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Evidence for strong synaptic coupling between single tactile afferents from the sole of the foot and motoneurones supplying leg muscles

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Reflex responses from tactile afferents

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Abstract

It has been known for some time that populations of cutaneous and muscle afferents can provide short latency facilitation of motoneurone pools. Recently, it has been shown that the input from individual low-threshold mechanoreceptors in the glabrous skin of the hand can modulate ongoing activity in muscles acting on the fingers via spinally mediated pathways. We have extended this work to examine whether such strong synaptic coupling exists between tactile afferents in the sole of the foot and motoneurones supplying muscles that act about the ankle. We recorded from 53 low threshold mechanoreceptors in the glabrous skin of the foot via microelectrodes inserted percutaneously into the tibial nerve of awake human subjects. Reflex modulation of ongoing whole muscle EMG was observed for each of the four classes of low-threshold cutaneous mechanoreceptors (17 of 21 FA I; 2 of 4 FA II; 7 of 18 SA I; and 4 of 10 SAII). Reflex modulation of the firing probability in single motor units (5 of 11) was also observed. These results indicate that strong synaptic coupling between tactile afferents and spinal motoneurones is not a specialization of the hand, and emphasizes the potential importance of cutaneous inputs from the sole of the foot in the control of gait and posture.
Introduction

Information from low-threshold mechanoreceptors in muscle and skin is important in the control of the foot in man, with reflexes involving both muscle and cutaneous afferents having been described in the lower limb (for review see Brooke et al. 1997). Yet, while it is well known that synchronous activation of a population of muscle afferents can excite homonymous spinal motoneurones at monosynaptic latencies, and that the asynchronous discharge of a population of muscle spindles facilitates the firing rates of homonymous spinal motoneurone at all levels of volitional drive (Macefield et al. 1993), it is also known that the input from an individual muscle spindle afferent is not sufficiently strong to modulate the ongoing voluntary EMG of leg muscles (Gandevia et al. 1986).

Using the same approach our laboratory has recently shown that in the hand, as in the leg, the synaptic strength of individual muscle spindle afferents is so weak that it cannot be identified using spike-triggered averaging of EMG (McNulty and Macefield 2001). However, we have also shown that individual low-threshold mechanoreceptors in the glabrous skin of the hand can cause reflex modulation of EMG of the muscles acting on the digits (McNulty and Macefield 2001; McNulty et al. 1999). Less than half of all the cutaneous mechanoreceptors exhibited any reflex coupling, with reflex coupling limited to the rapidly adapting type I (FA I), rapidly adapting type II (FA II) and slowly adapting type II (SA II) mechanoreceptors. Interestingly, no slowly adapting type I (SA I) mechanoreceptors exhibited any reflex coupling. It is possible that the capacity of individual cutaneous mechanoreceptors to modulate the firing of spinal motoneurones is unique to the hand, due to the fine motor control for which the hand is known, and the proposed highly interconnected sensorimotor elements (Wiesendanger 1999). Alternatively, it may be that individual cutaneous mechanoreceptors, including those in the hand, have strong reflex coupling to the motoneurone pools of muscles acting on their innervation territory.
In the present study we address this issue of specificity by investigating the reflex coupling between individual low-threshold mechanoreceptors in the glabrous skin of the foot and spinal motoneurones supplying muscles acting about the ankle. Electrical stimulation of populations of cutaneous afferents innervating the sole and toes has been shown to exert both excitatory and inhibitory reflex effects on on-going voluntary contractions of muscles acting about the ankle (Aniss et al. 1992; Gibbs et al. 1995); reflex modulation has also been observed in remote muscles (Gibbs et al. 1995; Zehr et al. 2001). However, these reflex effects are not immutable - they can be modulated by the gait cycle (De Serres et al. 1995; Duysens et al. 1990; Haridas and Zehr 2003; Yang and Stein 1990) and postural task (Burke et al. 1991). The pathways involved in the reflex coupling between cutaneous mechanoreceptors and muscles of the lower limb are proposed to include a range of pathways, from oligosynaptic spinal pathways (Aniss et al. 1992) to transcortical pathways (Christensen et al. 1999; Nielsen et al. 1997). By studying the reflex coupling between single tactile afferents from the foot and the spinal motoneurones supplying the leg, we hope to elucidate the role of different types of cutaneous mechanoreceptors in the reflex control of the lower leg muscles. Preliminary results from this study have appeared in abstract form (Fallon et al. 2004).

**Materials and methods**

**Subjects**

Thirty-one recording sessions were performed on 18 healthy volunteers (13 females and 5 males) aged 19 to 36 years. None of the subjects had any known neurological or motor disorders and all subjects gave written informed consent. The Committee on Experimental Procedures Involving Human Subjects, University of New South Wales, gave ethics approval to all procedures, which were carried out in accordance with the principles of the Declaration of Helsinki.
Experimental set-up

Subjects lay prone on an adjustable chair, with both legs extended and supported on a piece of high density foam at the ankle. In this position the knee was at an angle of approximately 15° flexion and the ankle at 100° extension. The experimental limb was then secured using a Velcro strap around the leg, approximately 50 mm proximal to the Achilles tendon. Surface EMG recordings were collected using disposable 10 mm² Ag/AgCl electrodes placed over the bellies of tibialis anterior (TA), lateral gastrocnemius (LG), medial gastrocnemius (MG) and soleus. The EMG activity was amplified and filtered (bandwidth 10 Hz – 1 kHz, 50 Hz notch) before being digitized at 2 kHz and stored for subsequent analysis (Powerlab and Chart, ADInstruments, Australia).

Transdermal electrical stimulation (0.2 ms, 0 – 20 mA, 1 Hz: DS3, Digitimer, UK) was used to locate the approximate position of the tibial nerve within the popliteal fossa. Stimulation of the tibial nerve was evident by twitch responses of the triceps surae muscle group and paraesthesiae in the glabrous skin of the foot. The insertion site was chosen as the location just proximal to the knee capsule that could elicit the greatest sensation and/or twitch at the lowest current. A low impedance reference electrode was placed under the skin, roughly 50 mm medial to the insertion site of the recording microelectrode. An insulated tungsten microelectrode (200µm diameter, 55 mm length, Frederick Haer Inc., ME, USA) was then inserted through the skin. Stimulation through the microelectrode via an optically-isolated constant current source (0.2 ms, 0 – 1.3 mA, 1 Hz: ML180, ADInstruments, Australia) was used to guide penetration of the neural sheath. Further fine manipulation of the microelectrode to isolate single cutaneous afferents was done using auditory feedback of the neural activity (gain 10⁴, bandwidth 300 Hz – 5 kHz: ISO-80, World Precision Instruments, USA) while providing mechanical stimuli to the foot. Neural activity was then digitized at 20 kHz and stored for subsequent analysis (Powerlab and Chart, ADInstruments, Australia).
Experimental procedure

Individual afferents were categorized as innervating fast-adapting (FA I or FA II) or slowly-adapting (SA I or SA II) mechanoreceptors using criteria previously described (Johansson 1978; Kennedy and Inglis 2002; Vallbo and Johansson 1984). The mechanical threshold for each afferent was measured using calibrated von Frey hairs (Simmes-Weinstein Esthesiometers, Stoelting, US). The receptive field of each mechanoreceptor was mechanically stimulated (rapid stroking across the receptive field for FA I, blowing across the receptive field for two of the four FA II and rapid stroking across the receptive field for the remaining two FA II, maintained indentation of a ‘hot spot’ for twelve of the eighteen SA I and rapid stroking across the receptive for the remaining six SA I and lateral skin stretch for SA II) while the subject performed a weak voluntary contraction (< 10% MVC). The rapid stroking across receptive fields was manually performed by the experimenter, using a blunt probe with a tip diameter of approximately 2 mm, and therefore varied in frequency, was typically with the range of 5 – 10 Hz. The subjects were instructed to gently plantar flex against the experimenter’s hand, which was held across the metatarsophalangeal (MTP) joints, and therefore the contractions were essentially isometric. Although the instructions were only to plantar flex, the majority of subjects also inadvertently cocontracted TA. Subjects were given verbal feedback to ensure the level of contraction remained constant, as assessed by the level of MG EMG. Each contraction was held for approximately 5 minutes while the cutaneous afferent was continuously activated, yielding on average 5,000 trigger spikes (range: 700 – 17,000) from each afferent. Subjects reported no difficulty in maintaining the weak voluntary contraction, nor did they report any feelings of fatigue.

Data analysis

The spike morphology of the recorded neural activity was analyzed using Spike 2 (Cambridge Electronics Design, UK) to confirm the unitary nature of each afferent recording. The EMG was processed by calculating a 5 ms sliding window root-mean-square (RMS) using custom written
software (Igor Pro 5, Wavemetrics, USA). The RMS EMG was then spike-trigger averaged to the neural activity to reveal any reflex coupling between the afferent discharge and the ongoing EMG activity. The criterion for identifying a reflex coupling response was a change in the ongoing EMG activity that projected beyond the 99.5% confidence interval (equivalent to the mean ± 3 standard deviations) for a minimum of 2 ms, giving a combined probability of detection of greater than 98% confidence. As cyclic modulation of ongoing EMG may occur (see McNulty and Macefield 2001; McNulty et al. 1999) a pseudo-random spike train was generated from the recorded afferent discharge and was subsequently used to create a second, ‘random’, spike-trigger averaged EMG response. The pseudo-random spike train was generated by randomly shifting the afferent spike by between –100 ms and 400 ms, equivalent to the period over which the spike-triggered averaging was calculated. The mean and standard deviation of this ‘random’ response was then used to construct the 99.5% confidence interval (see Figure 3B). The latency of the response was measured as the time to the 99.5% confidence interval crossing point, the earliest time at which a response was deemed to have occurred. The latency to the peak of the response was also measured to aid comparison with our previous results from the hand (see Discussion). Reflex couplings were classified as ‘short’ or ‘long’ latency based on the latency of the reflex response. ‘Short’ latency reflex coupling was defined as one that occurred at an appropriate latency (25 – 120 ms, see Reflex latency and reflex classification section in Discussion for rationale). Any statistically significant modulations of the ongoing EMG with a latency of more than 120 ms were classed as ‘long latency’ reflex couplings (see Results), and were not studied in detail. The amplitude of reflex responses were expressed as the peak percentage change in the on-going RMS EMG.

On occasion, single motor unit (SMU) activity could be identified from the surface EMG recordings; the unitary nature of the SMU was confirmed by examining the motor potential morphology. Confirmed SMUs were subsequently analyzed for reflex coupling following procedures similar to those used by Aniss et al. (1992); cross-correlograms of the afferent discharge
versus the SMU activity and the ‘random’ spike train versus the SMU activity were constructed. The ‘random’ cross-correlogram was then used to estimate a mean background level to subtract from the cross-correlogram when generating a cumulative sum (CUSUM). The criterion for identifying a reflex coupling response was that the CUSUM must project beyond the 95% confidence interval (calculated as 2 standard deviations of the ‘random’ CUSUM).

Statistics

χ² tests were used to examine any difference in the distribution of receptor types in the foot versus the hand, and proportions of receptor types that exhibited statistically significant EMG modulations versus those that did not. Differences in the properties of afferents that exhibited a reflex coupling, versus those that did not, were examined using t tests or Mann-Whitney U tests where appropriate. Comparisons of reflex latency and magnitude (expressed as peak modulation as a percentage of ongoing background EMG activity) were made using Kruskal-Wallis tests, with Bonferroni adjustments for post-hoc testing.

Results

Afferent sample

Fifty-three afferents innervating low threshold mechanoreceptors in the glabrous skin of the foot were recorded. The sample consisted of 21 (40%) FA I, 4 (7%) FA II, 18 (34%) SA I and 10 (19%) SA II afferents. The mechanical thresholds and approximate receptive field sizes for the afferents are summarized in Table 1. Additionally, one afferent innervating a high threshold (greater than the largest calibrated von Frey hair of 6.65), deep Ruffini-like ending mechanoreceptor in the mid-sole was recorded. The majority of cutaneous afferents innervated regions on the plantar surface of the foot (n = 47), with the remainder innervating the glabrous skin on the lateral (2 FA I and 1 SA I) or medial (2 SA I and 1 SA II) borders of the sole (see Figure 1).
Spike-triggered averaging revealed that 57 % of all afferents exhibited some form of reflex coupling. All four types of low threshold mechanoreceptors exhibited reflex coupling, with activation of 17 (81 %) FA I, 2 (50 %) FA II, 7 (39 %) SA I and 4 (40 %) SA II afferents resulting in statistically significant modulation of ongoing EMG activity (for details see Table 2). Stroking, in addition to blowing, was used to activate 2 of the 4 FA II afferents, to elicit a greater discharge than can normally be achieved by blowing over the receptive field. Similarly, 6 of the 18 SA I afferents received a combination of both stroking and maintained indentation, owing to their rapid adaptation to the maintained indentation. In neither case was there any evidence of a cyclic modulation of the autocorrelogram, which would have been expected to result in a cyclic modulation of the EMG, or a difference in the responses of the units that received the mixed activation compared to those that received either pure blowing or maintained indentation. As illustrated in Figure 1 no differences were apparent in the distributions of the receptive fields of mechanoreceptors that exhibited a reflex coupling compared with those that did not. There was also no significant difference in mechanical threshold (p = 0.11), receptive field size (p = 0.59) or the number of afferent spikes recorded (p = 0.24) between the two groups of afferents.

**Reflex responses**

The most commonly observed reflex coupling was between FA I mechanoreceptors in the glabrous skin of the foot and the muscles acting about the ankle. The FA I afferent illustrated in Figure 2 was located in the skin overlying the MTP joint of digit V, and had a receptive field of 86 mm$^2$ and a threshold of 3.22, which were intermediate and low for FA I mechanoreceptors respectively. The unit was activated by rapid manual repetitive stroking across its receptive field at a frequency of approximately 9.5 Hz, resulting in bursts of 2 – 4 spikes every 110 ms, as evident in the instantaneous frequency, neurogram and autocorrelogram in Figure 2. The afferent firing resulted in significant modulation of the ongoing EMG in TA, LG and soleus. While there was clear modulation of the ongoing EMG in MG, it did not reach our strict criterion for significance (i.e. the
modulation did not exceed the 99.5 % confidence levels for more than 2 ms). The EMG modulation in all muscles was cyclic, with the peak modulation being 2.4 %, 2.4 % and 1.2 % of the ongoing EMG for TA, LG and soleus respectively. The period of the cyclical modulation of the EMG corresponded to the inter-burst period of the afferent firing. Interestingly, the responses from the plantar flexors were all in-phase with the response in TA. As the response was cyclical it is not possible to determine the true latency of the response nor the true nature of the response (whether excitatory, inhibitory or both), however the latency to the first excitatory response (latency of the response exceeding the 99.5 % confidence level) after the afferent spike was 48 ms, 63 ms and 54 ms for TA, LG and soleus respectively.

It is possible that the reflex response illustrated in Figure 2 was the result of a population of mechanoreceptors all firing in a similar pattern, as many afferents would have been activated by the mechanical stimulus (see Discussion for further detail). However, mechanical stimulation of an SA I mechanoreceptor, via maintained indentation, results in asynchronous firing of adjacent activated units (although the same may not be true when stroking the receptive field): spike-triggered averaging of the unit illustrated in Figure 3 resulted in significant modulation of the ongoing EMG in TA, LG, MG and soleus. This afferent was located in the glabrous skin on the distal phalanx of digit II, and had a receptive field of 63 mm$^2$ and a threshold of 3.84, which were both characterized as intermediate for SA I mechanoreceptors. Sustained indentation within its receptive field produced complex reflexes in TA, LG, MG and soleus. In TA a ‘short’ latency decrease (3.0 % of the ongoing EMG) at 66 ms was followed by an increase (3.0 % of the ongoing EMG) at 119 ms. In LG a ‘short’ latency increase (3.2 % of the ongoing EMG) at 29 ms was followed by a decrease (3.9 % of the ongoing EMG) at 76 ms, and in MG a ‘short’ latency increase (1.9 %) at 33 ms was followed by a decrease (3.2 % of the ongoing EMG) at 71 ms. In soleus a ‘short’ latency increase (1.9 % of the ongoing EMG) at 39 ms was followed by an decrease (2.0 % of the ongoing EMG) at 87 ms.
Reflex amplitudes and latencies

A summary of the numbers of both ‘short’ and ‘long’ latency reflexes is given in Table 2. Reflex couplings between individual cutaneous mechanoreceptors and muscles acting about the ankle are summarized in Table 3 & Table 4. The histogram of the reflex latencies (Figure 4) suggests that the distribution of reflex latencies may be multi-modal, with possible peaks around latencies of 30 and 70 ms. There was no significant difference in the reflex latencies for different mechanoreceptor types (p = 0.13) or muscles (p = 0.63). There was also no significant difference in the magnitude of the reflexes for any of the muscles (p = 0.19). However, mechanical stimulation of rapidly adapting mechanoreceptors (pooled FA I and FA II results) resulted in significantly smaller reflexes than stimulation of slowly adapting mechanoreceptors (pooled SA I and SA II results; p ≤ 0.001). There was also a correlation between the periodicity observed in the reflex EMG response and the afferent interspike interval for all periodically activated mechanoreceptors (Figure 5).

Single motor units

The activity of 11 SMUs (3 LG, 7 MG and 1 soleus) could be identified from the surface EMG recordings, which were associated with 7 FA I and 4 SA I afferents. Analysis of the 11 pairs of single tactile afferents and SMUs revealed that 6 (46 %) of the pairs exhibited a reflex increase in the probability of the SMU firing (for details see Table 5). Figure 6 illustrates the reflex coupling between an SA I mechanoreceptor in the glabrous skin on the lateral aspect of the foot and a SMU in MG. The SA I mechanoreceptor had a receptive field of 20 mm² and a threshold of 4.08, which were small and intermediate respectively. Repeated maintained indentations of the mechanoreceptor’s receptive field resulted in periods of ongoing afferent discharge (lasting for several seconds) and an autocorrelogram with a single peak, indicative of asynchronous firing. The weak voluntary contraction resulted in the SMU firing at approximately 6 Hz throughout the duration of the contraction. The cross-correlogram and CUSUM illustrate that there was a
significant excitatory reflex coupling, with a latency of 51.4 ms, between the SA I mechanoreceptor and the SMU in MG.

Discussion

For the first time, the activity of individual low-threshold mechanoreceptors in the glabrous skin of the foot has been shown to cause a potent reflex modulation of ongoing EMG activity in muscles that act about the ankle. In contrast to the hand, all classes of tactile afferent (including SA I) were observed to exhibit some form of reflex coupling. Modulation of the timing of the firing of volitionally activated single motor units was also observed.

Afferent sample

In the only other report of the properties and distribution of low threshold mechanoreceptors in the sole of the foot, Kennedy & Inglis (2002) recently reported that the relative proportions of the four classes of receptor did not appear to be significantly different to those in the hand described by Johansson & Vallbo (1979). It has also been reported that there are no significant differences in the relative proportions of the different types of receptors in the area of glabrous skin innervated by the sural nerve, compared to the hand (Trulsson 2001). The proportions of different receptor types in our sample are in agreement with these reports, and were not significantly difference from the hand (p = 0.12). The distribution and size of the receptive fields, along with activation thresholds, of the different types of receptors examined in the present study were also similar to those reported by Kennedy & Inglis (2002). The most notable features of the afferent supply of the sole is the paucity of receptors on the arch of the foot, and the absence of any spontaneous discharge of the SA II endings in the absence of external mechanical stimulation of the foot.

Reflex latency and reflex classification

The distribution of reflex latencies found using whole nerve stimulation of the tibial and/or sural nerves at the level of the ankle is often divided into ‘early’ (less than 70 ms), ‘mid’ (70-120 ms) and
‘late’ (longer than 120 ms) responses (Aniss et al. 1992; Brooke et al. 1997; Burke et al. 1991; Gibbs et al. 1995; Kukulka 1994), with the earliest reflex responses seen after whole nerve stimulation at the ankle being in the order of 30 ms (Kukulka 1994). As our recordings of afferent activity were made at the level of the popliteal fossa (on average 0.25 m above the ankle), and the conduction velocity of large myelinated afferents in the tibial nerve is in the order of 50 ms\(^{-1}\) (Macefield et al. 1989), we would expect reflex latencies to be approximately 5 ms shorter than those reported in studies involving whole nerve stimulation at level of the ankle. Thus, the latency of the earliest reflex responses should be in the order of 25 ms.

Kukulka (1994) reported that the minimum reaction time latency, for voluntary modulation of the activation of soleus in response to electrical stimulation at the ankle, was in the order of 150 ms after training, although the latency was longer in naïve subjects. This raises the possibility that ‘late’ reflexes, with latencies greater than approximately 150 ms, may have been under volitional control. However, given the range of conduction velocities reported in the literature for single cutaneous afferent fibers, we cannot discount the possibility that latencies >150 ms could be associated with the combination of the slowest conducting cutaneous and motor fibers operating via a spinal reflex loop, particularly in tall subjects with a longer pathway. In fact, long-latency reflexes (130-450 ms) have been observed after sural nerve stimulation in patients with clinically complete spinal cord transection (Roby-Brami and Bussel 1987). Additionally, our experimental protocol of instructing subjects to maintain a constant level of contraction, and the use of spike-triggered averaging of RMS EMG using several thousand afferent spike triggers, would significantly reduce any inadvertent changes in the level of voluntary contraction performed by the subject. Therefore, although we are confident that any statistically significant modulations of ongoing EMG that we observed were most probably reflex in nature, any reflexes that occurred after 120 ms were classified as ‘long-latency’ reflexes and were not studied in detail.
Accordingly, we concentrated our analysis on reflexes with latencies in the ‘early’ to ‘mid’ range (i.e. those that occurred between 25 and 120 ms). This also down-played the effects of cyclic responses; because no periodically activated mechanoreceptors were activated at a frequency of more than 10 Hz, any cyclic effects would not be evident within our 95 ms window of interest (i.e. between 25 and 120 ms). ‘Early’ reflex responses, evoked via whole nerve stimulation, must be due to oligosynaptic spinal pathways, as the shortest possible latency for a transcortical reflex is in the order of 70 ms (Nielsen et al. 1997). Based on latency alone, whole nerve ‘mid’ latency responses could travel by oligosynaptic spinal, propriospinal or supraspinal pathways, and there is evidence that at least some of the ‘mid’ latency reflexes are “at least partly, mediated by a transcortical pathway (Nielsen et al. 1997)” While Figure 4 suggests a grouping of the present reflexes into ‘early’ and ‘mid’ latency components, there was no statistically significant difference in reflex magnitude (p = 0.46) between ‘early’ (25-70ms) and ‘mid’ (70-120ms) latency reflexes. The broad range of conduction velocities of individual cutaneous afferents, (20-91 m s⁻¹ in the upper limb Kakuda 1992; Knibestol 1973; Macefield et al. 1989; Mackel 1988; Vallbo 1971), further complicates any separation of individual reflexes based on latency differences alone. As our experimental techniques also did not allow us to determine whether transcortical pathways mediated any of the reflex responses, we had no firm basis on which to separate the ‘short’ latency reflex responses into ‘early’ or ‘mid’ latency reflexes.

We primarily reported latency measured as the time to our 99.5% confidence level threshold crossing following the recorded spike, as this was the earliest possible time that a response was deemed to have occurred. This commonly used method of measuring the latency of a response to a threshold crossing inevitably depends on exactly how the threshold has been defined, and will tend to emphasise the latency of the fastest possible pathways in reflex responses. We therefore also reported the latencies using another common method, which is to measure the latency to the peak of the reflex response. Measuring the latency with this method highlights the modal latency of the
reflex pathways, and has been shown to be a useful measure in the reflex responses to single cutaneous afferents in the hand (McNulty & Macefield, 2001). Both reflex measures, however, will be affected by how broad the reflex response is, particularly if afferents are activated in bursting patterns corresponding to stimulus movement across their receptive fields, and will inevitably tend to over estimate the minimum possible reflex latency.

**Individual or population response?**

The most commonly observed reflex couplings (55% of all reflexes) were expressed by the FA I mechanoreceptors. These afferents were mechanically stimulated by stroking across their receptive fields, which resulted in periodic bursts of afferent activity, and subsequent cyclic reflex modulation, at the frequency of the periodic activation (see Figure 2 and Figure 5). Apart from activating the FA I of interest, the mechanical stimulation would also have activated other FA I mechanoreceptors, as well as other types of low-threshold receptors, with overlapping receptive fields. Spike-triggered averaging reduces the effects of other afferents that are not firing synchronously with the afferent being recorded, however we cannot exclude the possibility that other afferents may have contributed to the reflex response. Given that other FA I afferents are likely to be firing in periodic bursts similar to those of the recorded afferent, the proportion of individual FA I mechanoreceptors exhibiting tight coupling may be an overestimate.

This line of reasoning is also pertinent for the 2 FA II and 6 SA I afferents activated by stroking across their receptive field; as this type of mechanical stimulation will activate multiple cutaneous receptors. Therefore, there is a high probability that the coincident discharge of additional receptors (particularly FA Is) will occur in close temporal proximity to the trigger unit, contributing to both the amplitude and morphology of the averaged EMG. However, the mechanical stimuli used to activate the majority of FA II (blowing), SA I (maintained indentation) and SA II (skin stretch) mechanoreceptors result in asynchronous afferent activity, as can be seen in the autocorrelogram of afferent activity (see Figure 3). Spike-triggered averaging from an asynchronous afferent discharge
reduces the possibility that other afferents contribute to the reflex response where there is a well defined peak projecting clearly beyond the background EMG activity, because the probability of another afferent consistently firing at the same instant as the recorded afferent is low. Therefore, we believe that the probability of the reflex modulation being due to the input from a single tactile afferent is higher for those units firing asynchronously, than for those (primarily FA I afferents) activated by stroking and exhibiting a cyclic autocorrelogram.

We can therefore conclude that reflex modulation of voluntary contractions of muscles acting about the ankle can be produced by the activation of individual low threshold mechanoreceptors, and that the input from many afferents is not required. We can also conclude that temporal summation of an afferent volley, as could occur following a burst of impulses in, for example, an FA I afferent is not required as single afferent spikes can modulate the ongoing EMG.

**Reflex responses**

The median magnitude of the reflex responses for all muscles was 2.3 % of the ongoing RMS EMG. Not surprisingly, given the much smaller afferent volley in this study, the response is markedly smaller than the reported magnitudes for whole nerve stimulation, which range from the order of 10 % (Gibbs et al. 1995) to 100 % (Aniss et al. 1992) of the ongoing EMG. However, the variety of reflexes observed from individual mechanoreceptors were similar to those observed with whole nerve stimulation. Interestingly, there were no significant differences in the reflexes observed for muscles, which is at odds with the report of Aniss *et al.* (1992), but in agreement with Gibbs *et al.* (1995) and the report of single motor unit reflexes of Kukulka (1994). However, given the relatively small number reflexes measured for each afferent type – muscle combination, it is possible that difference do exists that we simply did not observe.

Perhaps surprisingly, the magnitudes of the reflex responses to activation of individual mechanoreceptors from the foot were not statistically different (p = 0.70) from those in the hand (McNulty and Macefield 2001), the only other study to report the magnitude of the reflex effects of
individual, not populations of, mechanoreceptors. (The magnitude of the responses in the hand were reported as peak-to-peak modulations of predominantly cyclic responses (McNulty and Macefield 2001), and we therefore halved the reported magnitudes to allow comparison with the peak modulations reported here.) In fact, the median magnitude of reflex responses from individual cutaneous mechanoreceptors in the hand was also 2.0 %; although the amplitude of the response associated with FA I afferents was larger in the hand. Given the demands of fine motor control imposed on the hand, and the resulting proposed highly interconnected sensorimotor elements (Wiesendanger 1999), compared to the grosser motor control imposed on the foot due to the demands of locomotion, it is interesting that the magnitudes of the reflexes are the same. It suggests that perhaps these reflexes are not an adaptation for the fine motor control of the human hand but are a general property of individual cutaneous mechanoreceptors in glabrous skin reflex coupling to the motoneurone pools of muscles acting on their innervation territory.

Contrasting the similarity of the reflex magnitudes, the latencies of the reflexes from the foot were significantly longer than those from the hand (p < 0.05; for both threshold and peak latency measures). While the longer conduction segments in the leg may have influenced the longer reflex latencies, the contribution of ‘mid’ latency reflex responses observed in the foot, which were not observed in the hand, cannot be excluded. However, perhaps the most obvious difference between the reflexes in the foot and those in the hand is the proportions and types of units that exhibited reflex responses. In the hand, the majority of reflex responses were from SA II mechanoreceptors, with no SA I mechanoreceptors exhibiting any reflex coupling. In the foot, the majority of reflex responses were from FA I mechanoreceptors, followed by SA I mechanoreceptors. This difference in reflex coupling may be associated with the different functional demands placed on the hand and the foot (see below).
Single motor units

The types of reflex modulation of SMUs observed was within the spectrum of those reported in the literature (Aniss et al. 1992; Kukulka 1994), although, like the whole muscle reflexes, the reflex modulations were markedly smaller in magnitude, as would be expected. Interestingly, it was occasionally possible to observe reflex modulation of SMU activity without statistically significant modulation of the whole muscle response (Figure 6). This suggests that not all SMUs received the same amount of drive from each individual cutaneous afferent. It also highlights the point that the reported proportion of each type of mechanoreceptor that exhibits reflex coupling is likely to represent the lower limit of the actual proportion of couplings.

Functional Implications

Reflex modulation of lower limb muscles by cutaneous afferents had been proposed by Sherrington (1910), and more recent work has highlighted the important role of such reflexes in gait (Zehr et al. 1997; Zehr et al. 1998) and posture (Day and Cole 2002) and emphasized that the reflexes are not simple ‘limb flexion’/withdrawal reflexes (Burke et al. 1991). In fact, efforts are already underway to attempt to augment cutaneous feedback in an effort to increase postural control (Priplata et al. 2003). To date, however, none of the studies involving cutaneous reflexes from the foot have identified which types of low-threshold mechanoreceptor subserve the reflexes.

While the glabrous skin of the foot shares the same types of low-threshold mechanoreceptors as the hand (Kennedy and Inglis 2002; Trulsson 2001), as noted above, the SA II afferents supplying the sole of the foot are unusual in having no background discharge. Presumably these afferents would respond to remote skin stretch when the foot is subjected to load, and the absence of spontaneous activity is related to the different skin mechanics in the foot versus the hand. Interestingly, in the hand, it is the SA II mechanoreceptors that exhibited the most robust reflex coupling (52 %, McNulty and Macefield 2001), followed by the FA I mechanoreceptors. However, in the foot it is the FA I, followed by the SA I, afferents that exhibit the most robust reflex coupling. This
difference in reflex coupling is possibly associated with the different functional demands placed on the hand and the foot. In the hand, the reflexes are associated with information related to the conformation of the hand (SA II mechanoreceptors) and contact with objects that are being grasped (FA I mechanoreceptors), and may aid in the manipulation of objects. In the foot, the reflexes are likely to be associated with information related to foot contact (FA I mechanoreceptors) and the maintained contact of the foot on a support (SA I mechanoreceptors), and may aid in signaling different phases of the gait cycle and the reflex control of posture. Zehr et al. (1997) reported that stimulation of the tibial nerve causes reflex facilitation of the dorsi-flexors of the ankle during early swing / stance-swing transition, but that the reflex changes to become facilitation of plantar-flexors during late swing. We have reported reflex facilitation of both plantar- and dorsi-flexors, at similar latencies to those of Zehr et al. (1997). It therefore appears that in the prone position, both the plantar- and dorsi-flexor reflex pathways are accessible.

**Conclusion**

We have shown that the input from each type of low-threshold mechanoreceptor in the glabrous skin of the foot causes reflex modulation of the ongoing activity of muscles that act about the ankle. This is in contrast to the hand, where SA I mechanoreceptors do not cause reflex modulation of ongoing contractions of the hand muscles. The reflex modulation caused by individual low threshold mechanoreceptors in the foot is in agreement with reports for whole nerve stimulation, and is likely to be important in the control of gait and posture.

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References


Table 1. Response properties of cutaneous mechanoreceptors in the glabrous skin of the foot

<table>
<thead>
<tr>
<th>Type</th>
<th>Number (% of total)</th>
<th>Threshold Median (mN)</th>
<th>Range</th>
<th>Receptive field size (mm²) Median</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>FA I</td>
<td>21 (40)</td>
<td>3.84 (6.9)</td>
<td>2.83 – 4.56</td>
<td>86.4</td>
<td>15.7 – 636.2</td>
</tr>
<tr>
<td>FA II</td>
<td>4 (7)</td>
<td>3.84 (6.9)</td>
<td>3.84 – 4.08</td>
<td>2434</td>
<td>549.8 – 5222.9</td>
</tr>
<tr>
<td>SA I</td>
<td>18 (34)</td>
<td>4.08 (12.0)</td>
<td>3.22 – 4.74</td>
<td>55.4</td>
<td>18.8 – 176.7</td>
</tr>
<tr>
<td>SA II</td>
<td>10 (19)</td>
<td>4.53 (33.5)</td>
<td>3.84 – 5.46</td>
<td>117.8</td>
<td>7.1 – 490.9</td>
</tr>
</tbody>
</table>

The number of each type of cutaneous mechanoreceptor, along with mechanical threshold, measured with calibrated von Frey hairs, and receptive field size properties.
Table 2. Summary of afferents exhibiting reflex responses

<table>
<thead>
<tr>
<th></th>
<th>Significant EMG</th>
<th>‘Short’ latency</th>
<th>‘Long’ latency</th>
<th>None</th>
</tr>
</thead>
<tbody>
<tr>
<td>Modulation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FA I</td>
<td>17</td>
<td>8</td>
<td>9</td>
<td>4</td>
</tr>
<tr>
<td>FA II</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>SA I</td>
<td>7</td>
<td>6</td>
<td>1</td>
<td>11</td>
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<tr>
<td>SA II</td>
<td>4</td>
<td>3</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>Total</td>
<td>30</td>
<td>8</td>
<td>13</td>
<td>23</td>
</tr>
</tbody>
</table>

The number of each type of cutaneous mechanoreceptor exhibiting each class of reflex.
Table 3. Summary of reflex magnitudes

<table>
<thead>
<tr>
<th></th>
<th>FA I</th>
<th>SA I</th>
<th>SA II</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>TA</td>
<td>1.8</td>
<td>2.5</td>
<td>3.1</td>
<td>2.3</td>
</tr>
<tr>
<td></td>
<td>(0.8-3.2)</td>
<td>(2.0-3.0)</td>
<td></td>
<td>(1.4-3.2)</td>
</tr>
<tr>
<td></td>
<td>n=4</td>
<td>n=3</td>
<td>n=1</td>
<td>n=8</td>
</tr>
<tr>
<td>LG</td>
<td>1.5</td>
<td>3.2</td>
<td>2.3</td>
<td>2.0</td>
</tr>
<tr>
<td></td>
<td>(1.3-2.6)</td>
<td>(2.9-3.9)</td>
<td></td>
<td>(1.3-3.9)</td>
</tr>
<tr>
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</tr>
<tr>
<td>MG</td>
<td>1.7</td>
<td>3.2</td>
<td>2.9</td>
<td>2.8</td>
</tr>
<tr>
<td></td>
<td>(1.5-2.6)</td>
<td>(1.9-6.7)</td>
<td></td>
<td>(1.5-6.7)</td>
</tr>
<tr>
<td></td>
<td>n=3</td>
<td>n=9</td>
<td>n=1</td>
<td>n=13</td>
</tr>
<tr>
<td>Soleus</td>
<td>2.3</td>
<td>1.7</td>
<td></td>
<td>2.0</td>
</tr>
<tr>
<td></td>
<td>(0.8-2.8)</td>
<td>(1.3-2.0)</td>
<td></td>
<td>(0.8-2.8)</td>
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<td></td>
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<td>n=2</td>
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<td>n=7</td>
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<td>Total</td>
<td>1.8</td>
<td>2.9</td>
<td>2.9</td>
<td>2.3</td>
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<tr>
<td></td>
<td>(0.8-3.2)</td>
<td>(1.3-6.7)</td>
<td>(2.3-3.1)</td>
<td>(0.8-6.7)</td>
</tr>
<tr>
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<td>n=18</td>
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<td>n=3</td>
<td>n=38</td>
</tr>
</tbody>
</table>

Median (and range) of reflex magnitudes, expressed as percentage change in on-going RMS EMG, of ‘short’ latency reflex responses.
Table 4. Summary of reflex latencies

<table>
<thead>
<tr>
<th></th>
<th>FA I</th>
<th>SA I</th>
<th>SA II</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>TA</td>
<td>42.5 (29.8-59.7)</td>
<td>71.6 (67.3-76.1)</td>
<td>71.0</td>
<td>63.5 (29.8-76.1)</td>
</tr>
<tr>
<td></td>
<td>[48.5 (37.5-76)]</td>
<td>[77.5 (69-91.5)]</td>
<td>[75]</td>
<td>[72 (37.5-91.5)]</td>
</tr>
<tr>
<td></td>
<td>n=4</td>
<td>n=3</td>
<td>n=1</td>
<td>n=8</td>
</tr>
<tr>
<td>LG</td>
<td>47.3 (30.0-92.1)</td>
<td>77.1 (29.9-99.6)</td>
<td>70.5</td>
<td>60.2 (29.9-99.6)</td>
</tr>
<tr>
<td></td>
<td>[72.7 (31.5-114)]</td>
<td>[101 (34-102)]</td>
<td>[72]</td>
<td>[80.2 (31.5-114)]</td>
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<td></td>
<td>n=6</td>
<td>n=3</td>
<td>n=1</td>
<td>n=10</td>
</tr>
<tr>
<td>MG</td>
<td>50.6 (47.7-66.7)</td>
<td>55.2 (33.6-78.3)</td>
<td>72.3</td>
<td>55.2 (33.6-78.3)</td>
</tr>
<tr>
<td></td>
<td>[59.5 (50.5-69)]</td>
<td>[56.5 (34.5-88)]</td>
<td>[75]</td>
<td>[59.5 (34.5-88)]</td>
</tr>
<tr>
<td></td>
<td>n=3</td>
<td>n=9</td>
<td>n=1</td>
<td>n=13</td>
</tr>
<tr>
<td>Soleus</td>
<td>53.7 (27.2-65.1)</td>
<td>57.6 (29.7-85.4)</td>
<td>-</td>
<td>53.7 (27.2-85.4)</td>
</tr>
<tr>
<td></td>
<td>[56 (31-83.5)]</td>
<td>[63.2 (29.5-97)]</td>
<td>-</td>
<td>[56 (29.5-97)]</td>
</tr>
<tr>
<td></td>
<td>n=5</td>
<td>n=2</td>
<td>-</td>
<td>n=7</td>
</tr>
<tr>
<td>Total</td>
<td>48.9 (27.2-921.1)</td>
<td>67.3 (29.9-99.6)</td>
<td>71.0 (70.5-72.3)</td>
<td>57.4 (27.2-99.6)</td>
</tr>
<tr>
<td></td>
<td>[57 (31-114)]</td>
<td>[69 (29.5-102)]</td>
<td>[75 (72-75)]</td>
<td>[65.2 (29.5-114)]</td>
</tr>
<tr>
<td></td>
<td>n=18</td>
<td>n=17</td>
<td>n=3</td>
<td>n=38</td>
</tr>
</tbody>
</table>

Median (and range) of latencies, in milliseconds, measured to the 99.5% confidence level crossing, of ‘short’ latency reflex responses. Values shown in square brackets indicate the latency to the peak of the reflex response.
Table 5. Single motor unit reflex responses

<table>
<thead>
<tr>
<th>Unit Type</th>
<th>Muscle</th>
<th>Reflex Latency (ms)</th>
<th>Reflex Magnitude (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FA I</td>
<td>MG</td>
<td>41.9</td>
<td>3.0</td>
</tr>
<tr>
<td>FA I</td>
<td>MG</td>
<td>93.8</td>
<td>6.8</td>
</tr>
<tr>
<td>SA I</td>
<td>LG</td>
<td>57.8</td>
<td>13.2</td>
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<td>SA I</td>
<td>LG</td>
<td>97.5</td>
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<tr>
<td>SA I</td>
<td>MG</td>
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<tr>
<td>SA I</td>
<td>MG</td>
<td>51.8</td>
<td>39.9</td>
</tr>
</tbody>
</table>

The reflex latency and magnitude, expressed as percentage increase in the probability of firing above on-going activity, of each reflex coupling between a cutaneous mechanoreceptor and single motor unit.
Figure Legends

Figure 1. Distribution of cutaneous mechanoreceptors in the glabrous skin of the foot

The approximate receptive field location and size for each afferent recorded from the glabrous skin of the foot is illustrated. There was no difference in the distributions of afferents that exhibited ‘short’ latency (filled), ‘long latency’ (hatch) or no (open) reflex couplings.

Figure 2. Reflex coupling with an FA I afferent

A, raw data for an FA I afferent innervating the skin overlying the MTP joint of digit V, with instantaneous frequency (top trace), neurogram (second trace) and four EMG traces below; B, autocorrelogram (5 ms bins) of the afferent spikes illustrating cyclic firing, with superimposed spikes in inset; C, spike-triggered averaged nerve and RMS EMG (with 99.5 % confidence limits) illustrating cyclic modulation of ongoing EMG.

Figure 3. Reflex coupling with an SA I afferent

A, autocorrelogram (5 ms bins) of afferent spikes (with superimposed spikes in inset) illustrating asynchronous firing of an SA I afferent innervating the glabrous skin on the distal phalanx of digit II, with spike-triggered average RMS EMG illustrating reflex modulation of ongoing EMG (dashed lines illustrate 99.5 % confidence limits, see Materials and methods for details); B, autocorrelogram and spike-triggered average RMS EMG of ‘random’ spike train illustrating no significant modulation of ongoing EMG.

Figure 4. Reflex latencies

Histogram of reflex latencies for all ‘overt’ and ‘significant’ reflex responses.

Figure 5. Reflex periodicity

There is a strong ($r^2 = 0.94$) correlation between the reflex EMG periodicity and the mean interspike interval of the periodically active mechanoreceptor afferents (pooled data from 20 FA I (●) and 1 SA II (■) units).

Figure 6. Reflex coupling between an SA I afferent and a single motor unit

A, raw data for an SA I afferent innervating the skin on the lateral aspect of the foot, with instantaneous frequency (top trace), neurogram (second trace), single motor unit instantaneous frequency (third trace) and MG EMG (bottom trace); B, autocorrelogram (5 ms bins) of the afferent spikes illustrating asynchronous firing (with superimposed spikes in inset) and spike-triggered averaged nerve; C, cross-correlogram (with cumulative sum and 95 % confidence limits) of the
single motor unit and afferent spikes illustrating significant modulation of the single motor unit activity (with superimposed motor unit potentials in inset), and spike-triggered averaged RMS EMG (with 99.5 % confidence limits) illustrating no statistically significant modulation of the ongoing EMG.
Figure 1
Figure 2
Figure 4