table 4). These data suggest that as a population, SAI afferents preferentially encode for low frequencies below 30Hz, but some afferents can respond and entrain at higher frequencies.

SAII afferents had a limited capacity to entrain to the vibratory stimuli, and entrainment was only observed at amplitudes greater than 1mm over the 3-10Hz frequency range (Table 4). At 10Hz, all five of the SAII afferents tested fired at 1.5mm and 40% had reached entrainment threshold. Above 10Hz (and tested up to 30Hz), 1:1 firing was not achieved even at 2mm, so 10Hz represents the upper frequency at which SAII afferents were shown to consistently respond.

Overall, entrainment threshold was found to decrease with increasing frequency for both FAI and FAII afferents (Figure 8). From the regression of entrainment threshold on frequency, the ratio of median entrainment threshold of FAII afferents relative to that of

FAI afferents measured at the same frequency was estimated to be exp(-1.168)=0.311 (Table 5). In other words, the median entrainment threshold of FAII afferents tended to be just over 30% of the median entrainment threshold of FAI afferent for a given frequency ($p < 0.001$). Further, when comparing afferents of the same class but for which the frequency of the first group of afferents is, say, double that of the second group, then the ratio of median entrainment threshold for the two groups was estimated to be $2^{-0.151} = 0.901$. So, the doubling of frequency was associated with a 10% decrease in median entrainment threshold ($p = 0.069$). The trends for SA afferents were inconclusive, likely due to the fact that they were tested at fewer frequencies compared to FA afferents.

Firing threshold

The trends in firing thresholds across frequencies for each afferent class were similar to that of entrainment, but with responses at higher frequencies as well (Figure 9). From the GEE analysis, the ratio of median firing threshold of FAII afferents relative to that of FAI afferents measured at the same frequency was estimated to be exp(–1.454) = 0.233 (Table 6). In other words, the median firing threshold of FAII afferents was, again, much lower and tended to be just under 25% of the median firing threshold of FAI afferents for a given frequency ($p < 0.001$), i.e. more sensitive. Further, when comparing afferents of the same class but for which the frequency of the first group of afferents is, say, double that of the second group, then the ratio of median firing threshold for the two groups was estimated to be $2^{-0.245} = 0.844$ (Table 6). So, the doubling of frequency was associated with a just over 15% decrease in median firing threshold ($p = 0.008$).

Discussion

The present study was conducted to (i) identify and characterize the firing characteristics of foot sole cutaneous afferents in response to vibratory stimuli, and (ii) provide a reference to analogous studies performed in the hand. We have identified class specific vibration tuning frequencies for foot sole cutaneous afferents, demonstrated that there are (overlapping) ranges of individual afferent responses associated with each class, and shown how these responses differ from afferent responses in the hand.

Vibration tuning of foot sole cutaneous afferents

We vibrated the foot sole across a range of frequencies and amplitudes and found that FAII afferents demonstrated the most robust firing response compared to the other classes of cutaneous afferents. FAII afferents fired and entrained at relatively low vibration amplitudes, and had the highest ImpCycle responses across nearly all frequency-amplitude combinations. FAII afferents appear to be tuned across our range of vibration input; reaching entrainment threshold at similar amplitudes (0.05-0.25mm) across frequencies (5-150Hz). The relatively low firing threshold of FAII afferents suggests that FAIIs may be the only class of cutaneous afferents that can be isolated (respond without contamination from other afferents) with foot sole vibration. The functional significance (i.e. tactile perception, postural control) of FAII feedback cannot be determined from the present data; however it is clear that FAII afferent feedback will be present in most or all tactile responses.

In contrast to the ubiquitous firing of foot sole FAII afferents, overlapping entrainment frequency ranges were present for FAI, SAI, and SAII afferent classes. FAI afferents were found to have consistent ImpCycle responses between 8-60Hz, indicating a vibration range over which they most faithfully encode the frequency. As a population, SAI and SAII afferents most readily entrained to frequencies below 20Hz, where SAI afferents tended to have lower entrainment thresholds, and larger ImpCycle responses, compared to SAII afferents.

Comparison between body regions

The foot sole vibration tuning curves established in the present study are in partial agreement with data from the hand. Johansson et al. (1982) published optimum entrainment frequency ranges for hand cutaneous afferents and reported that SA afferents were most easily entrained at low frequencies (SAI 2-32hz, SAII <8Hz), while FA afferents were tuned to higher frequencies (FAI 8-64Hz, FAII >64Hz). In the foot sole, FA afferents are similarly found to respond and entrain to higher frequencies compared to SA afferents, however distinct tuning ranges are less apparent.

Interestingly, the foot sole and hand data demonstrate differences in the number of spikes evoked across frequencies when exposed to similar amplitudes. Hand afferents are found to be more sensitive, discharging an increased number of spikes at a given frequency amplitude combination. At 4Hz, 1mm peak-to-peak vibration evoked an average firing rate of 4:1 in FAI afferents, and 5:1 in FAII afferents in the hand (Johansson et al., 1982). In contrast, 5Hz-1mm vibration evoked 2:1 and 3:1 firing in FAI and FAII foot sole afferents respectively. Similarly, the firing rates of hand SA units were typically shown to be double that of foot sole SA afferents. It appears that cutaneous afferents in the hand have lower firing thresholds, entrain more easily, and discharge more spikes at a given amplitude compared to the same afferent classes in the foot sole. This disparity between the foot sole and hands may be due to distinct mechanoreceptor adaptations related to sensory function and skin mechanical property differences (Kekoni et al., 1989; Strzalkowski et al., 2015b).

The increased afferent firing in the hands relative to the feet may represent the requirement for texture and velocity perception necessary for handling objects. Conversely, the postural significance of foot sole feedback may not necessitate high individual afferent firing and rather rely on population characteristics across the foot sole.

Vibration responses of cutaneous afferents in the lower limb have been previously investigated; however, these data were limited to recordings from the lateral fibular nerve, with afferent receptive fields in the leg and foot dorsum (Vedel and Roll, 1982; Ribot-Ciscar et al., 1989). These early studies combined cutaneous afferents into FA and SA groups, and applied fewer frequency-amplitude combinations (10-300Hz, 0.2 and 0.5mm amplitude, 1mm diameter probe) than the present study. Despite these methodological differences, FA afferents were found capable of entraining to higher frequencies compared to SA afferents, which is in agreement with the present findings. Interestingly, firing rates greater than 1:1 were not observed in leg and foot dorsum afferents at the largest 0.5mm amplitude (Ribot-Ciscar et al., 1989). In contrast, 0.5mm vibration amplitude evoked firing rates greater than 1:1 in all foot sole afferent classes in the current work at class specific frequencies (Table 4). The elevated thresholds of afferents innervating the leg and foot dorsum compared to the plantar surface of the foot sole may reflect the functional significance of foot sole cutaneous feedback in controlling standing balance compared to feedback from other lower limb regions.

Functional implications of foot sole vibration

It is well established that cutaneous feedback from the soles of the feet is important in the control of upright stance and gait (Hayashi et al., 1988; Kavounoudias et al., 1998; Meyer et al., 2004; Kars et al., 2009). The application of subthreshold foot sole vibration, which is thought to increase the availability of foot sole feedback, has been shown to improve standing balance and gait, as evidenced by reductions in postural sway (Priplata et al., 2005; Lipsitz et al., 2015) and measures of gait variability (Galica et al., 2009; Lipsitz et al., 2015). In addition, suprathreshold foot sole vibration has been shown to modulate postural sway, where the magnitude and velocity of sway increases with higher frequencies (Kavounoudias et al., 1999). The present data demonstrate that the vibration frequencies and amplitudes (20, 60, 100 Hz and 0.2-0.5mm) employed by Kavououdias et al. (1999) can cause robust firing across afferent classes. However, what we have also shown is that it may not be possible to establish how each afferent class response contributes specifically to the observed postural responses. Importantly, these previous studies support the concept that altering foot sole cutaneous feedback via subthreshold and suprathreshold vibration does change afferent firing, and can ultimately modulate standing balance. However, since it is unknown how the cutaneous afferent firing characteristics change under different loading conditions, the present data should not be taken to represent afferent firing in different contexts.

The frequencies transmitted through the foot sole in association with gait and standing balance are not well understood. Regardless, our results demonstrate that foot sole cutaneous afferents respond and contribute tactile feedback in response to large amplitude vibrations. Further, foot sole cutaneous afferents are silent at rest but show robust responses, especially FA afferents, to dynamic mechanical perturbations of the skin. In fact, the heterogeneous vibration tuning we observed across classes suggests that the CNS may be primed for class specific firing rates. For example, we hypothesize that a high frequency burst from FA afferents will produce a different, perhaps muted, response in higher order neurons than that from a similar SA afferent input. We also conjecture that standing balance likely evokes robust firing across all foot sole afferent classes, and the relative contributions of each class in modulating motor output may depend on the postural context. Future work is still needed to investigate higher order class specific responses and their behaviour under different postures.

The functional significance and central weighting of feedback from different afferent classes is not clear. It is reasonable that low frequency response patterns from SA afferents would evoke a different, perhaps larger, postural response than a similar low frequency firing pattern from a population of FA afferents. The afferent signal is important in the modulation of muscle reflexes and postural control likely arises from a combination of afferent input, where external stimuli has been shown to evoke a range of firing across classes (Fallon et al., 2005). We found that afferent firing rate increased with high vibration amplitude; however, since the data were collected with the subject prone and the foot sole unloaded (2mm probe pre-indentation), these results cannot be extrapolated to an upright loaded posture. Future studies are needed to identify the firing characteristics of cutaneous afferents under prolonged loaded conditions and in older adult and/or diseased populations to further explore the functional significance of distinct afferent class vibration tuning.

Summary and conclusions

The present experiment provides an analysis of the vibration response characteristics of cutaneous afferents in the glabrous skin of the human foot sole. Tactile feedback from the feet plays an important role in the control of standing balance and gait, and the present findings expand upon how the foot sole is viewed as a sensory structure. We have demonstrated that cutaneous afferents in the foot sole display class specific tuning to vibratory stimuli, and that FAII afferents have the lowest firing and entrainment thresholds across frequencies. Optimal entrainment frequencies for FAI, SAI, and SAII afferents were found to overlap below 20Hz. Foot sole FAI afferents were tuned to faithfully encode frequencies between 8Hz and 60Hz, where similar ImpCycle responses were found at low vibration amplitudes. Afferent class vibration entrainment ranges provide a clear indication of the capacity of cutaneous afferents to faithfully encode a given vibration stimulus. Vibrations associated with natural stimuli are expected to evoke complex patterns of afferent firing that combine to inform perceptual experience and motor control. Ultimately this work provides a mechanistic look at the capacity of foot sole cutaneous afferents to respond to vibration, which may aid in the development of sensorimotor control models and design of balance enhancement interventions.

Grants: This project was funded by a NSERC Discovery Grant (400723) to Leah Bent and a NSERC PGSD to Nicholas Strzalkowski.

Conflict of interest: The authors declare that there are no conflicts of interest, financial or otherwise.

Author contributions: N.D.J.S and L.R.B conceived and designed the research; N.D.J.S and L.R.B performed the experiments; N.D.J.S, R.A.A and L.R.B interpreted results of experiments; N.D.J.S and R.A.A analyzed data and prepared figures; N.D.J.S drafted manuscript; N.D.J.S, R.A.A and L.R.B edited and revised manuscript; N.D.J.S, R.A.A and L.R.B approved final version of manuscript.

Acknowledgments: The authors would also like to thank Robyn Mildren for her contributions towards data collection, and Derek Zwambag for his help in preparing Figure 3.

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Table captions

Table 1: Characteristics of cutaneous afferents identified and tested in the foot sole

Table 2: Descriptive statistics for impulses per cycle, entrainment threshold and firing threshold, post stratified by afferent class

Table 3: Multivariate regression of impulses per cycle with exchangeable correlation structure

Table 4: Cutaneous afferent class firing characteristics, percent firing (% firing) and percent firing at or greater than 1:1 (% ≥1:1) as well as the sample number (n) across select frequency-amplitude combinations

Table 5: Regression of entrainment threshold on frequency and afferent class with exchangeable correlation structure

Table 6: Regression of firing threshold on frequency and afferent class with exchangeable correlation structure

Figure captions

Figure 1: A 30Hz vibration ramp (amplitude 0.005-1.25mm) with the firing response of a representative FAII afferent. The top panel presents from top to bottom: the instantaneous afferent firing frequency response (Hz), the 30Hz acceleration profile of the vibration ramp (ΔV/sec), the peak-to-peak amplitude of each vibration burst (mm), and the raw neurogram of the single unit recording (V). Within the afferent firing response panel the gray dashed and dotted lines indicate 60Hz and 30Hz responses respectively. With increasing vibration amplitude (left to right) the FAII afferent firing response increased. Low stimulus amplitudes (0.005-0.0075mm) did not evoke an afferent response. A) Highlights a 0.025mm vibration burst, and B) highlights a 0.5mm vibration burst. The two small panels present A) 1:1 at 0.025mm and B) 2:1 at 0.5mm ImpCycle responses over one second of vibration.

Figure 2: Afferent class; Fast Adapting Type I (FAI: 19) and Type II (FAII: 9), Slowly Adapting Type I (SAI: 14) and Type II (SAII: 10) receptive field locations. Circles represent receptive field (RF) and are drawn to represent difference in RF size.

Figure 3: The average impulses per cycles of FAI, FAII, SAI, and SAII afferents at each vibration frequency-amplitude combination in the foot A) and hand B). The foot data is from the present study while the hand data has been adapted from Johansson et al., 1982. The legend on the right indicates the magnitude of afferent responses, ranging from <0.5:1 impulses per cycle (dark blues) to >6:1 impulses per cycle (dark reds). Open circles indicate vibration stimuli that were delivered but did not evoke an afferent firing response.

Figure 4: Average FAI impulses per cycle responses across vibration frequency and amplitude combinations. 1:1 firing rate is highlighted by a grey line (A). Individual afferent ImpCycle responses of select FAI afferents across vibration frequency at 1mm (B) and 0.25mm (C) amplitudes. The average of each group is represented by the black stars.

Figure 5: Average FAII impulses per cycle responses across vibration frequency and amplitude combinations. 1:1 firing rate is highlighted by a grey line (A). Individual afferent ImpCycle responses of select FAII afferents across vibration frequency at 1mm (B) and 0.25mm (C) amplitudes. The average of each group is represented by the black stars.

Figure 6: Average SAI impulses per cycle responses across vibration frequency and amplitude combinations. 1:1 firing rate is highlighted by a grey line (A). Individual afferent ImpCycle responses of select SAI afferents across vibration frequency at 1mm (B) and 0.25mm (C) amplitudes. The average of each group is represented by the black stars.

Figure 7: Average SAII impulses per cycle responses across vibration frequency and amplitude combinations. 1:1 firing rate is highlighted by a grey line (A). Individual afferent ImpCycle responses of select SAII afferents across vibration frequency at 1mm (B) and 0.25mm (C) amplitudes. The average of each group is represented by the black stars.

Figure 8: Profile plots of entrainment threshold versus frequency, by afferent class. Entrainment is defined as a ratio of at least 0.9:1, meaning the afferent fires for each sine wave. Individual afferent data are presented; each dashed line represents a different afferent. Black stars are the average firing thresholds at each stimulation frequency.

Figure 9: Profile plots of firing threshold versus frequency, by afferent class. Each dashed line represents a different afferent. Black stars are the average firing thresholds at each stimulation frequency.