Title: Single low threshold afferents innervating the skin of the human foot modulate ongoing muscle activity in the upper limbs.

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Running Title: Skin on the foot influences upper limb muscle activity

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Abstract

We have shown for the first time that single cutaneous afferents in the foot dorsum have significant reflex coupling to motoneurons supplying muscles in the upper limb, particularly posterior deltoid and triceps brachii. These observations strengthen what we know from whole nerve stimulation that skin on the foot and ankle can contribute to the modulation of interlimb muscles in distant innervation territories. The current work provides evidence of the mechanism behind the reflex, where one single skin afferent can evoke a reflex response, rather than a population. Nineteen of forty one (46%) single cutaneous afferents isolated in the dorsum or plantar surface of the foot elicited a significant modulation of muscle activity in the upper limb. Identification of single afferents in this reflex indicates the strength of the connection and ultimately, the importance of foot skin in interlimb coordination. The median response magnitude was 2.29% of background EMG and the size of the evoked response did not significantly differ among the four mechanoreceptor classes (p>0.1). Interestingly, while the distribution of afferents types did not differ across the foot dorsum, there was a significantly greater coupling response from receptors located on the medial aspect of the foot dorsum (p<0.01). Furthermore, the most consistent coupling with upper limb muscles was demonstrated by Type I afferents (fast and slowly adapting). This work contributes to the current literature on receptor specificity, supporting the view that individual classes of cutaneous afferents may subserve specific roles in kinesthesia, reflexes and tactile perception.
Introduction

Recovery from a destabilization involves a multifaceted response of both lower (Zehr and Stein 1999; Haridas et al. 2005; Zehr et al. 1998) and upper limb (McCloy and Maki 1995; Misiaszek 2003; Haridas et al. 2006) reflex activation. During locomotion, rapid onset of lower limb muscles provides phase related excitation or inhibition responses to re-establish equilibrium (Zehr et al. 1998; Eng et al 1994, Duysens et al. 1990; Van Wezel et al 1997). Similar responses have been identified in the upper limb, where unexpected slips (Marigold et al. 2003) or platform perturbations (McCloy and Maki 1995) result in rapid and intentional arm movement at latencies as early as 88ms.

There is much speculation as to the trigger for the initiation of the postural response in the lower limb (muscle spindle, skin afferents, vestibular). Based on work in animals the onset of the lower limb automatic postural responses are unlikely to be related to vestibular contributions (Inglis and Macpherson 1995). This is corroborated by research investigating vestibular loss patients during whole body platform perturbations, where the initiation of the response remains intact (Horak et al. 1990). In contrast, a reduction in large diameter somatosensory input (skin and muscle), following changes related to diabetic neuropathy, is correlated with considerable delays in the onset latency of postural responses evoked in the lower limb, suggesting a role for these afferents in the postural response (Inglis et al. 1994).

Further segregation between muscle spindle and skin contributions has been investigated through the removal of skin information in perturbation studies. In particular, changes in dynamic postural recovery highlight a specific role for skin in automatic postural adjustments (Meyer et al. 2004; Perry et al. 2000). The removal of skin information through anesthesia
(Meyer et al. 2004) and foot sole cooling (Magnusson et al. 1990 a,b; Perry et al. 2000) has been shown to lead to large deficits in postural control and dynamic balance; with specific reductions in the occurrence and timing of lower limb responses to perturbations. While rapid initiation of upper arm movement has also been reported in response to unexpected perturbations (McIlroy and Maki 1995; Misiaszek 2003; Ghafouri et al. 2004; Marigold et al. 2003) such a standard dissection of sensory contributions to the onset of this upper limb response is lacking. So the question remains; ‘what sensory information is used to trigger the rapid onset of responses in the upper limb?’

Early work has identified the presence of upper limb reflex responses to high intensity stimulation over the foot sole skin (Kearney and Chan 1979), or ankle movement (Kearney and Chan 1981) leading to speculation that propriospinal pathways ensure limb coordination during specific tasks. Interlimb reflexes have also been evoked during electrical or mechanical perturbations of the foot (Dietz et al. 2001; Haridas and Zehr 2003). Following superficial peroneal stimulation (SP; a primarily cutaneous nerve at the ankle) in sitting (Zehr et al. 2001) cycling (Balter and Zehr 2007) and during locomotion (Lamont and Zehr 2006, Lamont and Zehr 2007) upper limb musculature has been shown not only to be influenced, but also phase dependently modulated (Lamont and Zehr 2007) as a result of lower limb cutaneous activation. Evidence of these cutaneous-evoked interlimb responses support the hypothesis that skin on the foot may serve as a potential trigger for rapid upper limb reflexes. Further support is provided by evidence of strong synaptic intralimb connections from single cutaneous afferents to motoneurons, which have been shown within the upper limb (McNulty and Macefield 2001; McNulty et al. 1999) and within the lower limbs (Fallon et al. 2005),
demonstrating an important role for skin to evoke, or modulate muscle activity within its own
innervation territory. What remains to be determined is the specificity of this synaptic coupling.
Can individual skin afferents from the foot modulate the upper limb motor neuron pool and
ultimately muscle activity?
The current work aims to address whether single low threshold mechanoreceptors in the skin
of the foot modulate on-going muscle activity in the upper arm. The purpose of the work is to
determine the strength of the synaptic connection; whether the synaptic coupling from one
receptor is sufficient, or whether a population of cutaneous receptors, as supported through
digital stimulation, is necessary to elicit this interlimb reflex response. By examining individual
cutaneous contributions we can also gain insight into receptor-specific contributions to the
reflex response.

Materials and methods
Subjects
Eighteen healthy volunteers participated in 26 recording sessions (7 females (average height
and weight 168cm, 60kg) and 11 males (average height and weight 180cm, 78.5kg); aged 20 to
38 years, average 25 years). None of the participants reported any neurological or skeletal-
motor deficiencies. All subjects gave written informed consent as approved by the University of
Guelph human ethics committee, and carried out in accordance with the principles of the
Declaration of Helsinki.

Experimental set-up
Subjects either sat in an adjustable chair, with both legs slightly flexed (common
peroneal/fibular recordings), or lay prone with the chair in a treatment table position (tibial
nerve recordings). In the sitting position the legs were supported with a piece of foam under
the distal thigh to maintain an adequate angle at the knee for palpation of the common
peroneal (fibular; CP) nerve and electrode insertion. In this position the knee was at an angle of
approximately 120° flexion (180° as horizontal) and the ankle relaxed at 100° extension. Versa
Form pillows were used to ensure subject comfort and subject position was adjusted to
maximize muscle relaxation to improve the recording success. This prevented disturbances to
neural sensory recordings via activation of motor units. In the prone position, subjects lay with
their head facing forward, legs extended and knees slightly flexed (10°). During data collection
from either nerve surface electromyography (EMG) recordings were collected using disposable
surface electrodes (Ag-AgCl bipolar electrodes, Kendall LTP, Chicopee, MA) placed over the
bellies of posterior deltoid (PD) and the lateral head of the triceps brachii (Tri). EMG signals
were differentially amplified (gain 500, band pass 10-1000Hz, AMT-8 Bortec Biomedical Ltd,
Calgary, Canada) and digitally sampled at 2000Hz (Model 1401 DAQ system, CED, Cambridge
UK).

To locate the site of the CP nerve at level of the fibular head or the tibial nerve in the popliteal
fossa, transdermal electrical stimulation (0.2 ms, 0 – 10 mA, 1 Hz: ) was performed using a
Grass SIU-C constant current stimulus isolation unit, and a S88X Grass stimulator (Astro-Med,
West Warwick, RI, USA). Successful stimulation of the CP nerve was demarcated by twitches in
the peronei muscles (Peroneus brevis and longus) as well as in extensor muscles of the ankle
and digits; extensor hallucis longus( EHL), extensor digitorum longus (EDL), tibialis anterior (TA).
Additionally paraesthesiae in the skin of the foot dorsum and front of the leg indicated CP
location. For the tibial nerve, the optimal site was chosen once paraesthesiae was experienced
into the heel and sole of the foot, accompanied by twitches in the triceps surae muscles (soleus, lateral and medial gastrocnemius). The site for electrode insertion was chosen when the greatest twitch/sensation could be elicited with the smallest current. Once the site was marked, a low impedance reference electrode was placed just under the skin, roughly 20-30 mm distal (CP) or medial (tibial) to the insertion site of the recording microelectrode. The recording electrode (insulated tungsten microelectrode, 200μm diameter, 30-35 mm length (CP), 55mm length (tibial), Frederick Haer Inc., ME, USA) was then inserted at the marked location. Manipulation of the electrode was done using auditory feedback of the neural activity. The neural signal was amplified (gain $10^4$, bandwidth 300 Hz – 3 kHz: ISO-80, World Precision Instruments, USA) to locate the nerve. Once the nerve was located, further fine manipulation of the microelectrode was performed to isolate single cutaneous afferents while providing mechanical stimuli to the skin in the area of interest (foot dorsum; CP, foot sole; tibial). Neural activity was then digitized at 20 kHz and stored for subsequent analysis (Spike 2 version 6 software; Cambridge Electronics Design, UK).

**Experimental procedure**

Once located, individual afferents were classified as innervating either fast-adapting (FAI or FAII) or slowly-adapting (SAI or SAII) low threshold mechanoreceptors using criteria that has been described previously (Johansson 1978, Vallbo and Johansson 1984; Kennedy and Inglis 2002; Edin 2001; Fallon et al 2005). The receptive field size, mechanical threshold and location of ‘hot spots’ within the receptive field were determined using calibrated monofilaments (Semmes-Weinstein monofilaments, North Coast, San Jose CA). The monofilament which exerted a force 4-5 times the threshold force was used to identify the receptive field. Mechanical
activation of the identified low threshold mechanoreceptor was then initiated using the handle of a paint brush over the receptive field (tip diameter approximately 2mm). For Ectopic units, the general location of the skin receptor was classified based on the location of the last fascicle from which cutaneous multiunit action potentials were elicited. For example, for the two ectopic units recorded palpation of the skin 1) near the great toe and 2) at the ankle midline elicited multiunit cutaneous action potentials from the fascicle prior to the single afferent recording. It is important to add here that we cannot say with 100% certainty that the single afferents were cutaneous due to inability to verify via receptive field stroking, however, we are confident based on auditory feedback that we remained within the same fascicle when the single afferent became apparent. Mixed afferents (skin and muscle) are found infrequently in the CP nerve. Based on the receptor type, different receptor activation methods were used; for identified FAI receptors a rapid ‘stroking’, for FAIIIs blowing across the receptive field was first employed (and activated three of four initially), however, all four FAIIIs located here were also activated with rapid stroking as blowing was unsuccessful to continuously activate the receptor. SAIIs were activated using skin stretch along the axis that evoked the greatest response or indentation. When the receptor adapted to the stimulus, the indentation was reinitiated. SAIIs were activated using skin stretch along the axis that evoked the greatest response or indentation. Occasionally firing from the SAIIs and SAIIs had to be supplemented with some stroking to ensure adequate collection of spikes. Additional stroking only occurred in 4 units (of 19) during the last minute of a 5 or 6 minute data collection. Of note, 2 of the 4 units which were supplemented by stroking did NOT demonstrate reflex coupling.
During mechanical activation of the cutaneous receptor participants were instructed to isometrically activate their deltoid muscle and lateral triceps simultaneously at approximately 10% (light contraction). A researcher gently supported the arm to facilitate controlled muscle activation. Contraction were held for 2-3 minutes before relaxation. An additional 2 minutes of contraction was then completed. These short duration contractions were designed to minimize the risk of fatigue. The total duration of contraction enabled adequate time to elicit a sufficient number of action potentials from the cutaneous unit for spike triggered averaging (average number of sweeps 4598 range 364-23,595 sweeps). Contraction level was calculated as a percentage of maximum voluntary effort (MVE), which was established as the peak EMG over 100ms across three trials of maximal effort. MVE was performed at the onset of the experiment prior to the microneurography session. During the experiment subjects were verbally instructed by the experimenter to maintain the 10% level.

For three receptors (SAI, FAI, FAII), in addition to the isometric contraction subjects were required, in a second data collection, to grasp a vertical pole to activate triceps and deltoid muscles. This provided a more posturally-relevant context for the activation of the upper limb muscles (Lamont and Zehr 2007). Of note, subjects were instructed not to lean against the back rest of the chair in order to increase their reliance on the arm musculature for postural support. Lamont and Zehr (2007) found that when subjects held on to an earth-referenced pole during locomotion, the cutaneous reflex response evoked in upper limb muscles was increased (specifically triceps brachii and posterior deltoid), compared to unsupported walking. They concluded that holding a pole results in an overall decrease in cutaneous threshold, which may facilitate the use of the upper limbs in a functional balance response. In the current study a
position was assumed that was identical to the isometric contraction and both muscles
(posterior deltoid and triceps) were monitored for comparable levels of background
contraction (10%).

**Data analysis**

Identification of unitary spikes was performed using Spike 2 software (Spike 2, version 6,
Cambridge Electronics, UK England). Spike morphology was used to generate a template and
spikes throughout the recording were classified based on shape. If a recording was found to
include multiple cutaneous afferent contributions, the recording was not included for further
analysis.

Surface EMG was analysed using a 5ms sliding window root mean square (RMS) using Spike 2
software. The RMS EMG was then spike-trigger averaged to the afferent firing to identify any
time locked modulation of upper limb activity related to cutaneous activation. A significant
response was identified when the EMG modulation surpassed two and a half standard
deviations (SD) about the mean for at least 5ms, which is equivalent to a 99% confidence
interval. Due to the cyclic nature of several recorded afferents it was necessary to generate a
control random spike. The random spike was generated using a custom made program (spike 2
software). The mean interval of the ‘real’ spike train was used to generate a Poisson
distribution of random intervals with the same mean interval. This random spike was then used
to generate the confidence interval, which determined significance.

**Latency and amplitude**

The latency of the reflex coupling was determined as the duration from spike onset to the time
the RMS EMG surpassed the confidence interval (99%). Significant reflex coupling between
single low threshold mechanoreceptors and the muscles of the upper limb were classified into
three categories based on latency. Reflex responses occurring at a latency between 25ms-70ms
were classified as short latency, 70-120ms as medium latency and >120ms as long latency
(Fallon et al. 2005; Aniss et al. 1992; Brooke et al. 1997; Burke et al. 1991; Gibbs et al. 1995;
Kukulka 1994; Zehr et al. 2001). The current paper focused on reflexes occurring in the early
and mid time frame (25ms to 120ms) to highlight responses with a greater probability of
classified as significant, with the shortest onset latency, and the cyclical nature recorded. The
amplitude of the reflex response was calculated as the peak percent change from background
RMS EMG and was identified as a reflex response if the latency was shorter than 120ms (Fallon
et al 2005, ). ‘Cyclic’ onsets latencies were not included in the overall average for onset latency
calculations.

Statistics

χ² tests were used to examine the distribution of receptor types across the foot and the
number of receptors in each category that exhibited a significant reflex coupling versus those
that did not. Receptive field and threshold were assessed using t-tests to compare receptor
characteristics of receptors that demonstrated a significant EMG modulation versus those that
did not. Reflex magnitude (measured as a % of background EMG) was examined between the four receptor types using a Kruskal-Wallis one-way ANOVA with Bonferroni adjustments for multiple comparisons.

**Results**

Forty one low threshold mechanoreceptors were sampled from the skin overlying the dorsum (32) and sole (9) of the foot. Of these units there were 15 FAIs (37%), 4 FAIIs (10%), 9 SAIs (22%), 10 SAIIIs (24%), 2 ectopic units (5%) and one hair unit (2%). Following spike triggered averaging of upper limb muscles a total of 18 of 41 (44%) single low threshold mechanoreceptors in the foot demonstrated significant synaptic coupling with motoneurons supplying muscles in the upper limb (Figure 1). The afferents that exhibited a significant modulation of the ongoing upper limb EMG activity included 10/15 (67%) innervated by FAI, 1/4 (25%) innervated by FAII, 4/9 (44%) innervated by SAI, and 2/10 (20%) innervated by SAII low threshold mechanoreceptors. Both ectopic units demonstrated a significant coupling response represented as a modulation of the upper limb muscle activity, while the hair receptor did not (Figure 1). An equal number of units demonstrated significant responses in the upper limb, whether they were located on the dorsal (47%) or plantar (50%) surface of the foot. One additional unit (SAI) did not show significant coupling during a ‘free’ isometric contraction, however, when an earth referenced pole was held during muscle activation significant modulation of both deltoid and triceps was observed (Figure 6 and earth vertical reference, below) increasing the total to 19 of 41 units with a significant coupling (46%).

**Receptor characteristics and distribution**
The average receptive field (RF) size and threshold for each of the four classes of receptor closely resembles current literature on cutaneous mechanoreceptors in the foot (Table 1). To assess differences relative to the literature data were compared using chi square analyses. Receptive field size for units on the dorsal or plantar surface of the foot did not differ significantly from those reported previously by Fallon et al (2005) on the plantar surface of the foot (p>0.05), nor did they differ significantly from foot dorsum receptors in a recent study by Aimonetti and colleagues (2007) (p>0.1). With respect to the proportions of afferents distributed among the four mechanoreceptor classes, the 41 single afferents recorded in the current study did not differ significantly from those recorded in the plantar surface of the foot (p>0.05; Fallon et al 2005). There was a predominance of afferents innervating fast adapting receptors identified in both the current study (37%) and Fallon et al. (40%). In contrast, the distribution of afferents among the four different classes did differ significantly from those reported by Aimonetti et al (2007) in the foot dorsum (p<0.01), where the greatest number of afferents were innervated by slowly adapting Type II (30%) mechanoreceptors (followed by FAI; 27%). The large representation of SAII afferents (with Aimonetti et al 2007) may result from the location of the recorded afferent RF; predominantly on the anterior surface of the leg, where no receptors were recorded in this location in the current data pool.

To identify the distribution of receptors that demonstrated a significant coupling response the foot was divided into quadrants (Figure 2). Twenty two and a half of the thirty two units on the dorsum of the foot were found on the medial aspect, with seven and a half on the lateral side. Within each quadrant reflex coupling with the upper limb was assessed to identify whether a specific location on the foot had a higher probability of eliciting a response. While the total
number of recorded afferents did not differ significantly between the four quadrants (p>0.05),
the distribution of receptors that demonstrated a significant reflex response were found to
differ across the foot dorsum, with significantly greater coupling shown in the two medial
quadrants (p<0.01). Additionally, the RF size was significantly different between receptors
demonstrating a coupling with upper limb motoneurons (smaller) and those which did not
modulate upper limb activity (larger) (p<0.001), whereas threshold levels of these receptor
groups did not differ (P>0.1).

Reflex response

The most common reflex coupling was seen between FAI mechanoreceptors and motoneurons
innervating muscles of the upper limb, where 67% of the recorded FAIs demonstrated a
significant modulation. This prevalence of coupling in the FAI receptors supports findings by
Fallon et al. (2005), where 81% of the FAIs in the foot sole demonstrated the ability to
modulate ongoing muscle activity in the lower limb. The second highest prevalence of coupling
in the current study was seen with SAI units (44%), also consistent with previous reports of
intralimb responses in the lower limb (Fallon et al 2005). In fact the proportion of units in each
class that demonstrated significant coupling did not differ from the results obtain by Fallon et al
(2005) (p>0.1), which is the only other study to date that has examined these reflex responses
from cutaneous receptors in the foot.

The average magnitude and latency of the reflex responses are given in Table 2. A total of 19
single units are represented including one unit in which a reflex was demonstrated only after
the subject held onto a vertical reference pole (see below). Six of the 19 units (including both
ectopic units) demonstrated synaptic coupling with motoneurons innervating both triceps and deltoid muscles. The median response magnitude was found to be 2.29% of background EMG (2.59% including the SAI pole unit) and did not significantly differ among the four receptor classes (p>0.1). Interestingly, the reflex strength (amplitude of response) was similar to the reflex magnitude reported by Fallon and colleagues for ‘within’ lower limb responses (2.3%) and for responses ‘within’ the upper limb of 2.05% reported by McNulty et al. (2001).

Cyclic response

All 15 of the FAI afferents were activated by stroking across the receptive field. Rapid stroking increases the number of action potentials during the data collection phase to enable observation of any present EMG modulation after spike triggered averaging. Figure 3 illustrates one FAI afferent that evoked cyclic activation in both PD and Tri EMG following high frequency activation across its receptive field (Figure 3 and Table 2). This single FAI afferent was located along the dorsal/medial border of the foot slightly proximal to the 1st metatarsal head. Its RF was small (2mm x 3mm) and threshold was 0.085g, which was low relative to the other recorded FAIs. This particular FAI was able to evoke significant and periodic modulation in both deltoid and triceps that followed the 10Hz activation of the afferent over its receptive field (Figure 3). As a result of the cyclic nature of this unit, the onset latency of the muscle response was calculated as 11ms relative to ‘time zero’. Clearly this muscle onset latency is too early to be associated with the spike trigger averaged units at time zero, and is more likely representative of a response to an earlier cycle of activation of the FAI unit (resulting in a reflex
latency closer to 110ms). The cyclic nature itself makes it impossible to predict which of the cycles of afferent firing corresponds to the muscle modulation. As a result, onset latencies calculated in afferents that clearly exhibited a cyclic response in nature were not included in the overall latency calculation. The remaining nine FAI units were all activated at a frequency which generated the periodic bursts at a frequency outside of the latency of interest for reflex coupling (periodicity > 300ms, reflex coupling < 120ms). All other types of receptors (FAII, SAI, SAII) can be activated by stroking across the receptive field, however, this type of activation was only applied if a response was not apparent using conventional approaches (only used for 4 additional units - see methods). Figure 4 illustrates a FAII mechanoreceptor (pacinian corpuscle) that was also activated through stroking, which resulted in a cyclical response from the afferent and a time locked response in the PD EMG following each afferent burst. Activation of this particular FAII was not possible through rigorous blowing across the RF. A strong correlation was found ($R^2 = 0.997$) between the mean inter-spike interval of the FAII afferent activation, and the reflex periodicity of the EMG response in Deltoid.

**Single afferent response**

The probability of representing reflex coupling related to one single afferent is increased in afferents that generate their own unique, and thus asynchronous firing pattern relative to other skin receptors located in the immediate anatomical region. SAI and SAII receptors demonstrate unique firing to constant indentation, and SAII also respond to skin stretch. When natural activation is used to generate action potentials from which to spike trigger average ongoing EMG there is an increased confidence that coupling is related to single afferent activation. Figure 5 is an example of an SAII receptor located slightly proximal to the ankle that
was activated by skin stretch along the ankle dorsum in a proximal-distal direction (Figure 5, inset). This single afferent demonstrated significant modulation of ongoing deltoid activity roughly every 60ms, which correlates well with the regular firing of the SAII afferent (16.7Hz).

**Earth-referenced pole**

In two participants, following the isometric contraction protocol, activation over the receptive field was repeated while subjects generated muscle activity by holding onto an earth referenced pole. The amplitude of the background EMG activity (100ms) was not statistically different between the two trials (isometric contraction vs. muscle activation to grasp the pole) (P=0.13). Both units (FAII and SAII) demonstrated significant coupling to motoneurons in the upper limb muscles during trials with the earth referenced pole. For the SAII unit, grasping the earth referenced pole resulted in a significant inhibition of ongoing muscle activity in both deltoid (61ms onset, 7.65% amplitude) and triceps (88ms onset, 6.38% amplitude) (Figure 6).

This afferent was located in the glabrous skin on the pad (plantar surface) of digit IV. The receptive field size (42 mm²) and threshold level (0.4g) were both low relative to the recorded population. This SAII unit was activated using constant indentation over its receptive field to avoid cyclical activation of the afferent, and therefore reduce the chances of contributions from neighbouring skin receptors (corroborated by the autocorrelogram). Also of note is that the number of sweeps during isometric contraction included 1000 more spikes than the trial with the pole, supporting an overt modulation when the arm grasped onto an earth vertical reference. The second unit (FAII) also demonstrated a significant inhibitory modulation; this response was in the triceps brachii (155ms, 2.27%). However, the modulation fell outside of our criteria for a short or medium latency response. This unit was located on the plantar surface of
the foot between digits III and IV. Interestingly, neither of these units (SAI, FAII) were able to initially (with isometric contraction alone) modulate the motor neuron pool of either upper limb muscle.

Discussion

We have, for the first time, demonstrated a reflex connection between single low threshold mechanoreceptors in the skin on the foot dorsum with motoneurons supplying muscles of the upper limb. Unique to other studies that have examined individual cutaneous contributions to muscle modulation, we have identified the presence of interlimb reflex responses. Similar to the foot sole, but in contrast to the hand we have shown that strong reflexes can be generated from all four types of cutaneous receptors in this hairy skin (including SAI).

Afferent response

The most commonly observed reflex coupling in the upper limb muscles was through activation of FAIs (67%) followed by SAIs (44%). Coupling within the remaining afferent classes was considerably less frequent (20% SAI, 25% FAII). FAIs from the foot sole in a previous study were also shown to elicit a strong coupling response within the lower limb (Fallon et al 2005) but were not as prominently featured in the reflex response within the arm (McNulty et al 1999). FAI afferents are believed to innervate Meissner corpuscles, which are most sensitive to forces tangential to the skin (shear) and are known to respond to incipient slips within the receptive field, primarily signaling external perturbation forces (Macefield 1998; Johansson et al. 1982).

Based on their rapidly adapting nature, they are also ideal for providing dynamic input (velocity). Skin on the foot is our direct interface with the environment and can provide valuable information regarding the support surface and obstacles in our path. Contact signaled
by the foot dorsum skin during dynamic tasks (tripping) provides critical and timely information that can influence upper limb responses to help move the centre of mass forward (Marigold et al. 2003), or grab for a support hold (McIlroy and Maki 1995).

It is notable that SAI receptors demonstrated strong reflex coupling between foot dorsum skin and muscles of the upper limb (second only to the frequency of FAI responses). In combined data across two papers, McNulty et al. (1999; 2001) reported a distinct lack of response from SAI receptors in reflex coupling within the upper limb. While FAIs can provide some input regarding shape (Philips et al. 1992), SAI receptors are known to be important in tactile discrimination, including the ability to encode raised dots, edges, curves and object orientation in the hand (Johansson and Vallbo 1980; Goodwin et al 1995; LaMotte and Srinivasan 1996; Johansson et al. 1982) and respond with high resolution to changes in pressure (Mountcastle 1966). Input from SAIIs regarding shape and indentation may not feature prominently for reflex responses when grasping objects (McNulty et al 1999), however, such input may be critical for facilitating appropriate cautionary strategies during locomotion. The ability of SAIIs to faithfully code compression may specifically highlight their role on the foot dorsum, to signal shape and size of contacted objects or changes in (expected) compression of the foot skin during gait.

Previous reports in the hand have indicated that SAIII afferents respond to movement of the digits (Burke et al. 1988) as well as skin stretch around joints (Knibestol and Vallbo 1970) suggesting a role in kinesthesa. Aimonetti et al. (2007) have also demonstrated pronounced firing of SAIII afferents in the skin of the ankle following movement of the foot. SAIIs in the current study, were the group of receptors to show the least amount of coupling (20%).
feasible that SAIIs information regarding skin stretch and potentially ankle joint position are most important in eliciting intralimb responses to activate muscles at the ankle, and not interlimb responses to distant arm muscles. Previous work, both within the upper limb (McNulty 1999; 2001) and lower limb (Fallon et al. 2005) have shown a strong coupling response of SAIIs within their own innervation territory (52% coupling within the upper limb, 40% coupling within the lower limb). Therefore, as a proprioceptor, SAIIs input can provide information regarding local skin stretch and is therefore ideal to modulate joint position for successful grasp, or foot placement around the local joint. Such modulations may not be appropriate or necessary for interlimb control.

Reflex amplitudes

We compared the reflex magnitudes of our upper limb responses to those reported in two previous papers, which examined coupling with motoneurons acting on muscles of a homonymous innervation territory (Fallon et al. 2005; McNulty et al. 1999, 2001). Our amplitude calculation was based on peak responses, and therefore we halved the amplitude responses reported previously by McNulty et al (2001; who used peak to peak) to enable a comparison. Perhaps surprisingly, the magnitude of the median reflex response in the current study (2.29% of background) did not differ from previous intralimb findings (2.3%; Fallon 2005 and 2.05%; McNulty 2001), despite our modulation of muscles of a distant innervation territory. These previous intralimb reflex magnitudes can no longer be attributed to a general cutaneous coupling response within a limb, rather it appears that interlimb cutaneous input displays an equally potent impact on muscle activity. Zehr and colleagues (2001), were the first group to report significant inter and intralimb reflexes following whole nerve stimulation of SP. Our
current observations from *single cutaneous afferents*, of prominent interlimb reflex coupling, adds further support that such links from physically distant cutaneous territories can influence and shape muscle activity for the reflex coordination of movement. Our results show EMG responses that are clearly time-locked to action potentials from a single afferent providing a strong indication that the recorded afferent is the primary contributor to the reflex. The possibility cannot be overlooked, however, that in some cases other afferents may contribute. Indeed, FAIs are often found in high densities in the skin and stroking over the receptive field of one receptor could simultaneously activate others, contributing to the overall EMG response. With SAI and SAII afferents, indentation and stretch activation of these afferents are more likely to segregate single receptor contribution, given that each receptor has a unique firing response to these natural stimuli. While the EMG response is time-locked to the recorded spike, increasing the probability that this single afferent is having the greatest (and potentially only) impact, it is also noteworthy that additional afferents may respond somewhat to these natural stimuli and fire at sub-harmonics to contribute to the EMG reflex response. Ultimately, the activation strategies used targeted skin over the receptive field and localized the response as much as possible and, we feel, succeeded in segregating individual afferent types and their responses. Compelling evidence that single afferents contribute to the reflex response is demonstrated by our data on ectopic units; where no external stimulation is applied. The lack of evoked stimulation argues definitively for single afferent contribution to the reflex response.

Pathways
Latencies for the interlimb reflex response evoked via single cutaneous afferents ranged from 38ms to 120ms, with a median of 59ms (mean 71ms). Interestingly, no differences were found in onset latency in our upper limb responses when compared to single afferent reflexes within the lower limb (Fallon et al 2005), where a median onset latency of 57.4ms was reported across all muscles and all receptor types. We propose that the reflexes evoked in the current study involve propriospinal pathways, however, the involvement of subcortical and even cortical structures cannot be ruled out. Conduction velocities in the upper and lower limb are known to vary considerably from 20m/s to 60m/s (Mackel et al. 1988) 26m/s to 91m/s, (Knibestol 1973) and 52m/s to 89m/s (Macefield et al 1989). Based on our shortest non-cyclical response (38ms) and a travel distance of roughly 1m (CP at knee, to shoulder), the conduction velocity of our fastest afferent is estimated at 30m/s, falling within the reported velocities for cutaneous afferents. Whole nerve SP, or foot skin stimulation to date has reported early, 45-50 ms (Zehr et al. 2001; Pieseiur-Strehlow and Meinck 1980) or middle, 75ms (Zehr et al 2001), 91-92ms (Kearney and Chan 1979) reflex latencies in the upper arm with these authors purporting connections via propriospinal pathways. Previous work has provided evidence that long-latency intralimb reflexes via SP stimulation (70-95ms) are at least partly mediated by a transcortical pathway (Nielsen et al 1997), however, it is noteworthy that these reflexes are within the lower limb. The range of our reflex latencies suggest they may fall into several categories, with evidence of both propriospinal (nine SL responses; 38ms-62ms) as well as coupling which may involve subcortical or cortical loops (eight ML responses; 71-120ms). Cutaneous reflexes reported by Pruszynski et al (2008) indicated the fastest voluntary activity in muscles across the
elbow to begin at 120ms, indicating our responses, for the most part, fall within a reflex latency.

The question remains, are these reflex responses fast enough to be involved in upper limb perturbation responses? Onset of activity in deltoid has been reported at 88ms following whole body perturbations (McIlroy and Maki 1995) and 143-150ms following slips (Marigold et al. 2003). With our median latency of 59ms, and maximum latencies of 120ms, it is feasible that skin receptors on the dorsum of the foot can contribute to these rapid upper limb responses, with FAIs and SAIs featuring prominently.

Regional differences and function

Fifteen FAI units were identified in the dorsum of the foot in the current study, despite a lack of evidence of fast adapting types of receptors in previous studies of hairy skin; in the face, forearm and thigh (Edin 2001; Vallbo et al. 1995). The authors suggested that receptor distribution (fewer FA I, more SA and hairy receptors) reflected regional differences in functional roles. SA receptors are known to provide rich information for proprioception, especially for joint position (Johnson KO 2001; Edin and Johansson 1995), which makes them suitably located across joints in the hairy skin of the body. FA receptors are able to provide information regarding contact timing and velocity of objects as they move along the skin (in grip or in slip), which is pertinent for the glabrous skin of the palms of the hands and soles of the feet. Here we found a large distribution of FAIs in the foot dorsum hairy skin (15 FAIs, 37% of all collected) as compared to 5% in knee and thigh (Edin 2001) and 0% in the forearm (Vallbo et al. 1995). In contrast, our distribution was very similar to reports in the glabrous skin (Fallon et al 2005; Kennedy and Inglis 2001), where the largest population of afferents innervated FAI
end organs. Aimonetti and colleagues (2007), who also sampled from skin on the foot dorsum and leg found the majority of afferents to innervate Type SAII. This may be due to receptor location, where 72% of the identified afferents were located on the leg and not the dorsum of the foot. Interestingly, when considering only receptors on the foot dorsum (25), Aimonetti found the greatest density of FAIs (nine; 36%), which corresponds well with the current study (37% FAIs). What appears to be emerging here is a regional difference across the foot dorsum from other regions of hairy skin. FAIs on the foot dorsum signal unexpected contact with objects in the path trajectory, and when coupled to motoneurons supplying muscles in the upper limb can subserve a protective balance role.

**Earth referenced contact**

Task dependent reflex modulation is not unique to a particular system. Coupling between cutaneous receptors and muscle spindles of the lower limb has been shown to be task dependent, with responses only emerging in a balance context (standing, Aniss et al. 1992). The technique of microneurography poses limitations on the ability to assess afferent responses in a posturally relevant position, as for the most part experiments are performed sitting or lying down. To enhance the balance context in the current study we provided an earth referenced pole.

Two subjects were instructed to hold an earth referenced rail while performing a level of contraction comparable to isometric contraction in a previous trial. Spike triggered averaging revealed no significant modulation during isometric contraction without the pole, however, while holding the vertical pole, there was an emergence of a significant response. These findings corroborate previous data of enhanced reflex responses in PD and Tri during a
locomotor study that examined gating of interlimb reflexes with an earth referenced handrail
(Lamont and Zehr 2007). The conclusion from these observations combined, is that a posturally
relevant context enhances the interlimb reflex response.

Conclusions

We have shown for the first time that single low threshold tactile afferents from the foot, and
particularly from the foot dorsum can modulate ongoing muscle activity in the upper limb.
Identification of each class of skin receptor also enables insight into those most closely coupled
to this interlimb reflex response (FAI, SAI). While our current data cannot determine whether
skin is the trigger for rapid upper limb responses (or simply a modulator) we have
demonstrated strong coupling that indicates the substrate does exist for skin to have a role in
these rapid perturbation responses. Modulation of ongoing muscle activity generated by the
activation of a single low threshold cutaneous afferent has previously been shown within the
upper limb (McNulty et al, 2001, 1999) and within the lower limb (Fallon et al. 2005). The
current work extends these findings to highlight cutaneous modulation of muscle activity across
innervation territories, arguing that cutaneous input is an important component in the
coordination of interlimb activity. Furthermore, testing of reflexes in a balance relevant context
(reference pole) demonstrated even larger responses, supporting a functional role for foot skin
in balance related upper limb reflexes.

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L.R.B and C.R.L. analyzed data. L.R.B. prepared figures; L.R.B. and C.R.L. interpreted results of
experiments; L.R.B. drafted manuscript; L.R.B. and C.R.L. edited and revised manuscript; L.R.B.
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Figure Captions

Figure 1: Distribution of identified low threshold cutaneous afferents in the dorsum and plantar surface skin of the foot. Representative receptive field size and location is illustrated. Cutaneous receptors that demonstrated a significant coupling response are shaded.

Figure 2: Distribution and reflex coupling response for cutaneous receptors A) on the dorsum and B) on the plantar surface of the foot. Afferents that demonstrated a significant coupling with muscles of the upper limb are represented by shaded receptive fields. Those showing no significant coupling remain unfilled. The foot is divided into quadrants to highlight receptor density and the relation of foot location to reflex coupling in the upper limb. Bar graphs indicate the percentage of each type of receptor with a coupling response in each quadrant.

Figure 3: Reflex coupling demonstrated with a fast adapting FAI afferent with receptive field located on the dorsal aspect of the foot proximal to digit I. This afferent was activated using rapid strokes across the receptive field. A significant reflex coupling was demonstrated in the triceps brachii muscle at 96ms. A) The cyclic nature of activation resulted in a cyclic response in the afferent autocorrelation as well as a reflex periodicity in both Deltoid and Triceps EMG response at similar latencies. No significant responses were shown in response to the randomly generated spike (B). The spike overlay is presented to illustrate unitary activity in the FAI.

Figure 4: Reflex coupling demonstrated by an FAII afferent. A) autocorrelation of the single afferent showing a 10Hz firing rate (generated by stroking across the receptive field) and significant reflex coupling in deltoid at 66, 166 and 263ms. B) Autocorrelation of random generated spike and absence of significant coupling with Deltoid. C) Correlation between reflex periodicity in Deltoid EMG and the interspike interval of the periodically activated FAII, R²=0.997. The spike overlay is presented to illustrate unitary activity in the FAII.

Figure 5: SAII unit found on the front aspect of the ankle (see photo inset). The SAII was activated by stretching along its preferred direction (proximal-distal along the leg). From bottom to top: a Raw neurogram of the SAII action potential, Interstimulus interval demonstrating an inherent firing frequency of 16.7 Hz in response to stretch, the spike trigger averaged Deltoid EMG with responses approximating a 16 Hz cycle (every 60ms) and an autocorrelogram of the SAII demonstrating a firing rate that corresponds (approximately 16 Hz). The spike overlay is presented to illustrate unitary activity in the SAII.

Figure 6: Low threshold cutaneous afferent innervating an SAI receptor located on the pad of the fourth digit. SAI was activated during A) isometric contraction of the upper arm or B) contraction of upper limb muscles while grasping an earth vertical referenced pole. The first row illustrates the autocorrelogram (count: 5 ms bins) of the afferent demonstrating its irregular firing behaviour (top; superimposed unitary spike in inset), followed by the spike-triggered averaged RMS EMG of deltoid and Triceps (2nd and bottom trace) (with 99% confidence limits). Despite the fewer number of sweeps in the earth referenced condition (666), there was a clear and significant modulation of both deltoid and triceps at latencies
indicating a potential spinal oligosynaptic, or subcortical reflex response. Average background EMG did not differ between the isometric contraction and the EMG generated while holding the earth reference (p=0.13)
Table 1: Summary of afferent median receptive field (mm²) and median threshold levels (N)

Median and Range are given for receptive field and activation threshold for each of the receptor classes. N represents the number of afferents contributing and the percent of the total population recorded (%).

<table>
<thead>
<tr>
<th>Class</th>
<th>n (%)</th>
<th>Threshold mN</th>
<th>Receptive Field mm²</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Median</td>
<td>Range</td>
</tr>
<tr>
<td>FAI</td>
<td>15 (37)</td>
<td>7.31</td>
<td>0.83-81.89</td>
</tr>
<tr>
<td>FAII</td>
<td>4 (10)</td>
<td>0.73</td>
<td>0.63-0.83</td>
</tr>
<tr>
<td>SAI</td>
<td>9 (22)</td>
<td>25.3</td>
<td>0.63-50.01</td>
</tr>
<tr>
<td>SAI1</td>
<td>10 (24)</td>
<td>1.42</td>
<td>0.63-32.36</td>
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Table 2. *Summary details of cutaneous afferents showing significant coupling*

Data from all units that demonstrated a significant coupling with motoneurons of upper limb muscles are presented by afferent type. Latency (ms) and amplitude (peak as a % of background EMG) are given for all units. Median latency is presented for all non-cyclical units. One unit (39) showed a significant coupling only when the participant held onto an earth referenced vertical pole (median in brackets for latency and amplitude includes this unit).

<table>
<thead>
<tr>
<th>MUSCLE</th>
<th>NERVE</th>
<th>UNIT</th>
<th>POLARITY</th>
<th>LATENCY (ms)</th>
<th>AMP (%background)</th>
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<tr>
<td>FAI</td>
<td>Deltoid</td>
<td>Cp</td>
<td>2</td>
<td>Inhibitory</td>
<td>72.3</td>
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<tr>
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<td>Deltoid</td>
<td>Cp</td>
<td>5</td>
<td>Cyclic</td>
<td>11</td>
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<tr>
<td></td>
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<td>Cp</td>
<td>9</td>
<td>Inhibitory</td>
<td>120</td>
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<tr>
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<td>Cp</td>
<td>11</td>
<td>Inhibitory</td>
<td>62</td>
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<td>Tibial</td>
<td>37</td>
<td>Inhibitory</td>
<td>105</td>
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<tr>
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<td>Triceps</td>
<td>Cp</td>
<td>5</td>
<td>Cyclic</td>
<td>96</td>
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<td>Triceps</td>
<td>Cp</td>
<td>8</td>
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<td>120</td>
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<td>Cp</td>
<td>10</td>
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<td>54</td>
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<td>102</td>
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<td>Tibial</td>
<td>39</td>
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<td>61</td>
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<td>56</td>
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<tr>
<td></td>
<td>Triceps</td>
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<td>Triceps</td>
<td>Cp</td>
<td>21</td>
<td>Cyclic</td>
<td>70</td>
</tr>
<tr>
<td>Median</td>
<td></td>
<td></td>
<td></td>
<td>Median</td>
<td>59 ms (61ms)</td>
</tr>
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</table>
A

50%

Total: FAI- 7                  4
FAII- 0                 0
SAI- 2.5              1
SAII- 1.5            0.5

Significant: FAI- 1                  0
FAII- 0                 0
SAI- 1.5              0
SAII- 0.5            0.5

B

50%

Total: FAI- 0                  0
FAII- 0                 0
SAI- 0                  0
SAII- 1.5              0

Significant: Total:
FAI- 3                  2
FAII- 1                 0
SAI- 1                  1
SAII- 1                 0

* two Ectopic not displayed due to uncertainty of location
A. FAI

B. Random generated Spike

Deltoid

Triceps

Time (s)
A  Isometric (10% MVC)

1662 sweeps

Deltoid

-0.1  0  0.1  0.2  0.3  0.4

Time (s)

30.9 uV

28.5 uV

51 uV

47 uV

Triceps

-0.1  0  0.1  0.2  0.3  0.4

Time (s)

B  Earth Reference (10% MVC)

666 sweeps

61 ms

99.8 uV

88.6 uV

100 uV

84 uV

10 uV

1 ms