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

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Bent LR, Lowrey CR. Single low-threshold afferents innervating the skin of the human foot modulate ongoing muscle activity in the upper limbs. J Neurophysiol 109: 1614–1625, 2013. First published December 28, 2012; doi:10.1152/jn.00608.2012.—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, although the distribution of afferent 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.

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

There is much speculation as to the trigger for the initiation of the postural response in the lower limb (muscle spindle, skin afferents, vestibular). On the basis of 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 (Magnussen et al. 1990a, 1990b; 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.

Although rapid initiation of upper arm movement also has been reported in response to unexpected perturbations (Ghafari et al. 2004; Marigold et al. 2003; McIlroy and Maki 1995; Misiaszek 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 also have 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) or cycling (Balter and Zehr 2007) and during locomotion (Lamont and Zehr 2006, 2007), upper limb musculature has been shown to be not only 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 et al. 1999; McNulty and Macefield 2001) 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 ongoing muscle activity in the upper arm. The purpose of this 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: 168 cm, 60 kg) and 11 males (average height and weight: 180 cm, 78.5 kg); ages 20–38 yr, average 25 yr]. 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 setup. 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 ~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 10–1,000 Hz, model AMT-8; Bortec Biomedical, Calgary, Canada) and digitally sampled at 2,000 Hz (model 1401 DAQ system; Cambridge Electronics Design, 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). Successful stimulation of the CP nerve was demarcated by twitches in the peroneal muscles (peroneus brevis and longus) as well as in extensor muscles of the ankle and digits; extensor hallucis longus, extensor digitorum longus, and tibialis anterior. In addition, paresthesia 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 paresthesia was experienced into the heel and sole of the foot, accompanied by twitches in the triceps surae muscles (soleus, lateral and medial gastrocnemii). 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, 200 μm in diameter, 30–35 mm (CP) or 55 mm long (tibial); Frederick Haer, Bowdoinham, ME] 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 104, bandwidth 300 Hz–3 kHz, model ISO-80; World Precision Instruments, Sarasota, FL) 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 (Spike2 software, version 6; Cambridge Electronic Design).

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 have been described previously (Edin 2001; Fallon et al. 2005; Johansson 1978; Kennedy and Inglis 2002; Vallbo and Johansson 1984). 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 four to five 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 ~2 mm). For cutaneous units, the general location of the skin receptor was classified on the basis of the location of the last fascicle from which cutaneous multifiber action potentials were elicited. For example, for the two cutaneous units recorded, palpation of the skin 1° near the great toe and 2° at the ankle midline elicited multifiber cutaneous action potentials from the fascicle prior to the afferent recording. It is important to add here that we cannot say with 100% certainty that the single afferents were cutaneous due to an 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” was used; for FAIIs, blowing across the receptive field was first employed (and activated 3 of 4 initially); however, all four FAIIs located here were also activated with rapid stroking because blowing was unable to continuously activate the receptor. SAIIs were activated using a constant indentation. When the receptor adapted to the stimulus, the indentation was reintiated. SAIIIs were activated using skin stretch along the axis that evoked the greatest response or indentation. Occasionally, firing from the SAIIs and SAIIIs 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-min data collection. Of note, two of the four units that were supplemented by stroking did not demonstrate reflex coupling.

During mechanical activation of the cutaneous receptor, participants were instructed to isometrically activate their tibial and soleus muscles with brief isometric contractions (1 s) for each. 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: 4,598; 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 100 ms across three trials of maximal effort. MVE was performed at the onset of the experiment before 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, 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 with that evoked during 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 Spike2 software (version 6; Cambridge Electronics Design). Spike morphology was used to generate a template, and spikes throughout the recording were classified on the basis of shape. If a recording was found to include multiple cutaneous afferent contributions, the recording was not included for further analysis.

Surface EMG was analyzed with the use of a 5-ms sliding window root mean square (RMS) using Spike2 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 2.5 standard deviations (SD) about the mean for at least 5 ms, which is equivalent to a 99% confidence interval. Because of 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 (Spike2 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 on the basis of latency. Reflex responses occurring at a latency between 25 and 70 ms were classified as short latency, 70–120 ms as medium latency, and >120 ms as long latency (Aniss et al. 1992; Brooke et al. 1997; Burke et al. 1991; Fallon et al. 2005; Gibbs et al. 1995; Kukulka 1994; Zehr et al. 2001). The current study focused on reflexes occurring in the early and mid-time frame (25 to 120 ms) to highlight responses with a greater probability of traveling within a spinal reflex loop (early latency), spinal oligosynaptic, or transcortical pathway (medium latency) (Nielsen et al. 1997). Activation of several of the single units was performed by manually scratching across the surface. Because of the cyclical nature of the afferent response, it was difficult to establish the latency of onset [i.e., within an analysis window the afferent may respond several times to activation (based on frequency of stroking), making it difficult to establish the timing of the response relative to the stimulus]. In these afferents the unit was classified as significant, with the shortest onset latency, and the cyclical nature was 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 120 ms (Fallon et al. 2005). “Cyclic” onset latencies were not included in the overall average for onset latency calculations.

Statistics. $\chi^2$ tests were used to examine the distribution of receptor types across the foot and to compare the number of receptors in each category that exhibited a significant reflex coupling with the number of 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 with those that did not. Reflex magnitude (measured as a percentage 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 overlaying the dorsum (32) and sole (9) of the foot. Of these units there were 15 FAIs (37%), 4 FAIIIs (10%), 9 SAIIs (22%), 10 SAIIS (24%), 2 ectopic units (5%), and 1 hair unit (2%). After 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 (Fig. 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 SAI low-threshold mechanoreceptors. Both ectopic units demonstrated a significant coupling response, represented as a modulation of the upper limb muscle activity, whereas the hair receptor did not (Fig. 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 (see Fig. 6 and Earth-referenced pole), increasing the total to 19 of 41 units with a significant coupling (46%).

Receptor characteristics and distribution. The average receptive field size and threshold for each of the four classes of receptor closely resemble those reported in current literature on cutaneous mechanoreceptors in the foot (Table 1). To assess differences relative to the literature, data were compared using $\chi^2$ analyses. Receptive field sizes 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 those for foot dorsum receptors in a recent study reported by Aimonetti et al. (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 that of 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 SAI afferents (with Aimonetti et al. 2007) may result from the location of the recorded afferent receptive field, 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 quad-
rants (Fig. 2). Of the 32 units on the dorsum of the foot, 22.5 were found on the medial aspect and 7.5 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. Whereas 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 was found to differ across the foot dorsum, with significantly greater coupling shown in the two medial quadrants ($P < 0.01$). In addition, the receptive field size was significantly different between receptors, demonstrating a coupling with upper limb motoneurons (smaller) and those that 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 obtained 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 1 unit in which a reflex was demonstrated only after the subject held onto a vertical reference pole (see Earth-referenced pole). 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,
earlier cycle of activation of the FAI unit (resulting in a reflex time of 0 ms relative to the onset latency of the muscle response was calculated as 11 ms) and Table 2). This single FAI afferent was located along the metatarsal head. Its receptive field was small (2 cm x 3 cm) and is more likely representative of a response to an additional approach (only used for 4 additional units, see MATERIALS AND METHODS). Figure 3 illustrates one FAI afferent that evoked cyclic activation in both PD and Tri EMG following high-frequency activation across its receptive field (Fig. 3 and Table 2). This single FAI afferent was located along the dorsal/medial border of the foot slightly proximal to the first metatarsal head. Its receptive field was small (2 cm x 3 cm) and its threshold was 0.085 g, 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 ~10-Hz activation of the afferent over its receptive field (Fig. 3). As a result of the cyclic nature of this unit, the onset latency of the muscle response was calculated as 11 ms relative to time 0. Clearly, this muscle onset latency is too early to be associated with the spike-trigger-averaged units at time 0 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 110 ms). 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 that generated the periodic bursts at a frequency outside of the latency of interest for reflex coupling (periodicity >300 ms, reflex coupling <120 ms). 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 with the use of conventional approaches (only used for 4 additional units, see MATERIALS AND 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 receptive field. A strong correlation was found ($R^2 = 0.997$) between the mean interspike 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 asynchro-
Table 2. Summary details of cutaneous afferents showing significant coupling

<table>
<thead>
<tr>
<th>Muscle</th>
<th>Nerve</th>
<th>Unit</th>
<th>Polarity</th>
<th>Latency, ms</th>
<th>Amplitude, % background</th>
</tr>
</thead>
<tbody>
<tr>
<td>FAI</td>
<td>Deltoid</td>
<td>CP 2</td>
<td>Inhibitory</td>
<td>72.3</td>
<td>3.5</td>
</tr>
<tr>
<td></td>
<td>CP 5</td>
<td>Cyclic</td>
<td>11</td>
<td>2.86</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CP 9</td>
<td>Inhibitory</td>
<td>120</td>
<td>2.59</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CP 11</td>
<td>Inhibitory</td>
<td>62</td>
<td>1.12</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tibial</td>
<td>37 Inhibitory</td>
<td>105</td>
<td>3.66</td>
<td></td>
</tr>
<tr>
<td>Triceps</td>
<td>CP 5</td>
<td>Cyclic</td>
<td>96</td>
<td>1.68</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CP 8</td>
<td>Excitatory</td>
<td>120</td>
<td>1.21</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CP 10</td>
<td>Excitatory</td>
<td>54</td>
<td>1.43</td>
<td></td>
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<tr>
<td></td>
<td>CP 13</td>
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<td>46</td>
<td>1.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CP 16</td>
<td>Excitatory</td>
<td>38</td>
<td>3.48</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tibial</td>
<td>40 Inhibitory</td>
<td>55</td>
<td>1.99</td>
<td></td>
</tr>
<tr>
<td>SAI</td>
<td>Deltoid</td>
<td>CP 28</td>
<td>Excitatory</td>
<td>71</td>
<td>0.97</td>
</tr>
<tr>
<td></td>
<td>CP 7</td>
<td>Inhibitory</td>
<td>48</td>
<td>3.16</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CP 22</td>
<td>Cyclic</td>
<td>35</td>
<td>1.85</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CP 25</td>
<td>Inhibitory</td>
<td>102</td>
<td>1.18</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tibial</td>
<td>39 Inhibitory</td>
<td>61</td>
<td>7.65 (pole)</td>
<td></td>
</tr>
<tr>
<td>Triceps</td>
<td>CP 7</td>
<td>Inhibitory</td>
<td>56</td>
<td>2.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tibial</td>
<td>39 Inhibitory</td>
<td>80</td>
<td>6.38 (pole)</td>
<td></td>
</tr>
<tr>
<td>SAI</td>
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<td></td>
<td>CP 35</td>
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<td>30</td>
<td>1.21</td>
<td></td>
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<tr>
<td></td>
<td>CP 35</td>
<td>Cyclic</td>
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<td>1.87</td>
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<tr>
<td>Ectopic</td>
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<tr>
<td></td>
<td>CP 21</td>
<td>Cyclic</td>
<td>65</td>
<td>2.99</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CP 20</td>
<td>Cyclic</td>
<td>121</td>
<td>1.73</td>
<td></td>
</tr>
<tr>
<td>Triceps</td>
<td>CP 21</td>
<td>Cyclic</td>
<td>70</td>
<td>3.2</td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td></td>
<td></td>
<td>59 (61)</td>
<td>2.29 (2.59)</td>
<td></td>
</tr>
</tbody>
</table>

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 percentage of background EMG) are given for all units. Median latency is presented for noncyclical units. One unit (unit 39) showed a significant coupling only when the participant held onto an earth-referenced vertical pole (medians given in parentheses for latency and amplitude include values for this unit). CP, common peroneal (fibular) nerve.

nous, firing pattern relative to other skin receptors located in the immediate anatomic region. SAI and SAI receptors demonstrate unique firing to constant indentation, and SAI 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 SAI receptor located slightly proximal to the ankle that was activated by stretch along the ankle dorsum in a proximal direction (inset). This single afferent demonstrated significant modulation of ongoing deltoid activity roughly every 60 ms, which correlates well with the regular firing of the SAI afferent (16.7 Hz).

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 (100 ms) was not statistically different between the two trials (isometric contraction vs. muscle activation to grasp the pole; \( P = 0.13 \)). Both units (FAII and SAI) demonstrated significant coupling to motoneurons in the upper limb muscles during trials with the earth-referenced pole. For the SAI unit, grasping the earth-referenced pole resulted in a significant inhibition of ongoing muscle activity in both deltoid (61-ms onset, 7.65% amplitude) and triceps (88-ms onset, 6.38% amplitude) (Fig. 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 (0.4 g) were both low relative to the recorded population. This SAI 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 neighboring skin receptors (corroborated by the autocorrelogram). Also of note is that the number of sweeps during isometric contraction included 1,000 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 (155 ms, 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. Uniquely, compared with other studies that have examined individual cutaneous contributions to muscle modulation, we have identified the presence of interlimb reflex responses. Similarly 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 SAIIs (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 (Johansson et al. 1982; Macefield 1998). On the basis of 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 center 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 reports, McNulty and colleagues (1999, 2001) reported a distinct lack of response from SAI receptors in reflex coupling within the upper limb. Whereas FAIs can provide some input regarding shape (Philips et al.
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 (Goodwin et al. 1995; Johansson and Vallbo 1980; Johansson et al. 1982; LaMotte and Srinivasan 1996) and respond with high resolution to changes in pressure (Mountcastle 1966). Input from SAI receptors regarding shape and indentation may not feature prominently for reflex responses when objects are being grasped (McNulty et al. 1999); however, such input may be critical for facilitating appropriate cautionary strategies during locomotion. The ability of SAI receptors 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.

SAII receptors. Previous reports in the hand have indicated that SAII afferents respond to movement of the digits (Burke et al. 1988) as well as skin stretch around joints (Knibestöl and Vallbo 1970), suggesting a role in kinesthesia. Aimonetti et al. (2007) have also demonstrated pronounced firing of SAII afferents in the skin of the ankle following movement of the foot. SAII receptors in the current study were the group of receptors to show the least amount of coupling (20%). It is feasible that SAII 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, within both the upper limb (McNulty et al. 1999; McNulty and Macefield 2001) and lower limb (Fallon et al. 2005) have shown a strong coupling response of SAII receptors with their own innervation territory (52% coupling within the upper limb, 40% coupling within the lower limb). Therefore, as a proprioceptor, SAII input can provide information regarding local skin stretch and is therefore ideal to modulate joint position for successful grasp or for 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; McNulty and Macefield 2001). Our amplitude calculation was based on peak responses, and therefore we halved the amplitude responses reported previously by McNulty and Macefield (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 et al. 2005; and 2.05%, McNulty and Macefield 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 et al. (2001) were the first group to report significant inter- and intralimb reflexes following whole nerve stimulation of SP. Our current observations of prominent interlimb reflex coupling from single cutaneous afferents adds further support...
to the premise 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. Although 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 subharmonics 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 38 to 120 ms, with a median of 59 ms (mean: 71 ms). Interestingly, no differences were found in onset latency in our upper limb responses compared with single afferent reflexes within the lower limb (Fallon et al. 2005), where a median onset latency of 57.4 ms 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, reportedly from 20 to 60 m/s (Mackel 1988), 26 to 91 m/s, (Knibestöl 1973), and 52 to 89 m/s (Macefield et al. 1989). Based on our shortest noncyclical response (38 ms) and a travel distance of roughly 1 m (CP at knee, to shoulder), the conduction velocity of our fastest afferent is estimated at 30 m/s, falling within the reported velocities for cutaneous afferents. Whole nerve SP or foot skin stimulation to date has
reported early, 45- to 50-ms (Pieseiur-Strehlow and Meinck 1980; Zehr et al. 2001) or middle, 75-ms (Zehr et al. 2001) or 91- to 92-ms (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 –95 ms) 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 suggests they may fall into several categories, with evidence of both propriospinal pathways (9 SL responses; 38 – 62 ms) and coupling that may involve subcortical or cortical loops (8 ML responses; 71–120 ms). Cutaneous reflexes reported by Pruszynski et al. (2008) indicated the fastest voluntary activity in muscles across the elbow to begin at 120 ms, 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 88 ms following whole body perturbations (McIlroy and Maki 1995) and 143–150 ms following slips (Marigold et al. 2003). With our median latency of 59 ms and maximum latencies of 120 ms, 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 FAI, 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 (Edin and Johansson 1995; Johanson 2001), 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. In the current study we found a large distribution of FAIs in the foot dorsum hairy skin (15 FAIs, 37% of all collected) compared with 5% in knee and thigh (Edin 2001) and 0% in the forearm (Vallbo et al. 1995). In contrast, our distribution was
very similar to those reported in the glabrous skin (Fallon et al. 2005; Kennedy and Inglis 2001), where the largest population of afferents innervated FAI end organs. Aimonetti et al. (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 et al. found the greatest density of FAIs (9/25, 36%), which corresponds well with findings in 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, they 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, since for the most part experiments are performed by subjects 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 subjects were 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 (25), 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, SAII). Although 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. Modula-
tion 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. 1999; McNulty and Macefield 2001) 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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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

L.R.B. conception and design of research; L.R.B. and C.R.L. performed experiments; L.R.B. and C.R.L. analyzed data; L.R.B. and C.R.L. interpreted results of experiments; L.R.B. prepared figures; L.R.B. drafted manuscript; L.R.B. and C.R.L. edited and revised manuscript; L.R.B. and C.R.L. approved final version of manuscript.

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