Firing Properties of Muscle Spindles Supplying the Intrinsic Muscles of the Foot in Unloaded and Free-standing Humans

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STATEMENT OF AUTHENTICATION

I, **Thomas Knellwolf**, declare that this thesis is based entirely on my own independent work, except for sections which were performed in collaboration with colleagues as acknowledged in the study and resulted in the publication of the journal articles shown below. To the best of my knowledge this project does not contain material previously submitted in fulfilment of the guidelines and requirements for the award of Doctor of Philosophy in the School of Medicine, Western Sydney University, and has not been submitted for qualification at any other academic institution.

Thomas Knellwolf

ACKNOWLEDGEMENTS

It is with no hyperbole that I declare, writing this thesis was *the* most challenging feat of my life so far and that it may hold that title for some time to come. Unlike previous goals I have set, the complexity and prestige of being able to contribute to the noble field of neurophysiology put all of my knowledge, nerve and character to the test. Truly, it was only with the support of those around me that I have managed to overcome my fears and thoughts of self-doubt to now proudly present this body of work.

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All of my co-supervisors have played instrumental roles in shaping my research experience. Both Alexander Burton and Elie Hammam were incredible role models, supporting so much of my data collection, analysis and writing but also setting the standard for professional achievements and I will always strive to achieve what they have. I had the pleasure of working with Professor Kenny Kwok for several weeks in Hong Kong and he continued to provide his insight into the force plate technology that was used. It was a great experience collaborating with

him and learning more about his work. Truly I am blessed to have worked with such a supportive, positive group of professionals as well as the countless others who have offered me advice and given me great learning experiences.

To my parents Paul and Kylie, my sister Rachel, and my partner Sunisa, you have all been there for me (when I would allow you to) and provided me with an unconditional love that I depended heavily on when facing the trials and tribulations of the final stages of writing. Without your close, emotional support, I am not sure I would have found the personal strength myself and I love you all for being there.

Finally, it would be remiss if I did not acknowledge my mentor Peter Khnana, who provided words of wisdom and dissected and addressed my personal conflicts keeping me from reaching this goal. I hope this work stands as a testament that I will continue to apply the lessons I have learnt in my future work, whatever it may entail.

Thank you all.

ABSTRACT

Human posture and locomotion are dependent on the sensory apparatus – involving muscle spindles, cutaneous afferents and the vestibular system – that provides proprioception. In my previous work with my Bachelor of Medical Research, I investigated the relationship between galvanic vestibular stimulation and the sensitivity of muscle spindles of the long muscles of the leg. While that study showed no correlation between these systems it was limited by the lack of subject postural threat. In order to record from muscle spindles directly during unsupported free-standing, a new methodology for microneurographic recording from the posterior tibial nerve at the ankle was developed. For the first time, we have been able to identify the firing properties of muscle spindle endings in the small (intrinsic) muscles of the foot, as well as mechanoreceptors in the skin of the sole, while the participant is standing unsupported. This thesis presents this methodology along with the recordings made. In **Study 1**, the firing properties of 26 muscle spindles supplying the intrinsic muscles of the foot are described in unloaded conditions. Their responsiveness to stretch and related joint movements is shown to be similar to those in the short muscles in the hand and the long leg muscles. Only 27% were spontaneously active, of which there was no consistent resting firing rate or discharge variability. In **Study 2**, activity from 12 muscle spindles supplying the intrinsic foot muscles in unsupported free-standing conditions is described. In this group 50% were spontaneously firing and 67% had activity correlated with changes of centre of pressure recorded by a force plate, primarily (88%) along the anteroposterior axis. In **Study 3**, the activity of 28

multiunit cutaneous afferent recordings, as well as of 15 single-unit cutaneous afferents, supplying the sole of the foot in unsupported free standing is described. Activity of cutaneous afferents was found to be dependent on receptor type and location of receptive field. The data presented in this report is proof of this novel methodology's suitability for detailed study into the sensory sources in the foot contributing to maintaining the upright posture.

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1 INTRODUCTION

1.1 Proprioception

1.1.1 General definitions and history

Proprioception ("proprio" = self) is the sense of position and movement of parts of the body relative to one another, and the sense of force and effort associated with muscle contraction; kinaesthesia is also used to describe this, but strictly speaking it only refers to the sense of movement. It is also clear that the sense of balance, while subserved by the vestibular apparatus, is dependent on input from the somatosensory system to allow head position relative to the body to be determined (Lackner & DiZio, 2005). Proprioception is achieved through a summation of peripheral sensory input describing the degree of, and changes in, muscle length and tension, joint angle, and stretch of skin (Proske and Gandevia 2009, 2012). This sensory input originates from a number of sources. It is known that low-threshold mechanoreceptive units in the glabrous skin of the hand respond to isotonic movements without incidental excitation via direct touch (Hulliger et al. 1979). The sense of muscle tension has been largely attributed to Golgi tendon organs, responding to force generated by the muscle (Houk and Henneman 1967). While joint receptors were initially viewed as important contributors to joint position sense at all angles (Mountcastle and Powell 1959), it has since been shown that their contribution to kinaesthesia only extends to that of being limit detectors, detecting only extreme positions within the normal range in both the cat (Burgess and Clark 1969; Clark and Burgess 1975) and humans (Burke et al. 1988; Macefield et al. 1990). The most important contribution to proprioception is made by muscle spindles, stretch sensitive mechanoreceptors found in virtually all skeletal muscles. Interestingly, they are particularly dense in

muscles where accurate proprioception is important. For instance, in the intrinsic muscles of hands of the bonnet monkey, which are responsible for fine manipulative tasks, up to 42.3 spindles per gram (wet weight) were identified (Devanandan et al. 1983). They are also abundant in the neck of the cat (Richmond and Abrahams 1975) and human (Cooper and Daniel 1963), where they play important roles in integrating information on the position of the head with respect to the body. It is beyond the scope of the present review to deal with the many psychophysical studies on human proprioception, and the relative roles of muscle spindles and cutaneous afferents in proprioception, and the reader is directed to two excellent reviews on proprioception, referred to above (Proske and Gandevia 2009, 2012).

1.2 Muscle Spindles

1.2.1 Structure of the mammalian muscle spindle

Much of what we have learnt about the structure of the mammalian muscle spindle comes from detailed observations made in the cat (Barker 1948; Boyd 1962; Hulliger 1984). The spindle's function as a length sensor arises essentially from its anatomical relationship with its parent muscle. It consists of a bundle of specialized, striated, intrafusal ("fusiform" = spindle-shaped) muscle fibres lying in parallel with the fascicles of the regular, force-producing, extrafusal (skeletomotor) muscle fibres. Any length changes in the muscle therefore results in stretch of intrafusal fibres that is then detected by sensory receptors located on the equatorial and polar regions of the muscle spindle. For a detailed treatise on how muscle spindles transduce stretch into action potentials, the reader is

referred to a recent review by Bewick & Banks (2015). While the majority of each intrafusal fibre is contractile, the equatorial regions are less so. This divides the spindles into two, independently acting, polar regions. The small cross-sectional area of the intrafusal fibres in the adult means that their contraction makes an insignificant contribution to the total external force of the muscle, while being sufficient to deform the sensory terminals (Kuffler et al., 1951). It is by this means that, through the motor innervation normally supplied to both polar regions of the spindle, via γ motoneurones (fusimotor neurones) the central nervous system is able to actively modulate the muscle spindle's stretch sensitivity. Figure 1.1 shows a transverse section of a cat muscle spindle, illustrating the relationships between the primary afferent, the intrafusal muscle fibres and capsular elements.

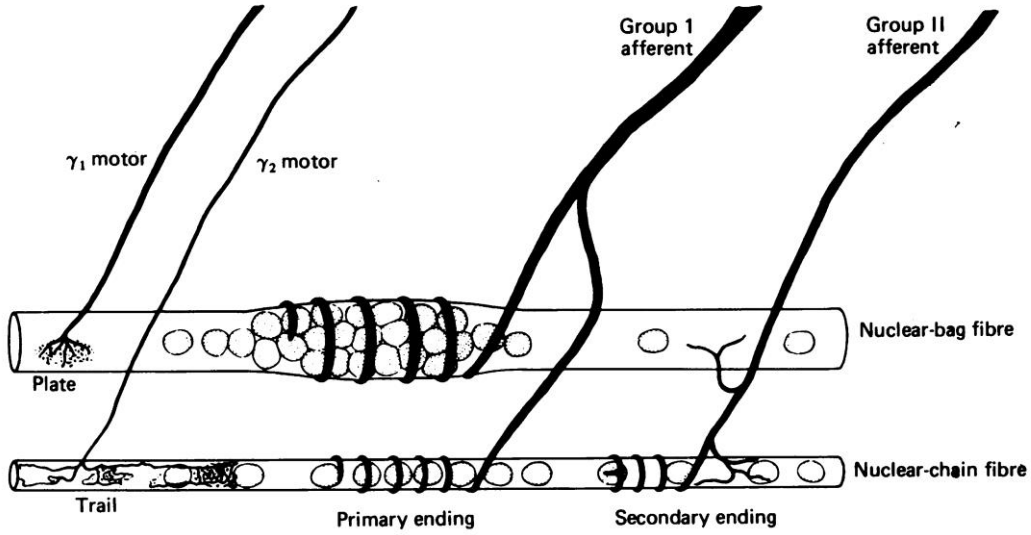

Figure 1.1: *A simplified diagram of the central region of a muscle spindle in the cat, showing a single "bag" intrafusal muscle fibre and a single "chain" intrafusal muscle fibre. Typically, muscle spindles in the cat contain two or three bag fibres and four to six chain fibres, and a complex motor innervation. Reproduced from Matthews (1964).*

Detailed anatomical studies of human muscle spindles were first performed on excised human intercostal muscles, largely corroborating what has been documented in the cat (Kennedy 1970). However, human primary endings appear not to possess an annulospiral structure, and the secondary ending innervates nuclear bag as well as nuclear chain muscle fibres. An example is shown in Figure 1.2. Detailed investigations of spindle structure have also been made from intrinsic and extrinsic finger muscles (Sahinen and Kennedy 1972; van Gorp and Kennedy 1974).

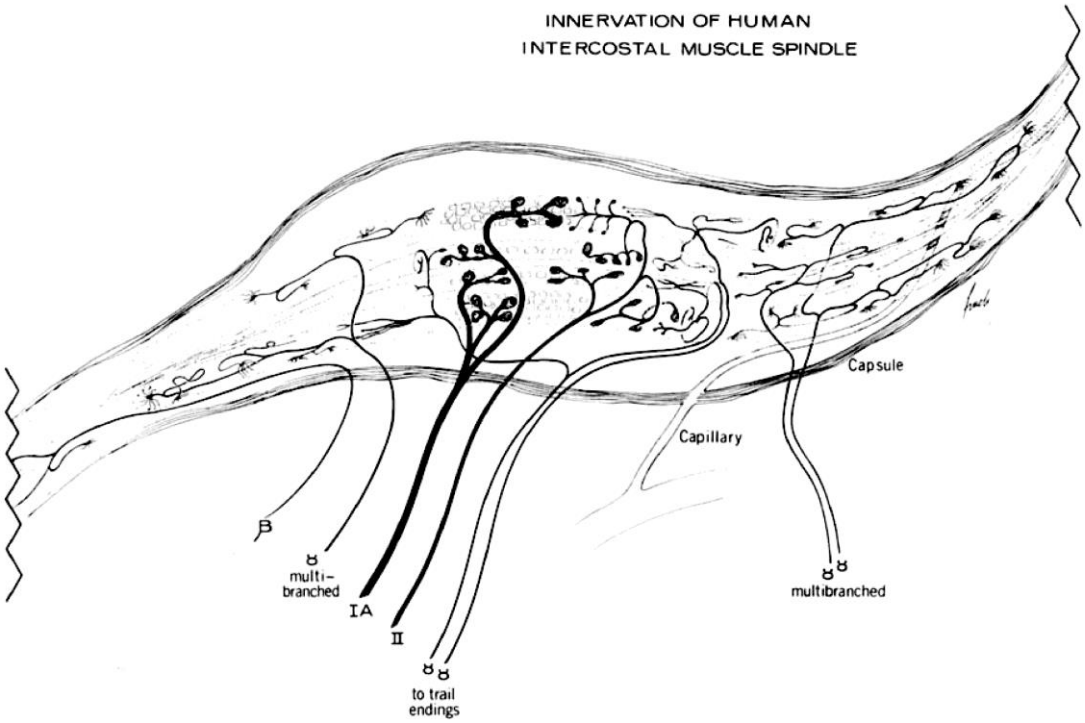

Figure 1.2: *Drawing from a histological specimen of a human muscle spindle, sampled from an intercostal muscle. Only two bag and two chain intrafusal muscle fibers are represented. The number of entering nerves, particularly the degree of branching and*

number of endings, has been limited to a few of each type for clarity. Reproduced with permission from Kennedy (1970).

1.2.1.1 Subdivision of intrafusal muscle fibres

The muscle fibres of the spindle are subdivided both structurally and functionally. The fibres were first distinguished histologically in the cat, into nuclear bag fibres and nuclear chain fibres, by the arrangement of nuclei in the equatorial cross-section: bag fibres reveal multiple visible nuclei while chain fibres would consistently have only one, due to the fact that chain fibre nuclei would be arranged in a single-file "chain" (Barker 1948). Bag fibres are of appreciably greater diameter and length than chain fibres, often extending beyond the spindle capsule (Boyd 1962; Cooper and Daniel 1963). Additionally, the fibres have been distinguished by the band structure of their myofilaments (Adal 1969; Corvaja et al, 1969) and through histochemical testing (James 1968; Spiro and Beilin 1969). Later studies further subdivided bag fibres into bag_1 and bag_2 types based variations in their ultrastructure and histochemical response profile (Ovalle & Smith 1972; Banks et al. 1977). An important observation was the variability between all three fibres in their reaction to staining for myofibrillar ATPase (mATPase). Indeed, the structural differences between the fibres are enough to suggest some functional differences. Typically bag_1 fibres have the slowest contraction rates followed by bag_2 and finally chain fibres. These differences in response at different frequencies were explored in depth by Boyd (1976). Human muscle spindle fibres were later histochemically distinguished by the presence of variants of myosin heavy chains and M-band proteins (Eriksson et al. 1988). For

more detail on the specific delineations and techniques used, the recent review by Thornell et al. (2015) should be observed.

The suggestion of functional duality is supported by the variations in afferent and efferent innervation, the properties of which will be discussed below. In terms of afferent innervation, primary afferents fibres innervate all three fibre types, while secondary fibres supply only bag_2 and chain fibres. Static fusimotor fibres also only supply bag_2 and chain fibres, while dynamic fusimotor axons innervate bag_1 fibres exclusively (Boyd et al. 1977; Proske 1997). Considering the significant functional differences between these γ-motoneurones (see below) it can be appreciated that the subdivisions of intrafusal fibre types work largely independently of one another.

1.2.1.2 Sensory innervation of the muscle spindle

There are two types of afferents that innervate muscle spindles. They are, like intrafusal fibre types, distinguished by structure and function into primary (type Ia) and secondary (type II) endings. As documented in the cat, primary endings have a larger diameter than secondary fibres (12-20 μm and 4-12 μm, respectively; Eccles and Sherrington 1930) as well as a faster conduction velocity (Hunt 1954). Our understanding of the anatomical differences comes largely from the work of Boyd (1962). Each spindle has only one primary sensory nerve ending (group Ia afferent), with the exception of muscle spindles of the neck (tandem spindles) which may have multiple. The ending consists of number of spirals, supplying all of the intrafusal fibres in the spindle at the equatorial region, with

one spiral per fibre. It is deformations of this ending that allow the detection of changes in length of the parent muscle. The number of secondary (group II) afferent endings in a spindle varies from zero to five and often supply one fibre each. They predominately terminate on nuclear chain intrafusal fibres. The endings consist of smaller spirals than primary endings that terminate on the polar ends of the spindle.

Muscle spindle afferents have been contrasted functionally by examining the response of mean firing rate to various changes in muscle length, commonly involving ramp-and-hold tests. This involves a muscle being held at a constant length (static component) before being stretched at a constant velocity (dynamic component) to a new length, at which it is again held constant. Primary afferents are sensitive to dynamic stretch, demonstrate an irregular spontaneous or volitionally maintained discharge, and exhibit an off-response at the point of relaxation (i.e. muscle stretch) following a slow ramping isometric contraction. They are silenced during rapid voluntary contraction when the fusimotor drive (discussed below) is insufficient to overcome the unloading effects of the extrafusal contractions. Secondary afferents generally exhibit a regular tonic discharge, decelerate during an unloading contraction and do not exhibit an off-response at the termination of a voluntary ramp-and-hold contraction (Edin and Vallbo 1990a).

1.2.1.3 Motor innervation of the muscle spindle

Muscle spindles are unique among proprioceptors for possessing their own motor supply in addition to afferent innervation. This motor innervation may originate from small diameter (4-8 μm), myelinated gamma (γ) motoneurones – also referred to as fusimotor fibres – or from axons branching from larger fibres that also supply extrafusal muscle known as beta (β), or skeletofusimotor fibres. These fibres supply both intrafusal fibres via trail endings at the polar ends. Similarly in humans, extensively branched small-diameter axons were found to innervate the intrafusal fibres at the poles of the muscle spindle (Kennedy 1970). Stimulation of γ-motoneurones produces no overt tension in the muscle, but does result in considerable excitation of both primary and secondary spindle afferents. It is known from the cat that high-intensity electrical stimulation of motoneurones supplying regular, extrafusal fibres known as alpha (α) motoneurones results in coactivation of γ-motoneurones (Severin et al. 1967). Hunt and Kuffler (1951) outlined the role of fusimotor drive for spindle function. During extrafusal muscle contraction the muscle shortens, leading to an unloading of the spindles, which reduces their spontaneous discharge. As stated above, γ-motoneurone activity is co-activated. This results in contraction of the polar ends of the intrafusal fibres, restoring tension and therefore sensitivity of the spindle to stretch. Thus, it can be said that one of the major functions of γ-motoneurones is in controlling the sensitivity of muscle spindle afferents as length detectors.

Fusimotor motoneurones have been differentiated into static and dynamic fusimotor axons, from ramp-and-hold experiments performed by Matthews

(1962). Dynamic axons have a weak effect on primary afferent firing in muscles held at a constant length, but are markedly sensitised to dynamic stretch, resulting in bursts at the beginning and end of the dynamic phase. Conversely, static axons are largely ineffective during the dynamic phase but create a powerful excitatory action while the muscle is at constant length, increasing the overall response to a change in length. Static axons have a great influence on both primary and secondary fibres, whereas dynamic axons show little to no effect on secondary endings, even if a primary ending of the same fibre is stimulated (Appelberg et al. 1966). In the decerebrate cat there is significant resting fusimotor outflow, primarily in static γ motoneurones (Matthews and Stein 1969b).

1.2.2 Functional properties of human muscle spindles

The first neurophysiological recordings from human muscle spindle afferents were made in vitro, again from excised intercostal muscles, and appeared in a brief report (Davis 1973); more detailed investigations of excised human spindles were undertaken by Kennedy and colleagues (Poppele and Kennedy 1974; Kennedy et al. 1974). As expected, human spindle afferents responded to direct passive stretch of the organ with a velocity-dependent increase in firing rate, their behavior largely paralleling that observed in the anaesthetized cat. However, it was not until the development of microneurography - by Karl-Erik Hagbarth and Åke Vallbo at the Academic Hospital in Uppsala, Sweden - in the mid-60s, that it became possible to study the functional properties of human muscle spindles in vivo, and during active contractions as well as passive conditions (Hagbarth and Vallbo 1968). Indeed, the primary motivation of Hagbarth and Vallbo to use metal

microelectrodes to record from peripheral nerves in awake human subjects was to study muscle spindles during voluntary contractions, to examine their potential role in the servo-loop model of motor control, postulated by Merton (1953).

Microneurography allows one to record from single myelinated axons, both afferent and efferent, applying reductionist principles (single-unit analyses of type-identified neurons) in a holistic fashion (intact nervous system, no anaesthesia); one can also record from single unmyelinated sensory and motor (sympathetic) axons, both afferent and efferent. However, it is worth pointing out that microneurography is limited to peripheral nerves that are accessible via insertion through the skin, and are not too deep, such as the median and ulnar nerves at the wrist or upper arm, the radial nerve in the upper arm, and the tibial and common peroneal nerves in the lower limb. In the context of the present review, this also limits the muscles that can be accessed; while distal muscles in the forearm and hand, and the lower leg and foot, can be examined, proximal and axial muscles are out of reach. Moreover, the types of active movements that can be studied are also limited, owing to the risk of losing the recording site during brisk and/or forceful movements, a criticism that has been raised when comparing data from conscious cats and conscious humans (Prochazka, 1989). The microneurographic approach is shown in Figure 1.3. Myelinated axons generate positive-going spikes when the tip of the microelectrode is located close to (or embedded within) the myelin sheath of an axon, while those of C-fibres are negative-going because the action potentials are recorded from the exposed axolemma. Accordingly, one would think it would be possible to record directly

from fusimotor neurons in humans, given that they have a larger diameter than C-fibres. Indeed, γ motoneurons are myelinated, so should - like other myelinated axons - generate positive-going spikes. However, surprisingly little is known about them in humans, and only one study claims to have recorded from γ motoneurons directly, identifying them as motor because they did not respond to vibration and their activity could be modified according to criteria established in experimental animals (Ribot et al. 1986). Nevertheless, changes in fusimotor drive can be reliably inferred from changes in the firing of muscle spindle afferents, from which single-unit recordings can readily be obtained in awake humans. This will be considered further below.

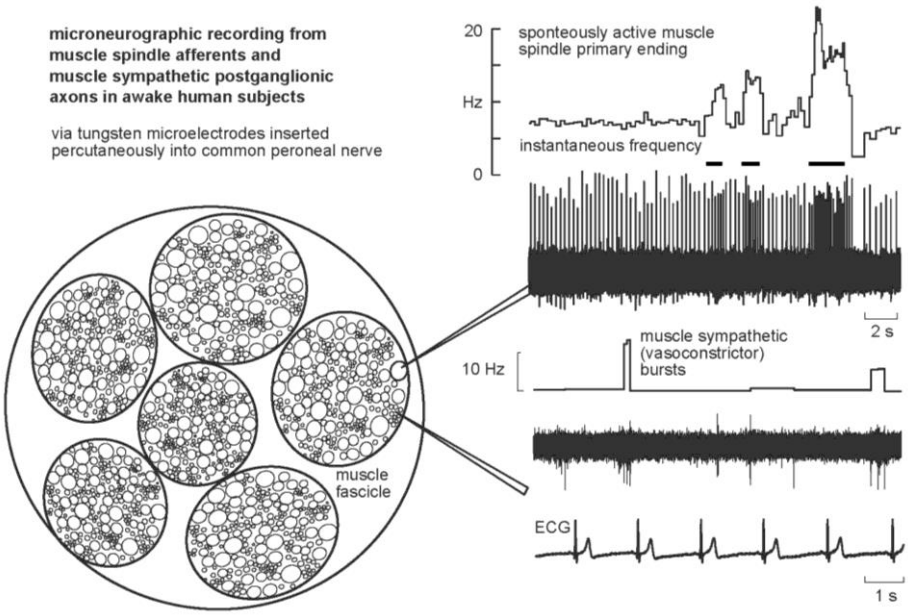

Figure 1.3: *Schematic representation of a cross-section of a human peripheral nerve. The nerve is composed of distinct bundles of nerve fibres, some innervating muscle (motor fascicles), others supplying skin (cutaneous fascicles). Within each fascicle are large- and small-diameter sensory fibres (afferents) and small-diameter*

(unmyelinated) postganglionic sympathetic fibres. Motor fascicles also contain large-diameter (alpha) motor axons supplying muscle fibres and smaller-diameter (gamma) fibres to the muscle spindles. In the top right is an example of a recording from a spontaneously active muscle spindle afferent responding located in extensor digitorum longus to passive plantarflexion of the toes, indicated by the horizontal bars. In the bottom right is a unitary recording from a postganglionic muscle sympathetic axon and concurrently recorded electrocardiographic activity (ECG). Reproduced from Macefield (2005) with permission.

1.2.2.1 Identification of muscle spindles

Muscle spindle afferents are found in muscle fascicles of peripheral nerves and supply a specific muscle. Identification is based on behavioural properties, similar to those used in studies in experimental animals. Many are spontaneously active at rest, with the mean frequency of the tonic discharge increasing during stretch of the receptor-bearing muscle and decreasing during passive shortening (unloading) of the parent muscle. These spontaneously active spindles are further subdivided into presumed primary or secondary endings: primary endings show a characteristic silent period following release of the stretch (passive shortening) and have a high dynamic sensitivity to passive stretch. Conversely, secondary endings typically decelerate, without ceasing completely, when the stretched muscle is returned to its resting length. In addition, Ia afferents typically show an increase in firing (off-discharge) during the relaxation phase of a volitionally-generated ramping contraction and brisk relaxation; this 'off-discharge' is absent for II afferents (Edin and Vallbo 1990a,b). For example, for recordings from the

common peroneal nerve, silent spindle endings are discovered by passively stretching the receptor-bearing muscle by plantarflexion of the ankle (for muscle spindles in tibialis anterior) or toes (extensor hallucis longus or extensor digitorum longus), or by inversion of the foot (for spindles in the peronei muscles). Subjects are asked to intermittently dorsiflex the foot or toes during the search procedure to facilitate the isolation of fusimotor-driven spindle afferents.

Initially, human muscle spindles were only classified as such if they were silenced during an electrically-evoked brief contraction (twitch) of the parent muscle – in which the intrafusal muscle fibres are unloaded – and increased their background discharge (or were recruited) during the subsequent relaxation phase of the twitch. Although the "twitch-test" is considered the gold-standard for identification of human muscle spindles, it is now not used routinely. It does require an amplifier that allows intraneural stimulation to be delivered without interrupting the recording (Burke 1997), although transcutaneous electrical stimulation over the muscle is also effective at inducing muscle twitches without disturbing the spindle afferent recording (Edin and Vallbo 1987). However, while muscle spindles respond during the relaxation (stretch) phase of the twitch, and Golgi tendon organ (Ib) afferents respond during the contraction phase, some muscle spindles do behave in an in-series fashion (i.e. like Golgi tendon organs) during a twitch contraction (Burke et al. 1987) and some alpha motoneurons may be reflexly recruited during the twitch (Inglis et al. 1995). As will be discussed below, many spindles are active during a voluntary contraction, which also recruits Golgi tendon organ afferents and, of course, motor axons. Spike-triggered

averaging of surface EMG has been used to differentiate between Ib sensory and α motor axons during active contractions, with motor axons being defined as such if the spike-triggered average EMG shows a clear time-locked response that follows the spike; conversely, Ib afferents either exhibit no time-locked EMG response or the EMG response precedes the spike (Fallon and Macefield 2007).

1.2.2.2 Sensitivity of muscle spindles to stretch

Spindle primary endings in the cat are exquisitely sensitive to small changes in length of their parent muscle (Brown et al. 1967); some endings can be driven up to 500 Hz with vibrations as small as 5 μm in peak-to-peak amplitude. Given their high dynamic sensitivity, Ia afferents in the cat respond ~90° in advance of the peak of the vibration cycle, which fits with their role as velocity receptors (Matthews and Stein 1969); the same has been shown for human primary afferents (Roll and Vedel 1982; Kakuda 2000). Accordingly, many argue that Ia afferents primarily encode changes in *relative muscle length*, with the II afferents serving as *absolute length detectors* owing to their lower sensitivity to small-amplitude high-frequency vibration. However, we know that there is considerable overlap between the two types of spindle endings when the muscles are relaxed, and especially when the muscles are contracting (Burke et al. 1976a,b; Fallon and Macefield 2007). Indeed, when muscles are active, the Golgi tendon organs, which are insensitive to length changes in the passive state but respond to the increase in muscle force, now become very sensitive to low-amplitude vibration (Fallon and Macefield 2007). A recording from a primary afferent located in the extensor hallucis longus (EHL) muscle is shown in Figure 1.4. It can be seen that the ending

exhibits a high dynamic sensitivity to brisk stretch of the muscle, produced by passive plantarflexion of the big toe at the metatarsophalangeal joint (Figure 1.4A). Moreover, applying vibration to the toe nail showed that the ending was sensitive to the small amplitude vibrations transmitted through the tendon to its location within the muscle belly (Figure 1.4C), although it was only partially entrained to the vibration cycle. Depending on their location in the muscle, spontaneously active muscle spindles, both primaries and secondaries, may exhibit a modulation of their discharge that is related to the pulsation of blood vessels in their immediate vicinity (McKeon and Burke 1981; Birznieks et al. 2012); some primary endings can even be driven or phase-locked to the pulse, generating one or two spikes that are time-locked to the heart beat (Birznieks et al. 2012).

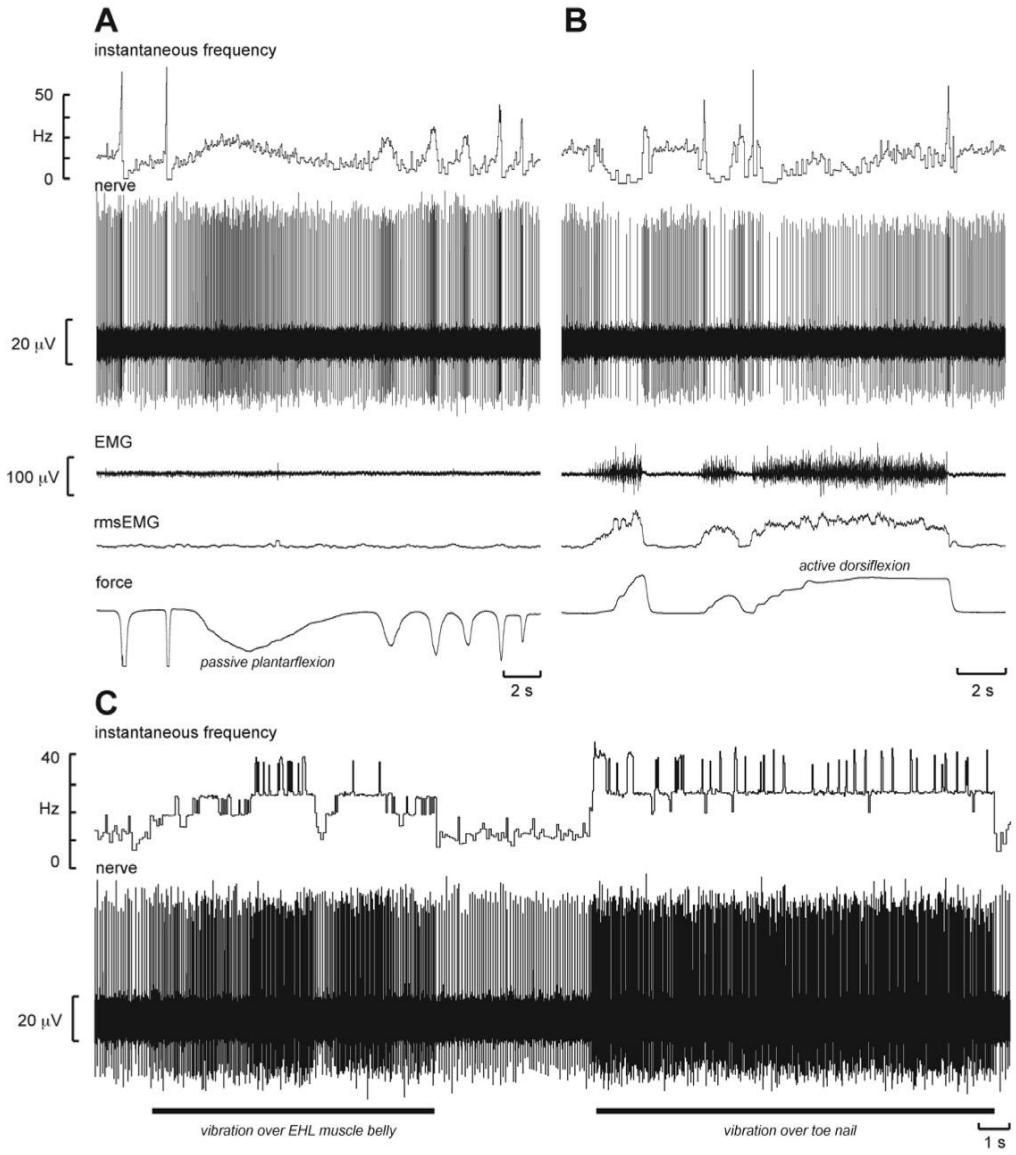

Figure 1.4: *Microelectrode recording from a spontaneously-active muscle spindle primary ending located in the extensor hallucis longus (EHL) muscle. Force was measured via a force transducer applied to the nail of the first digit, such that changes in force correlate with toe movement (labelled). The afferent increased its firing during stretch of the receptor-bearing muscle induced by passive plantarflexion of the digit at the metatarsophalangeal joint* ***(A)****. During a weak voluntary contraction the spindle was unloaded during the first two contractions but overcame this unloading during the*

*third contraction **(B)**. The spindle responded to vibration (~90 Hz) applied via a hand-held stimulator over the recptove field within the muscle belly, as well as over the toe nail; the latter indicates that the vibratory stimulus was transmitted adequately through the digit and, via the tendon, to the muscle **(C)**. Reproduced from Macefield (2005) with permission.*

All muscle spindles in humans, regardless of their identity as primary or secondary endings, are dynamically sensitive to imposed stretch of the parent muscle, usually produced by rotation about the joint on which the muscle acts. Muscle spindles faithfully encode joint angle when the muscles are relaxed, there being a linear relationship between joint angle and firing rate; (Edin and Vallbo 1990a; Kakuda 2000; Cordo et al. 2002; Day et al. 2017; Peters et al. 2017), as well as between joint angle velocity and firing rate (Grill and Hallett 1995), but there are no such relationships when the parent muscles are actively holding a joint position (Hulliger et al. 1982, 1985; Vallbo et al. 1981). It has recently been argued that muscle spindles also can encode, to a certain extent, force within the muscle. Blum and colleagues (2017) showed that the initial burst response of muscle spindles in the cat to an imposed passive stretch followed changes in the rate of rise in force (dF/dt) as well as acceleration, though later components correlated better to dF/dt. They conclude that the transient increase in dF/dt at the onset of lengthening reflects an increase in short-range stiffness due to actin-myosin cross-bridge dynamics. However, it should be pointed out that, like most investigations of muscle spindles, changes in muscle fascicle length were not recorded directly in this study and had to be estimated from changes in joint angle.

Recently, ultrasonography has been used to monitor changes in muscle fascicle length of the tibialis anterior muscle during passive sinusoidal rotations of the ankle joint in humans (Day et al. 2017). An example of stretch-evoked firing of a primary ending is shown in Figure 1.5. It can be seen that the spindle discharge approximated the sinusoidal changes in joint angle and muscle fascicle length and velocity. Across all spindles examined, the firing of the spindle could be approximated by a sine wave that had the same characteristics of the underlying fascicle length changes, regardless of the amplitude or velocity of stretch imposed by sinusoidal rotation of the ankle. While this has been observed directly in the cat (Matthews and Stein 1969a; Poppele and Bowman 1970; Hasan and Houk 1975a; 1975b) and in human spindles studied *in vitro* (Poppele and Kennedy 1974), as well as being inferred from studies that estimated muscle length from joint angle in humans (Kakuda 2000), until this recent study using ultrasonography it had never been directly measured in humans. During passive rotations of the ankle the firing of muscle spindles in tibialis anterior muscle is highly correlated with changes in fascicle length, occurring at relatively small, physiological changes in muscle length, and being independent of changes in the muscle-tendon unit. Given that the tibialis anterior muscle is essentially quiescent during standing, muscle spindles in this muscle would serve as ideal proprioceptors, given that they are signaling changes in length of passive muscle (Day et al. 2017). Muscle spindles in the calf muscles, which are actively engaged in keeping us upright when standing without support, have also been shown to be

sensitive to the types of low-frequency, low-amplitude angular excursions associated with standing (Peters et al. 2017).

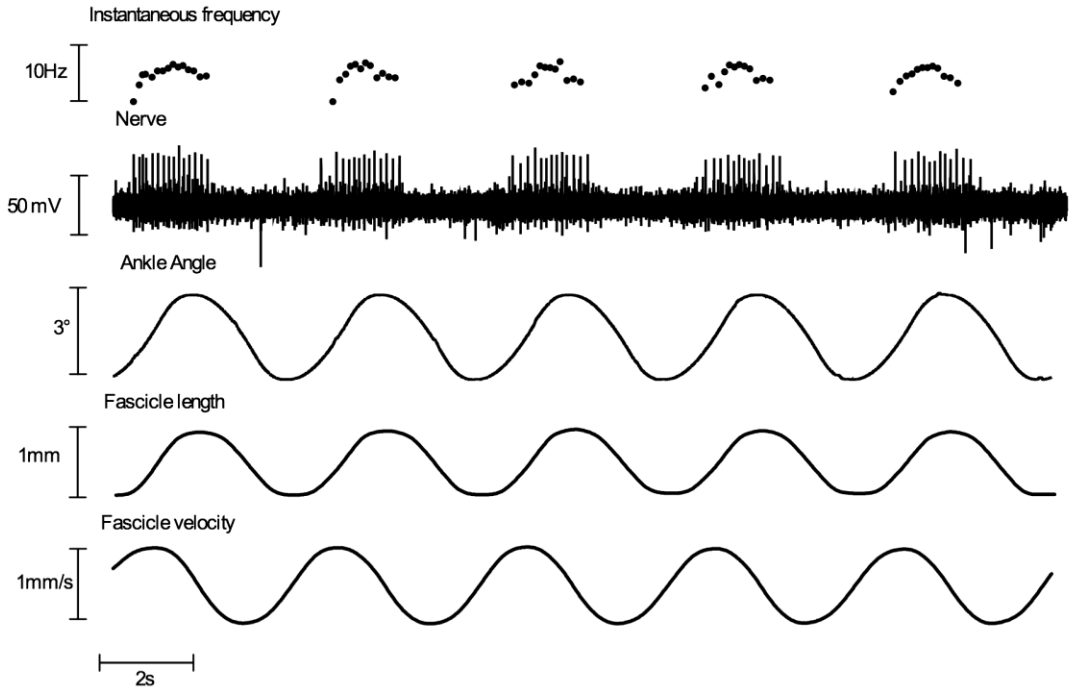

Figure 1.5: *Representative tibialis anterior spindle and fascicle behaviour during passive rotations of the ankle. Recording is from a spontaneously active primary muscle spindle ending from the tibialis anterior during 5 passive rotations of the ankle. The instantaneous firing rate of the identified unit is shown along with ankle angle and fascicle length and velocity of a tracked muscle fascicle from the same tibialis anterior. Reproduced with permission from Day et al. (2007)*

Muscle spindles, unlike cutaneous and joint receptors, respond preferentially along one axis of joint rotation (Burke et al. 1987) – that which causes stretch of the receptor-bearing muscle. Individual muscle spindles possess a directional sensitivity that allows them to encode changes in muscle length even during active contractions (Jones et al. 2001b). Moreover, fitting the firing of human muscle

spindle afferents to various models demonstrated that the information content in the velocity signal was ~10x higher than that of the displacement signal (Malik et al. 2016). Indeed, it has been argued that, given that the discharge of human primary endings precedes the rate of change in muscle length, they essentially act in the capacity of a "forward sensory model," influenced both by fusimotor drive during a contraction and future kinematics of the muscle (Dimitriou and Edin 2010). As a population, muscle spindles in the different muscles acting about the wrist joint, each of which has a preferred directional sensitivity, are able to encode multidirectional changes in joint angle (Jones et al., 2001b). The same is true for the ankle joint (Bergenheim et al. 2000; Ribot-Ciscar et al. 2002, 2003; Roll et al. 2000, 2004). This is shown for the ankle joint in Figure 1.6, in which mean spindle firing in each muscle was fitted to a cosine (A) and the individual vectors for each muscle computed (B, C). Moreover, muscle spindles in these muscles can faithfully encode complex movements (Roll et al. 2000), including those emulating cursive writing (Roll et al. 2004; Albert et al. 2005).

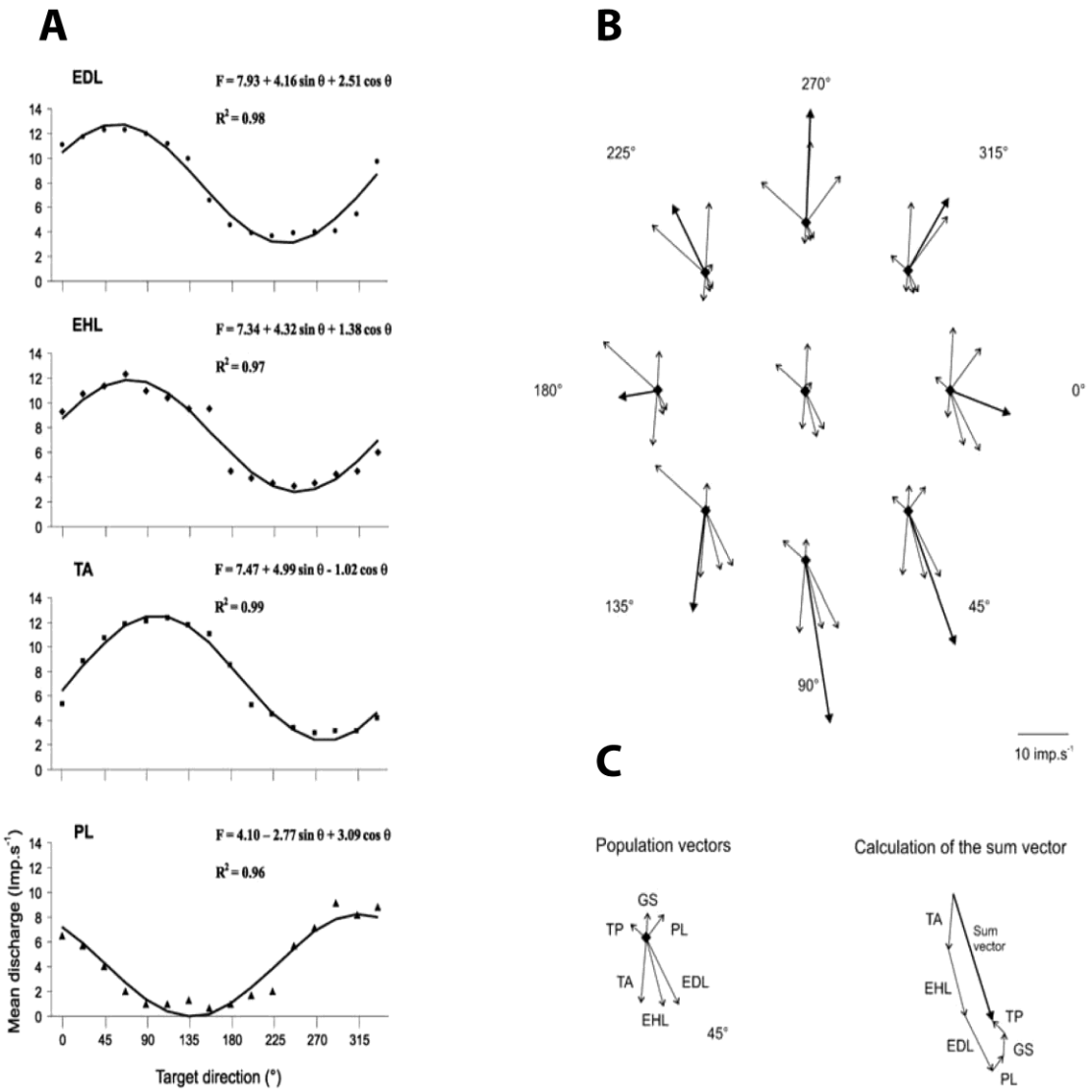

Figure 1.6: *mean discharge frequency of the whole sample of muscle spindle afferents for each differently oriented and maintained target position and for each recorded muscle. The direction of the target relative to the home position is given on the abscissa. Each of the muscle spindle populations coded the different positions according to a cosine-tuned function whose equation and coefficient of determination are given above each graph. B: the neuronal population vector model. Each diagram gives the result in one maintained position. The origin point of the vectors represents the target. Thin lines correspond to the population vector of each muscle; i.e., the direction of each vector corresponds to the preferred sensory direction of the muscle,*

and the length corresponds to the mean firing rate of the population of afferents of that muscle. The sum vector of all 6 population vectors is shown in bold. The center diagram gives the mean activity of each muscle population at the home position (foot in neutral position). C: individual and sum population vectors for all muscles. EDL, extensor digitorum longus; EHL, extensor hallucis longus; TA, tibialis anterior; PL, peroneus lateralis; GS, gastrocnemius soleus; TP, tibialis posterior. Combined from Figs. 3 and 4 of Ribot-Ciscar et al. (2003) and reproduced with permission.

1.2.2.3 Effects of immediate history on spindle discharge

It is well established that the discharge of muscle spindle endings is affected by previous stretch of the receptor bearing muscle (Edin and Vallbo 1990b) and by previous fusimotor activation during a voluntary contraction (Proske et al. 2000; Ribot-Ciscar et al. 1991; Macefield et al. 1991; Wilson et al. 1997. Muscle spindle discharge can remain elevated for long after a voluntary contraction (Wilson et al. 1997). This is not evidence of on-going fusimotor drive but of "thixotropy", the persistence of actin-myosin bonds formed in intrafusal fibres by the fusimotor activity that accompanied the contraction but ceased with it. As noted above, persistence of actin-myosin bonds may allow muscle spindles to encode the rate of change of intramuscular force (Blum et al. 2017). Muscle thixotropy has been shown to have significant effects on proprioception, with knowledge of joint position being affected by whether the muscle had previously contracted or was passively held in a lengthened or shortened position (Proske et al. 2000; Proske and Gandevia 2009, 2012). Sustained vibration applied to the muscle tendon has been shown to decrease resting spindle firing rate as well as reduce sensitivity to

passive stretch immediately after the cessation of vibration (Ribot-Ciscar et al. 1998b). This phenomenon lasted up to 40 seconds before there was a complete recovery. It was posited that the reduced post-vibratory muscle spindle activity accounted for the returning sensation observed following vibratory stimuli (Roll and Vedel 1982).

1.2.2.4 Resting fusimotor outflow to human muscle spindles

Because many muscle spindles are active at rest, owing to the prevailing degree of muscle stretch in the receptor-bearing muscle, a fall in firing rate during a voluntary contraction is interpreted as unloading of the spindle by shortening of the extrafusal muscle. Conversely, if the firing rate is maintained or increases - or a muscle spindle is recruited - during a voluntary contraction, it can be concluded that fusimotor neurones have been activated. In addition to firing rate, discharge variability is a useful measure of the effects of fusimotor drive on muscle spindles. In the decerebrate cat there is significant resting fusimotor outflow, primarily in static gamma motoneurones, that causes an increase in discharge variability of both primary (25%) and secondary (6.4%) endings; when the ventral roots have been cut, and hence fusimotor outflow interrupted, discharge variability decreases to 5.8% and 2.0%, respectively (Matthews and Stein 1969b). As in the cat, discharge variability is higher for the primary than for the secondary endings in humans, but there is overlap (Burke et al. 1979b; Nordh et al. 1983); mean variability is 3.6% and 8.3% for the secondary and primary spindle endings, respectively (Nordh et al. 1983). Matthews and Stein (1969b) noted that there is little difference between the discharge variability of de-efferented muscle spindles

in decerebrate cats and that in humans, suggesting that resting fusimotor drive to spindles is absent in relaxed human muscles (Burke et al. 1979b, 1981a; Nordh et al. 1983). Muscle spindle discharge and the response to stretch do not change significantly following complete nerve block (Burke et al. 1979a,). There is some evidence of resting activity in dynamic γ efferents, which may be increased by reflex action of cutaneous afferents (Aniss et al. 1990; Gandevia et al. 1994). However, the spontaneous firing rates of muscle spindles recorded from paralysed leg muscles in people with spinal cord injury (~10 Hz) were no different from those recorded from intact individuals; the same was true for discharge variability (~7 %; Macefield 2013). Again, this supports the conclusion that there is negligible fusimotor drive to human muscle spindles at rest. Whether this can be changed in a task-dependent manner will be discussed below.

1.2.2.5 Muscle spindle behaviour during voluntary contractions

During a nerve block that preferentially affects α-motor axons, a greater central drive (effort) is required to contract the paralyzed muscle; this increases the discharge of spindle endings, presumably because it activates γ-efferents (which are intact during the selective block) directed to the paralyzed muscle (Burke et al. 1979a). This supports the idea that static γ-motoneurones are activated when subjects volitionally contract a muscle (Vallbo 1974a; Vallbo et al. 1979). When the contraction is isometric, the fusimotor activation is usually sufficient to enhance the background firing of spontaneously active spindles, increase their discharge variability, increase their static response to stretch, and reduce the pause in discharge that occurs on muscle shortening (Vallbo 1971, 1973, 1974;

Burke et al. 1979b). There is also a suggestion that voluntary effort also activates dynamic γ-efferents (Kakuda and Nagaoka 1998), and some indirect evidence that β (skeletofusimotor) efferents can be activated by both voluntary effort (Aniss et al. 1988) as well as during transcranial stimulation of the motor cortex (Rothwell et al. 1987).

When voluntary contractions produce muscle shortening, the enhanced fusimotor drive can be sufficient to maintain or even increase spindle discharge, but this occurs only if the movement is slow or if the muscle is contracting against a load (Burke et al. 1978a,b). This can be seen in Figure 1.3B, in which the muscle spindle is unloaded during the slow contraction on the left but maintains its discharge during the longer contraction on the right. The increase in spindle discharge usually occurs after the onset of EMG activity in the contracting muscle, at a latency of some 20-50 ms when the contractions are rapid and phasic (Vallbo 1971; Hagbarth et al. 1975c). The latency of spindle acceleration can vary with contraction speed, and is consistent if the contraction is performed precisely the same way each time (Burke et al. 1978c, 1980; Wilson et al. 1997). In slow ramp contractions, it is likely that most spindle endings activated in the contraction will increase their discharge relatively early in the contraction (Edin and Vallbo 1990a; Wilson et al. 1997). This implies that at least some of the fusimotor neurones innervating a spindle are recruited early in the contraction. In isometric contractions, spindle discharge increases in proportion to contraction strength (Vallbo 1974a), largely because the discharge rates of the activated spindles increase with contraction strength. While there has been clear evidence of α-γ co-

activation in all voluntary contactions examined so far, there has been some evidence that the balance between the α and γ drives can be varied (Hulliger et al. 1985; Burke et al. 1980). This would be expected given that different descending pathways have quantitatively different effects on α and γ motoneurones, and many peripheral afferent inputs have different reflex effects on α and γ motoneurones (Aniss et al. 1990; Gandevia et al. 1994). However, one thing is clear: there is no evidence of spindles being recruited, or increasing their background discharge, *before* the onset of EMG – a pattern that would be expected from the servo-control theory of Merton (1953). Indeed, it was the seminal work of Vallbo in 1971 that conclusively demonstrated that spindle discharge *lags behind* the actvation of α-motoneurons, as evidenced from the latency of firing from the onset of EMG in the contracting muscle.

As noted above, in passive conditions human muscle spindles can faithfully encode angular position. However, during voluntary contractions this sensitivity to length changes is essentially lost, particularly during movements that cause shortening of the muscle but even during lengthening movements (Jahnke and Struppler 1990). When asked to actively hold a constant position, there is no relationship between joint angle and spindle firing (Vallbo et al. 1981; Hulliger et al. 1982), with spindle firing being better related to load rather than angular position (Vallbo et al. 1981). Moreover, during fast voluntary movements, most spindles are silenced during shortening contractions, though some exhibited an increase in firing that reflected an increase in fusimotor drive as force built up in the contracting muscle (al-Falahe et al. 1990a, 1991). Nevertheless, while muscle

spindles in the agonist muscle may be poor at encoding position during contraction, those in the antagonist muscle are not: as the agonist muscle shortens the antagonist lengthens, providing spindles in the stretched muscle with length and velocity information that they can faithfully encode (Ribot-Ciscar and Roll 1998; Dimitriou 2014). When the agonist and antagonist co-contract, however, spindle firing is higher; this may possibly reflect the smaller length changes (to which muscle spindles are more sensitive) in this condition (Nielsen et al. 1994).

1.2.2.6 Muscle spindle behavior during complex motor tasks

While most studies of human muscle spindles have used simple, and usually isometric, contractions of the receptor-bearing muscle, there have been some studies in which more complex volitional movements have been examined. When subjects were asked to grip an instrumented manipulandum between finger and thumb using the precision grip, randomly-occurring increases in tangential force at difference amplitudes and rates generated automatic long-latency increases in grip force that prevent slip and loss of grip of the manipulandum: this caused increases in muscle spindle firing in the finger flexor muscles during the evoked increases in grip force, increases that reflected the rate of rise in grip force, but there was no evidence of an increase in spindle firing in anticipation of the movement and no increases in spindle firing as the imposed tangential load increased prior to the generation of the grip response (Macefield and Johansson 1996). When subjects were asked to reach, grasp and lift objects (blocks of different size), both primary and secondary muscle spindle endings in the finger extensor muscles increased their activity in a manner that reflected the velocity of

the finger kinematics, with primary afferents also signaling acceleration, but neither class of spindle encoded the changes in muscle length or provided information on the size of the grasped object (Dimitrou and Edin 2008a).

Likewise, primary spindle endings in the wrist extensors encoded velocity and acceleration of the wrist kinematics during a key-pressing task, with secondary endings signalling velocity, but neither class of spindle afferent encoded changes in muscle length (Dimitrou and Edin 2008b). Mean data from 15 primary and 8 secondary spindles endings, located in the extensor carpi radialis muscle, are shown in Figure 1.7. Subjects used their middle finger to press keys on a 3 x 3 keyboard, resulting in the parent muscle being either long or short during the task. For the key sequences shown, it can be seen that both acceleration and velocity influenced the primary afferents, but only velocity affected the secondary afferents. The authors suggest that for the central nervous system to estimate the length or velocity of a muscle from the firing of muscle spindles, would require additional information about not only the central command to the extrafusal and intrafusal muscle fibres but also about the mechanical properties of the load, including muscle-tendon compliance on which the muscle acts. Indeed, volitional contraction of a finger against a viscous load revealed no specific signals in the spindle afferents that could encode the decrease in compliance (McNulty et al. 2008). Again, the central nervous system presumably disambiguates information on load by comparing the central command required to perform the movement with the resultant sensory feedback and the expected sensory feedback.

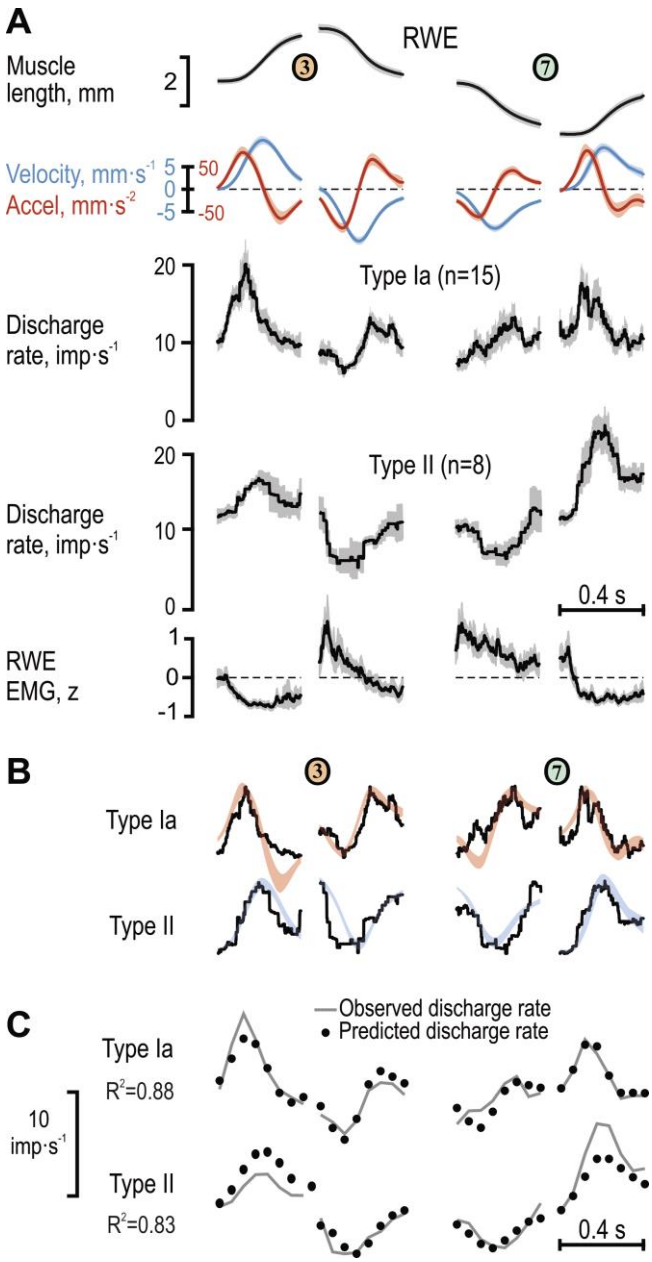

Figure 1.7: *Ensemble responses of muscle spindle afferents from the radial wrist extensor (RWE) muscle. A: averaged muscle length, velocity, acceleration (Accel), and EMG signals, along with the corresponding ensemble discharge rates (impulses/s) of type Ia (n =15) and type II afferents (n = 8) from the RWE, for a "long" key 3 and a "short" key 7. Shaded areas around means represent ± SD. B: qualitative comparisons of ensemble discharge profiles and acceleration (red) and velocity (blue). C: reconstructions of the observed ensemble discharge rates shown in A. Continuous lines*

represent the observed values, and circles represent the values predicted from linear regressions using the kinematics signals and RWE EMG as independent predictors. Reproduced with permission from Dimitriou and Edin (2008a).

1.2.2.7 Independent control of human fusimotor neurones

Despite the obvious links established in animal studies, including the elegant chronic recordings from muscle spindle afferents in awake behaving cats (Prochazka and Gorassini 1998a,b), there are relatively few studies in humans that support significant independent modulation of spindle gain via the fusimotor system. Nevertheless, reflex connections of human fusimotor neurons with cutaneous and muscle afferents (Aniss et al. 1990; Gandevia et al. 1994), as well as visual inputs (Jones et al. 2001a), have indicated the potential substrate for such independent control to exist. Vestibular stimulation is a potent stimulus for fusimotor neurons in the hindlimbs of the cat (Carli et al. 1967; Diete-Spiff et al. 1967), but studies in relaxed (Gandevia et al. 1994; Ribot-Ciscar et al. 2000; Bent et al. 2007) and active (Aniss et al. 1990; Jones et al. 2001a; Nafati et al. 2004; Bent et al. 2013) muscles have yet to demonstrate independent fusimotor changes in response to vestibular stimulation. Even in the near-vertical position, in the presence of ongoing EMG in the leg muscles – and presumably an increase in vestibulospinal drive – no evidence of independent fusimotor-driven changes in spindle sensitivity has been found (Knellwolf et al. 2016), though whether the same will be true when subjects are standing freely and exposed to postural perturbations remains to be seen. Recently, a small change in dynamic sensitivity of muscle spindles in the leg to passive rotations about the ankle joint was found

when subjects listened to emotionally-charged but not emotionally-neutral music (Ackerley et al. 2017). A recent immunohistochemical study provided morphological evidence for the presence of sympathetic innervation of human muscle spindles, identifying neuropeptide Y receptors on intrafusal fibres (Radovanovic et al. 2015). However, experimentally it has been shown that sustained increases in muscle sympathetic outflow have no effect on spindle firing rate or discharge variability (Macefield et al. 2003). There have also been investigations into the effect of noxious stimuli on fusimotor drive. A model for chronic musculoskeletal pain syndromes was established in anaesthetized animals based on the reflex activation of fusimotor neurons by nociceptor response (Johansson & Sojka, 1991). Unfortunately, following the same trend as other candidates for independent control, when this potential reflex excitation of fusimotor drive was investigated in awake human subjects, during the deep pain produced by injection or infusion of hypertonic saline into the tibialis anterior muscle, no net change in the spindle firing was seen – either with the muscle relaxed or with the fusimotor system engaged during a weak isometric contraction (Birznieks et al. 2008; Fazalbhoy et al. 2013).

It is generally accepted that fusimotor and skeletomotor neurones are coactivated during voluntary contractions in humans, with little evidence of independent control of the two motor systems – a point first made by Vallbo (1971). Many studies have since shown that the γ and α motoneurones are co-activated (Al-Falahe et al. 1988, 1990a,b; Kakuda et al. 1996; Wilson et al. 1997). However, it is reasonable to think that changes in the gain of human muscle spindles are likely

to occur in a task-dependent manner. Indeed, there has been some evidence to suggest that fusimotor neurones can change the sensitivity of muscle spindles independently of changes in EMG, and hence the activity of α motoneurons. When subjects perform a precision aiming task that had to be adapted to changes in visual feedback, the firing of muscle spindle afferents did not increase, but rather decreased, which the authors suggest allows the central nervous system to resolve the conflict between proprioceptive and visual feedback during the task (Jones et al. 2001a). While some investigators found no evidence of selective recruitment of fusimotor neurones (Wessberg and Vallbo 1995; Kakuda et al. 1996, 1997; Gandevia et al. 1997) others have, when attention to the task is required (Hospod et al. 2007; Ribot-Ciscar et al. 2000, 2009). Kakuda and colleagues (1996, 1997) found that fusimotor activity was higher during a precision task involving the fingers, but that the effect was not independent of the increase in skeletomotor activity. Conversely, Ribot-Ciscar and colleagues found evidence for an increase in spindle sensitivity during reinforment manouevres (Ribot-Ciscar et al. 2000) and as a function of task requirement (Ribot-Ciscar et al. 2009). Ribot-Ciscar and colleagues (2009) examined the effects of attending to either the movement or final position of an imposed ankle movement on spindle firing, showing that the balance of dynamic and static fusimotor drive to the spindle was shifted by directing one's attention to the foot. However, it must be emphasised that the changes seen that support independent fusimotor drive were very small, and certainly not as impressive as those documented in the cat. There is evidence that human muscle spindles also receive β innervation, i.e. a common axon that supplies both extrafusal and intrafusal muscle fibres (Kakuda et al. 1998), a

mechanism that ensures coactivation of α and γ motoneurones. Nevertheless, modelling does suggest that selective control of the dynamic and static gamma motoneurones is required to fully generate the repertoire of human movement and posture (Li et al. 2015). Indeed, most recently, Dimitriou (2016) showed that changes in spindle behavior during a visuomotor learning task cannot be fully explained by differences in muscle activity, but rather reflect an increase in fusimotor drive in the stage at which subjects have learnt (adapted) to the task.

1.2.2.8 Effects of muscle spindle activity on motor activity

It is well known that muscle spindles exert short-latency postive feedback onto α motoneurons in the spinal cord, although the synaptic strength of individual muscle spindles is weak; the same is true for individual muscle spindles in humans, as assessed using spike-triggered averaging of EMG, whereas the synaptic coupling of tactile afferents to motoneurons is strong (McNulty and Macefield 2001, 2002; Fallon et al. 2005). In long fatiguing contractions muscle spindle discharge is maximal initially at the beginning of a contraction and then decreases by about one-third at the end, which implies that feedback support to the contraction is maximal initially but subsequently falls (Macefield et al. 1991). A further implication is that, contrary to initial views, the γ efferent system is not mobilized to compensate for fatigue. When a motor nerve is blocked distal to the recording site, recordings can be made from α motoneurones deprived of feedback support from endings in the now-paralyzed muscle (Gandevia et al. 1990, 1993; Macefield et al. 1993). The discharge rates of motor axons reach roughly two-thirds of those of normally intact motor units, a finding that suggests significant feedback support to the contracting motoneurone pool. However, whether

this occurs through segmental, suprasegmental or cerebral pathways (or all) is not known. Nevertheless, despite it being more difficult to maintain motor unit firing in the absence of muscle afferents feedback, subjects can still recruit and de-recruit motoneurones and modulate their firing rates, given only knowledge of the central command (effort) that they are sending to the muscle.

What happens to motor control when spindle input is absent? Patients with complete large-fibre sensory neuropathy are critically dependent on vision to control the positions of their limbs in space, having lost input from large-diameter afferents originating in both muscle and skin (Cole and Sedgwick, 1992; Lajoie et al. 1996; Rothwell et al. 1982). It was recently shown that patients with Hereditary Sensory and Autonomic Neuropathy type III (HSAN III), also known as Familial Dysautonomia or Riley-Day syndrome, are devoid of functional muscle spindles in the legs, which fits with the loss of tendon and H-reflexes in these individuals (Macefield et al. 2011). They also have greatly elevated pain thresholds. Conversely, patients with another congenital sensory and autonomic neuropathy (HSAN IV), also known as congenital insensitivity to pain with anhidrosis (CIPA), have intact muscle spindles, as shown in Figure 1.8. Unlike individuals with large-fibre sensory neuropathy, those with HSAN III and HSAN IV have preserved large-diameter cutaneous afferents, though small-diameter cutaneous afferents are greatly reduced (Macefield et al. 2011). Nevertheless, their proprioception is greatly affected in these individuals, as is their gait and capacity to point at a target (Macefield et al. 2013), arguing for an important role of muscle spindles in many aspects of human motor control.

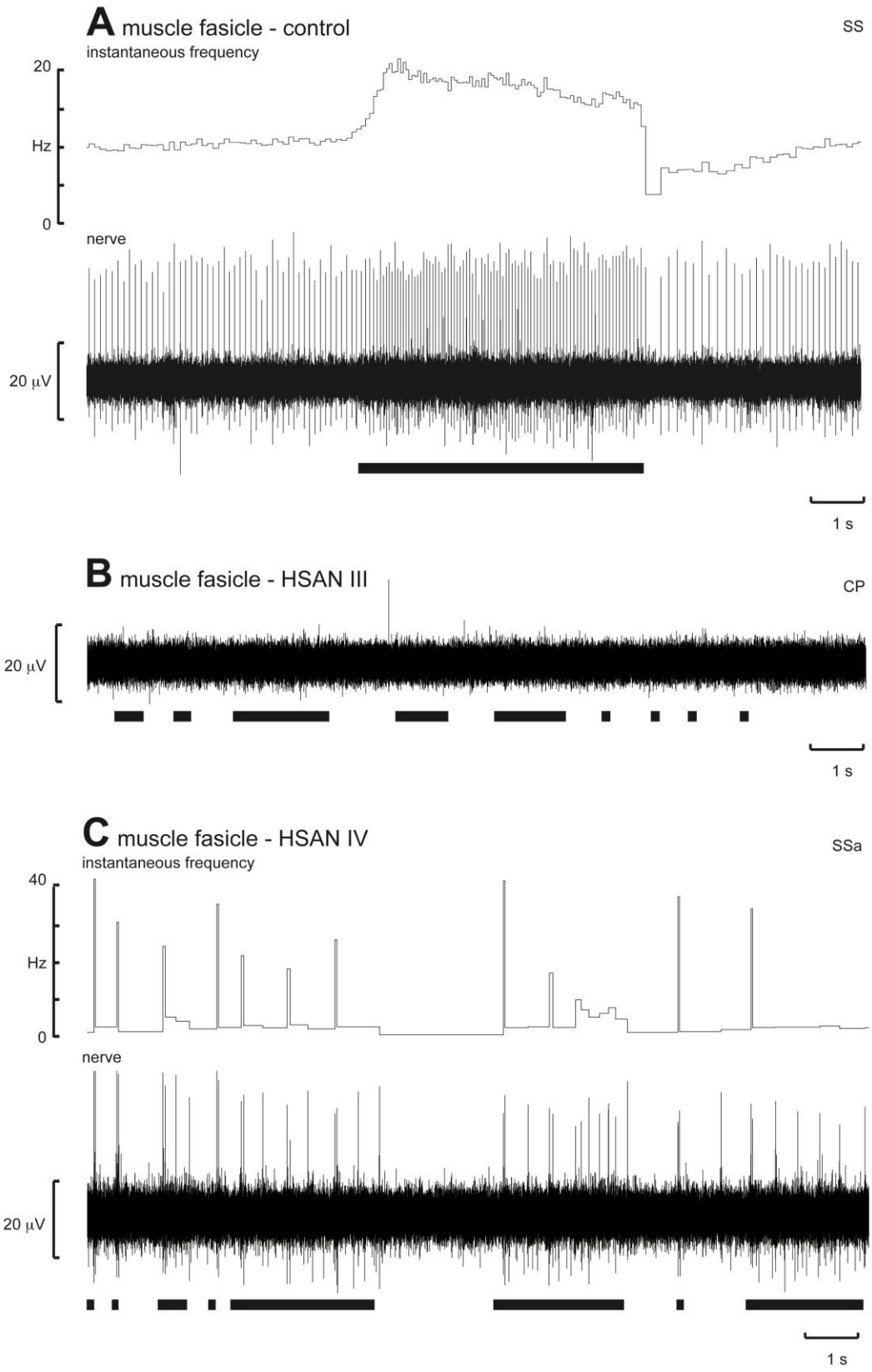

Figure 1.8: *Intraneural recordings from muscle fascicles of the common peroneal nerve (CP) during muscle stretch in a control subject (A), a patient with hereditary*

sensory and autonomic neuropathy type III (HSAN III; B), and a patient with HSAN IV (C). A: single-unit recording from a muscle spindle secondary ending located in the extensor hallucis longus muscle; the afferent responded during passive plantarflexions of the big toe. B: complete absence of muscle spindle afferent activity in the peronei fascicle during muscle stretch induced by passive inversion of the foot. C: recording from a muscle spindle primary ending located in the extensor digitorum longus muscle; the afferent responded dynamically during passive plantarflexions of the toes. In all traces, black horizontal bars indicate periods of passive stretch of the receptor-bearing muscle. Subject ID is represented by initials in top right corner of each panel. Reproduced with permission from Macefield et al. (2011).

1.3 Cutaneous Afferents

Our skin provides our primary mechanical interface with our environment. We are able to detect changes to our skin due to the environment via the specialized cutaneous mechanoreceptors embedded in the dermal and epidermal layers. These sensory units transmit tactile feedback to the CNS via the dorsal root ganglia (Johnson 2001). The perceived sensations allow us to characterize surfaces or materials we come in contact with as well as the quality of these stimuli. This is vital for fine manipulation of objects and controlled application of force onto the environment. In concert with our vestibular system and input from muscle afferents, these sensations contribute to proprioception and therefore our postural maintenance and standing.

1.3.1 Classification of afferent types

In order for a series of complex mechanical stimuli, experienced in a range of areas, forces and frequencies, to be discriminated between and later integrated, several receptor types, each with discrete specialised roles, are necessary. In the glabrous skin in humans, four distinct terminal endings and their associated afferent fibres satisfy this condition. Through microelectrode recordings from the median and ulnar nerves, the firing behaviour of these afferents in response to mechanical stimuli of the palm and fingers of the hand has been used to classify them, which will be briefly summated here and expanded upon in later sections.

Primarily, cutaneous afferents are classified by their response to a sustained stimulus and the characteristics of their receptive field, the area over which the afferent is excited (Knibestöl and Vallbo 1970; Macefield 1998). A sustained stimulus, usually an indentation, can be divided into dynamic and static phases, the dynamic phases occurring during increases or decreases in pressure and the static phase during constant pressure. Sensory units that respond only during the dynamic phases are termed fast adapting (FA) units (Knibestöl 1973; Iggo 1977). As the discharge rate correlates to the rate of change of the indentation they are able to encode the acceleration component of applied mechanical stimuli. During static phases of the indentation the rate of change is zero so they cease to fire. Activity of FA units to the dynamic phases at the beginning and end of a mechanical stimulus is referred to as an on-off response. While FA units encode stimulus acceleration, the magnitude of a sustained indentation is reported by slowly adapting (SA) units (Iggo 1977; Knibestöl 1975). The firing rates of SA

afferents are proportional to the force of stimuli, increasing and decreasing during dynamic phases and constant during static phases. However, while exquisitely sensitive, they will only fire once a force of sufficient magnitude is applied to the receptive field, this criterion being termed the firing threshold. Recruitment of silent FA and SA units also contributes to encoding the magnitude of an applied force.

The second major dichotomy of sensory units is the characteristics of their receptive field, borne from morphological disparity and resulting in significant contrast in stimuli response. The conventionally accepted criterion for identifying the receptive field size is the area in which a response to a stimulus intensity of 4-5 times the firing threshold is elicited (Vallbo and Johansson 1984). Across the four unit types identified, there is enough disparity in receptive fields that they can be divided into type I and type II afferents. Type I afferents typically have small receptive fields (~12 mm^2 in the palm of the hand) with clear borders (Johansson and Vallbo 1980). The sensory axons branch as they enter the skin, each branch terminating in a discreet mechanoreceptor ending. As a result, the small type I receptive fields usually have multiple points or "hot-spots" which elicit a larger response than the surrounding area. Conversely, type II afferents have significantly larger receptive fields (~88mm^2 in the palm of the hand) with poorly defined borders (Johansson and Vallbo 1980). These units do not branch but rather terminate within a large mechanoreceptor ending which in turn innervates a broad area, meaning they present a single hot spot.

1.3.1.1 Histology of the mechanoreceptor endings

Type I and II afferent units each terminate at two of the four unique mechanoreceptor endings. For each receptive field type, there is a corresponding FA and SA unit. Type I units terminate in Merkel cell-neurite complexes (SAI) and Meissner corpuscles (FAI) while type II units terminate in Pacinian corpuscles (FAII) and Ruffini endings (SAII) (Macefield 1998; Abraira and Ginty, 2013). These four units each have a unique morphology and firing properties that present different functional roles in the transduction of tactile stimuli. Thanks to the work of Miller and colleagues (1958), we have an understanding of the histology of these units.

Associated with specialized cells in the basal epidermis (stratum germinativum), Merkel cells are expanded disk-like endings that arise from branched axons and innervate a small, well-defined receptive field. In a single group there may be as many as 25-75 endings encompassing an area of ~25,000 μm^2. At a similar depth to Merkel cells, Meissner corpuscles are located in roughly half of the intradermal papillae, with either a single or up to 7 or 8 endings within one papilla. They have larger ellipsoidal, encapsulated endings that are directed towards the surface of the skin supplied by 2-6 myelinated axons (Darian-Smith 1984). They are responsive to light stroking across the skin, local shear forces and incipient or overt slips within the receptive field (Macefield 1988).

Morphologically similar to Golgi tendon organs, Ruffini corpuscles are oriented parallel to the skin with longitudinally arranged collagen fibres that permeate a

large area of the dermis. Because of this, they often exhibit directional sensitivity, the preferred response to stretch being determined by the orientation of the mechanoreceptor ending. This feature imparts an important role to SAII afferents: the capacity to contribute to proprioception by encoding the degree of skin stretch related to joint movement.

Located deeper in the dermis as well as the subcutaneous tissue, Pacinian corpuscles are composed of concentric lamellae around a central core that act as high-pass filters: slowly changing mechanical forces do not lead to deformation of the generator region in the central core, and only very brisk mechanical events lead to excitation. They may be found singly or in groups of four. These units respond to brisk perturbations, most strongly stimulated by gentle tapping over a large receptive field area as well as to tapping over areas remote to the site. They are also very sensitive to blowing over the skin, a very weak yet effective stimulus, resulting from the fricative quality of passing air over the pursed lips, highlighting the exquisite sensitivity of these units. Their instantaneous firing rates are typically higher than Ruffini corpuscles.

The density of each ending type can vary greatly. From recordings from the median nerve which supplies much of the glabrous skin of the hand, 43% of recorded units were FA I, 25% were SA I, 19% were SA II and 13% were FA II classes (Johansson and Vallbo, 1979). This disparity, particularly for FA I units, accounts for the relatively higher density of type I fibres with their smaller receptive fields in the finger pads.

1.3.1.2 Receptive fields

The area of skin in which an afferent response is elicited by a mechanical indentation is commonly referred to as the "receptive field". Typically these receptive fields have low mechanical thresholds at one or multiple points and decrease in sensitivity with the distance from these points, giving the responsive area a circular or ovoid shape. As mentioned previously, the size, shape and responsiveness of these fields is a result of the histology of the afferent branching pattern and mechanoreceptor ending.

Type I afferents branch multiple times before terminating in the superficial dermis. Both Merkel cell-neurite complexes and Meissner corpuscles are associated with the dermal papillae resulting in small circular or ovoid receptive fields with distinct borders. The distribution of these receptive fields is known to vary across the monkey (Darian-Smith and Kenins, 1980) and human hand (Johansson and Vallbo, 1979), with innervation density increasing in the digits compared to more proximal regions. As the density increases, the mean receptive field size of both FA I and SA I afferents has been seen to decrease, with units the distal phalanx (FA I: 39.3 ± 7.5 mm^2; SA I: 20.1 ± 3.1 mm^2) being significantly smaller than those on the palm (FA I: 75.3 ± 24.1 mm^2; SA I: 87.8 ± 28.4 mm^2) (Knibestol, 1975).

1.3.1.3 Mechanical Thresholds

An important measure of unit sensitivity for cutaneous afferents is the mechanical threshold of the unit, that is, the minimum stimulus applied to an area within the

receptive field required to elicit a response, either perceptual or neural. Johansson and Vallbo (1979) investigated both psychophysical and neural thresholds using a small probe that applied an increasing indentation at a constant velocity. From this study they determined a significant difference between unit types. Median indentation thresholds for rapidly adapting units were decidedly lower (FA II: 9.2 mm; FA I: 13.8mm) reflecting their higher sensitivity. SA I units median threshold was considerably higher (56.5mm) while SA II units required extensive indentation for activation (331 mm). Von Frey hairs (nylon filaments probes calibrated to deliver discrete forces to small surface areas) have also been used to provide the punctate stimulus (Johansson et al. 1980). FA II, FA I, SA I and SA II have median thresholds of 0.54 mN, 0.58 mN, 1.3 mN, and 7.5 mN respectively, this similar trend indicating the correlation between indentation amplitude and force.

The mechanical threshold is not consistent within the receptive field of a particular unit. Johansson (1978) used a servo-controlled stimulator that moved across the skin to map this variation. From this study, iso-sensitivity profiles produced demonstrate zones of maximal sensitivity (having the lowest mechanical threshold). As noted above, type II afferents are limited to one of these "hot-spots" within their receptive fields with a fairly uniform sensitivity steadily decreasing as distance from this point increases, resulting in poorly defined borders. Conversely, type I afferents have been shown to possess many points of increased sensitivity per receptive field: 4-7 for SA I afferents and up to 12-17 for

FA I afferents. This has been understood as a reflection of the multiple sensory endings resulting from the terminal branching of type I afferents.

1.3.2 Firing properties

1.3.2.1 General

The unique firing patterns that occur in response to specific stimuli are used as a primary criterion for characterization of cutaneous afferent types. A general description of the distinguishing characteristics of each unit will be provided, followed by an in depth comparison to specific stimuli in later sections.

Type I afferents both respond to distinct phases of indentation stimuli. The rapidly adapting afferents (FA I) are sensitive to dynamic changes in their receptive fields, responding to increasing and decreasing pressure, particularly at the onset and offset of a stimulus. This means that transient stimuli such as light stroking, shear forces and incipient or overt slips are effective for eliciting responses. Slowly adapting units (SA I) provide even more information, firing consistently during static stimuli within the receptive field above their mechanical threshold, but with their firing rate being modulated by accelerations in force magnitude. In this way, SA I units are able to provide both static and dynamic components of indentation stimuli.

Type II afferents respond to more generalized stimuli in the surrounding areas. FA II afferents are highly sensitive, responding particularly well to blowing over the receptive field or tapping on the limb or supporting surface, remote to the receptive field. Their instantaneous firing rates are typically higher than FA I

afferents. SA II afferents respond to lateral skin stretch with directional sensitivity, meaning that stretching is some directions increases the firing rate, while doing so in other directions would decrease it (Knibestol and Vallbo 1970; Knibestol 1975; Johansson 1978). Compared to SA I units, SA II units have a characteristically regular discharge, a feature which is used to distinguish between the two types.

1.3.2.2 Punctate Stimulus

Rapidly adapting afferents (FA I and FA II) can be grouped in their encoding of dynamic changes to the skin induced by punctate stimuli. A hyperbolic log tangent function has been demonstrated by their firing rate when graphed against different indentation *velocities* (Knibestol 1973). In the case of slowly adapting afferents (SA I and SA II) a similar logarithmic relationship is seen between the discharge frequency and the indentation *amplitude* (both static and dynamic phases) (Knibestol 1975). These studies highlight the key distinction between these units' behaviour and functional value.

Type I afferents appear to be more important in tactile discrimination of an uneven surface (Phillips et al. 1992). When stimulated by specific patterns of embossed dot arrays SAI and FAI units have the capacity of discriminating two dots <1.5 mm apart. FAII and SAII afferents when faced with the same task could only discriminate up to 3.5mm. This discrepancy is likely due to the smaller receptive field of the type I afferents and highlights their importance for tactile discrimination.

1.3.2.3 Vibratory Stimulus

Due to their higher dynamic sensitivity, rapidly adapting units are typically more responsive to vibration. This can be seen when a cylindrical probe applies a range of vibration frequencies in a sinusoidal pattern within the receptive fields of each unit class (Johansson et al. 1982). At an indentation amplitude of 1 mm, FA I afferents responded better to lower frequencies than FA II units (8-64 Hz and 64-400 Hz respectively). However, as the indentation amplitude increases beyond 1 mm the sensitivity of the two classes overlap as sensitivity to higher frequencies decreased. This is also true for slowly adapting units, both types being more responsive to low frequencies.

Interestingly for FA I and SA I afferents, vibration sensitivity is not uniform throughout the units' receptive fields. When a stimulator probe is applied to the edges of the receptive field, as opposed to the centre, the discharge frequency of the unit increases. This is particularly notable in SA I afferents (Johansson et al. 1982). This sensitivity gradient makes these units potent edge detectors which is potentially valuable for both manipulation of objects with the hands and responding to postural disturbances with the feet.

1.3.3 Role of cutaneous afferents in standing and locomotion

There is a wealth of evidence supporting the role of cutaneous afferents in free-standing balance as well as in locomotion. While it is known that the receptor subclasses can provide stretch and pressure feedback to the CNS, a more

important contribution to posture stability is reflex modulation of motoneuronal activity at the level of the spinal cord. Both noxious (Shahani and Young 1971) and non-noxious (Delwaide et al. 1981) stimuli to the sural nerve have been shown to modulate responses of the soleus and tibialis anterior muscles. The substrate for this pathway has been suggested by Rossi et al. (1996), who identified that nociceptive and non-nociceptive afferents of the medial plantar nerve share spinal pathways and converge on motorneurones of the tibialis anterior muscle. This has also been achieved by stimulation of single cutaneous afferents (Fallon et al. 2005). In this study, all afferent subtypes were coupled with EMG activity changes, with FAI followed by SAI having the most significant response. Compared to the hand where SAII then FAI units were the more significantly coupled (McNulty and Macefield 2001), they posit that this contrast could be reflective of a central prioritisation of stimulus type. For the purpose of weight bearing both surface-contact detection and continuous assessment of pressure are likely more valued, while stretch and contact detection are more important for detecting conformational changes of the hand with manipulating objects.

This modulation has been shown to be task dependent through studies in standing and walking. The previously described sural stimulation resulted in low-latency inhibition of EMG of the leg and thigh muscles but only during active contraction (Burke et al. 1991). In particular, the TA response was shown to be increased the more precarious the posture (seated vs free-standing vs unstable free-standing). Stimulation of the posterior tibial nerve resulted in facilitation of the soleus while prone but inhibition during free-standing or contraction while prone (Abbruzzeso

et al. 1996). Cutaneous stimulation has also shown some modulation of pretibial muscle spindle activity via the fusimotor system in freestanding (Aniss et al. 1990), an effect that previously was not achieved in recumbent subjects with relaxed (Gandevia et al. 1986) or contracting (Aniss et al. 1988) muscles.

There appears to be a complex relationship between the cutaneous stimuli and gait response during walking which is both phase- and nerve-dependent. Zehr et al. (1997) attempted to summarise the functionally relevant reflex responses, being those that modify the gait pattern, represented by ankle and knee angle. Transcutaneous stimulation of the posterior tibial nerve via the skin posterior to the medial malleolus was found to induce knee flexion and ankle dorsiflexion during the stance-to-swing phase but ankle plantarflexion during late swing phase. The former can be interpreted as a stumble correction response to an unexpected obstacle while the latter may represent a placing reaction when the ground is detected earlier than expected. Reflex modulation of EMG activity by stimulation of single cutaneous afferents has also been seen in muscles of the upper limb (Bent and Lowry 2013) which makes the concept of a global stabilisation response to cutaneous stimuli alone plausible.

There is some evidence that cutaneous activity encodes proprioceptive information in free-standing. Vibration of the sole of foot, similar to muscle afferent studies has been used to trigger predictable directional body tilts (Kavounoudias et al. 1998). The authors claim that by using a frequency of 100 Hz that this would selectively activate the cutaneous afferents, based on the

understanding that human muscle spindle primary endings primarily respond (in a phase-locked fashion) at 80 Hz sinusoidal stimulation, but given that until now recordings from spindles in the intrinsic muscles of the foot have never been performed such a conclusion is risky.

To further support the relationship between postural control and cutaneous afferent feedback many studies exist in which this sense is impaired by experimental protocol, age or disease. Immersing the foot in ice to reduce plantar sensation leads to significant changes in gait patterns, affecting joints of the ankle, knee and hip (Eils et al. 2004). Anaesthesia of the metatarsal heads of both feet via iontophoretic application of lidocaine results in predominately mediolateral balance deficits, while whole foot anaesthesia causes anteroposterior deficits (Meyer et al 2004). However, in both cases this occurs only when vision is occluded. Decreasing cutaneous reflexes and increasing plantar detection thresholds have been shown to correlate with age and this is associated with increased postural sway (Peters et al. 2016). In addition, chronic disease that results in reduced peripheral sensation. This has been seen in diabetic neuropathy and Charcot-Marie-Tooth disease (Kars et al. 2009). Diabetic neuropathy has been shown to impact balance in free-standing increasing area of CoP, velocity of CoP and CoP trace length (Uccioli et al. 1995). Both type 2 and severe type 1a variants of Charcot-Marie-Tooth disease have been seen to increase sway area compared to a healthy population (Nardone et al. 2000, Nardone et al. 2006).

1.3.4 Previous microneurographic work from skin afferents in the sole of the foot

Single unit recordings from cutaneous afferents of the sole of the foot have previously been obtained from the tibial nerve using the microneurographic technique (Fallon et al. 2005; Kennedy and Inglis 2002; Lowrey et al. 2013; Strzalkowski et al. 2015a, b). Strzalkowski et al. (2018) combined this data and other unpublished data to provide an overview of 401 cutaneous afferents. These units were characterised, with firing thresholds, receptive field areas and anatomical location described. FAI and SAI units were found to have significantly higher unit density in the toes when compared to the arch and heel as well as the lateral arch and metatarsals compared with the medial counterpart. They posit that the significance of these regions as the limits of the base of support suggest that these type I afferents play a unique role in postural maintenance to muscle afferents. An earlier microneurographic study of the sural nerve by Trullson (2001) characterised 104 cutaneous afferents, determining unit type and receptive field but finding no significant difference between units of the same class on glabrous and non-glabrous skin on the lateral aspect of the foot. Importantly, all previous recordings were made in prone subjects, which allows access to the tibial nerve in the popliteal fossa and the sural nerve 15cm distal to this. While this positioning is ideal for identifying receptive fields and other mechanical properties, due to the exposure of the sole of the foot, it is not suitable for assessing the physiological activity of these units in a functional role, such as during free standing.

1.4 The posterior tibial nerve

1.4.1 Why the posterior tibial nerve?

Invasive microelectrode recordings from human peripheral (and some cranial) nerves have contributed much to our understanding of the somatosensory nervous system and of sensorimotor control in humans. It has been 50 years since the first microneurographic recordings from awake human subjects were published by Karl-Erik Hagbarth and Åke Vallbo at the Academic Hospital in Uppsala, Sweden. Although they thought they would only ever be able to record multiunit activity (Hagbarth and Vallbo 1967), to their surprise they managed to impale sensory axons of individual muscle spindle endings in the median nerve and to record action potentials from single afferents during both passive muscle stretch and active contractions (Hagbarth and Vallbo 1968, 1969; Vallbo and Hagbarth 1967). Moreover, they also managed to record from individual mechanoreceptor afferents supplying skin (Vallbo and Hagbarth 1968). Much has been learned since these first recordings, with many laboratories throughout the world detailing the firing properties of individual somatosensory afferents, but a major limitation to recording from single axons via a microelectrode inserted through the skin has been the requirement to limit movements that could dislodge the microelectrode. Given that the microelectrode is held in place only by virtue of the skin and underlying tissues that support it, it is quite remarkable that single-unit recordings can be made at all.

Most unitary recordings during passive and active movements are obtained from peripheral nerves proximal to the site of action, such as the median, ulnar, or

radial nerves in the upper limb for movements of the hand (Burke et al. 1988; Vallbo 1971, 1973, 1974), which, given that these nerves are located two joints (wrist and elbow) away, distinct from the supplied muscles, afford great flexibility of the hand to perform behavioral tasks, such as grasping or lifting an object. For the foot, the common peroneal (fibular) nerve is most commonly used, but given that the fibular head is only one joint away (i.e., the ankle), this limits the scope of action; the long (extrinsic) muscles acting on the foot originate from the tibia and fibula, close to the intraneural recording site and hence susceptible to movement of the microelectrode during movements of the ankle. Moreover, unlike recordings from the median, ulnar, or radial nerves in the upper limb, which can target fascicles supplying the skin and intrinsic muscles of the hand, those from the common peroneal nerve can only target muscle afferents from the pretibial flexors and skin on the dorsum of the foot or lateral aspect of the leg. Conversely, the intrinsic muscles of the foot and the skin of the sole are supplied by the tibial nerve, but accessing this nerve in the popliteal fossa means that the knee needs to remain extended at all times: the subject cannot flex the knee to sit down and rest. Recording distal to the ankle joint, from the posterior tibial nerve, avoids some of these issues.

Although recordings from muscle spindle afferents have been made from the common peroneal nerve in freely standing humans (Burke and Eklund 1977), and we know that stretch reflexes in the extrinsic muscles of the foot, those acting about the ankle, are essential for maintaining ankle stiffness (Fitzpatrick et al. 1992), these data alone cannot provide a complete picture of the somatosensory

neural substrates required for maintenance of the upright posture. Mauritz and Dietz (1980) noticed increased body sway in subjects with eyes closed following ischemia of the lower legs by pneumatic cuffs, which blocked muscle and cutaneous afferents. Although we also know that anaesthesia of the sole of the foot (Meyer et al. 2004) or of the entire foot (Fitzpatrick et al. 1994) still allows one to maintain an upright stance, albeit with some disturbances in force distribution (Meyer et al. 2004), we know very little about the potential roles of muscle afferents in the intrinsic muscles of the foot, or of cutaneous afferents in the sole of the foot, in the fine sensorimotor control of upright stance. Given that the brain has rapid access to the sensory information provided by muscle and cutaneous afferents in the foot (Macefield et al. 1989b), it is likely that these inputs contribute importantly to our ability to stand upright on our feet.

Recordings from individual cutaneous mechanoreceptors supplying the sole of the foot have been made from the tibial nerve (Kennedy and Inglis 2002; Strzalkowski et al. 2015a, 2015b, 2017) or sural nerve (Trulsson 2001) in the popliteal fossa in passive conditions, or from the tibial nerve during weak static contractions of the dorsiflexors and plantar flexors (Fallon et al. 2005), but, until now, there have been no recordings made from sensory endings located in the small muscles of the foot. The posterior tibial nerve, located behind the medial malleolus of the ankle, is a mixed nerve that is used routinely in clinical neurophysiological assessments to measure, for example, conduction velocities in the lower limb (Macefield et al. 1989b) or somatosensory evoked potentials from muscle and cutaneous afferents in the foot (Macefield et al. 1989a).

1.4.2 Anatomy of the posterior tibial nerve

Common to any nerve selected for study via microneurography, a comprehensive understanding of the course of the nerve and the anatomy of its surrounding structures is required. Favorable characteristics for microneurography include low anatomic variability of the course of the nerve (which improves experimental reliability), low mobility of both the nerve and its surrounding structures, proximity to the skin, and few intervening structures (consisting only of connective tissue). With appropriate knowledge, the posterior tibial nerve can be approached at a site that satisfies these criteria. Anatomic details were obtained from *Sarrafian's Anatomy of the Foot and Ankle* (Kalikian and Sarrafian 2011).

The posterior tibial nerve extends from the arcade of the soleus muscle to the tibiotalocalcaneal canal as it travels along the posteromedial aspect of the ankle. This region is defined anteriorly by the medial malleolus and posteriorly by the calcaneal tendon, both of which are easily palpable. Within this portal, there are few intervening structures between the skin surface and the nerve (Figure 1.9). The subcutaneous tissue has a lamellar constitution with superficial veins and nerves. Insertion of the microelectrode may cause slight discomfort at this stage. Deep to this, the posterior tibial nerve descends on the posterior aspect of the medial malleolus. At its distal third, the tibial vessels and tendons of flexor digitorum longus and tibialis posterior lie anteromedially while the tendon of the flexor hallucis longus is lateral. Posterolaterally is the calcaneal tendon, its medial border approximately on the same sagittal plane as the lateral border of the

posterior tibial nerve. As the calcaneal tendon is easily palpable, this can be a good guidepost to avoiding the surrounding structures.

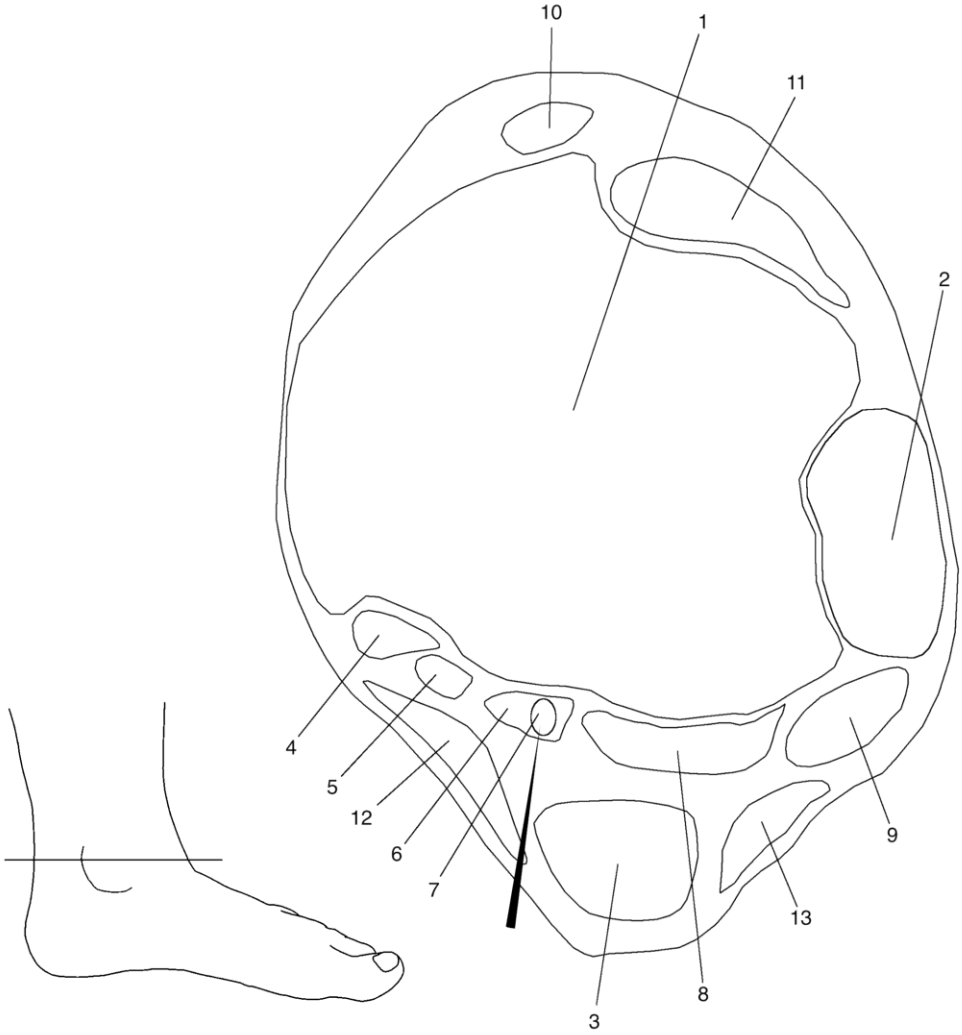

Figure 1.9: *Diagrammatic representation of cross-section of the ankle at the tip of the medial malleolus demonstrating the path a microelectrode to the posterior tibial nerve. (**1**, tibia; **2**, fibula; **3**, posterior superficial compartment (for calcaneal tendon); **4**, tunnel of tibialis posterior tendon; **5**, tunnel of flexor digitorum longus tendon; **6**, tunnel of posterior tibial neurovascular bundle; **7**, posterior tibial nerve, impaled by microelectrode; **8**, tunnel of flexor hallucis longus tendon; **9**, lateral compartment (for*

peronei muscles); ***10****, tunnel of the tibialis anterior tendon;* ***11****, anterior compartment;* ***12****, medial calcaneal compartment;* ***13****; lateral calcaneal compartment*

Two layers of aponeuroses support these structures. The superficial layer occurs at the level of the triceps surae and contains the calcaneal tendon. The deep aponeurosis covers the aforementioned tendons and neurovascular bundle. After the tendons cross, each tendon has its own separate compartment deep to this layer. The posterior tibial nerve, artery, and vein all share a common compartment. Between the deep and superficial layers is adipose tissue, termed the posteromedial "safe" zone. Distally, these layers fuse to form the flexor retinaculum, which forms a triangle. Most relevantly, its posterior edge follows an oblique line drawn from the anterior border of the medial malleolus to the posterosuperior corner of the os calcis. The experimenter should expect two moments of brief resistance during advancement of the electrode pieces each aponeurosis. Because of the thickness of the flexor retinaculum, electrode insertion distal to the oblique line described above should be avoided.

It should be highlighted that the intervening structures described above offer a distinct mechanical advantage to the stability of the electrode when performing microneurography in comparison with the sites used in previous studies. Whereas the median or common peroneal nerve lies typically 5–15 mm deep to the insertion site, the posterior tibial nerve is at a depth of <20 mm. The increased frictional resistance provided by these tissues, while restricting fine manipulation of the electrode during searching, improves the stability of the electrode against

gravitational and inertial forces, which is vital in recording in a free-standing context, particularly one with postural perturbations.

Within the posteromedial aspect of the ankle, the posterior tibial nerve gives off vascular and articular branches that supply the surrounding area and ankle as well as cutaneous branches. The medial malleolar branch innervates the skin over the malleolus, whereas the medial calcaneal nerve innervates the medial and central segments of the heel. Of particular significance, the medial calcaneal nerve arises in one or multiple branches and descends posterior to the nerve proper (Havel et al. 1988). Although extremely difficult to impale, it is often the first nerve to be stimulated by electrical current from the microelectrode during internal searching. Subject-reported sensations on the medial or underside of the heel provide a strong indication that the electrode is facing the nerve proper and will reach it with further advancement.

Distally, the posterior tibial nerve bifurcates into the lateral plantar nerve and medial plantar nerve. There is a high physiological variability as to the level at which this division occurs. Dellon and Mackinnon (1984) studied 31 cadaveric feet to determine the distribution of the division. The “malleolar-calcaneal axis,” a line extending from the centre of the medial malleolus to the centre of the calcaneus, was defined as a reference. In 55% of feet, the bifurcation was on the axis, and in 90% of feet, it was within 1 cm. Although the bifurcation was also found in one case to be 5 cm proximal to the axis, it can be used as a good measure for impaling the nerve before its division.

1.4.2.1 Branches of the posterior tibial nerve

The lateral plantar nerve and medial plantar nerve that result from the division of the posterior tibial nerve supply the majority of the cutaneous innervation of the plantar surface of the foot, suggesting their importance in detecting changes in posture and stability. Specifically, the lateral plantar nerve supplies the lateral aspect of the sole, lateral half of the fourth toe, and plantar and distal dorsal aspects of the fifth toe. The medial plantar nerve supplies the major central and medial segment of the sole, the medial half of the fourth toe, and the plantar and distal dorsal aspects of toes 1–3. Also of significance is the aforementioned medial calcaneal nerve, which supplies the central and medial segments of the heel.

Many of these nerves also provide extensive motor innervation for the foot. The lateral plantar nerve supplies abductor digiti minimi muscle, flexor digiti minimi brevis muscle, quadratus plantae muscle, transverse and oblique heads of adductor hallucis muscle, interossei muscles, and second to fourth lumbrical muscles. The medial plantar nerve supplies the abductor hallucis, flexor hallucis muscle, flexor digitorum brevis muscle, and first lumbrical muscle. This innervation contributes to movement of all toes, excluding extension, as well as assisting with maintenance of the longitudinal arches of the foot.

1.5 Aims

During my candidature I explored the potential of recording muscle spindle activity from the posterior tibial nerve, which had never been attempted. From the body of work previously presented, it is clear that these units are likely to contribute proprioceptive information from the foot in free-standing conditions.

1.5.1 Study 1: Characterisation of muscle spindles supplying the intrinsic muscles of the foot in unloaded conditions

As this is the first body of work to record from muscle spindles supplying the intrinsic muscles of the foot it was necessary to first characterise the firing properties of these units in the absence of any applied load. The aim of this study was to report on the firing characteristics, namely frequency range and variability of single unit muscle spindles of subjects in an unloaded position, seated with their foot suspended. The passive foot movements used to elicit a response or change in frequency were also noted so that role of spindles supplying a specific intrinsic muscle could be better understood.

1.5.2 Study 2: Behaviour of muscle spindles supplying the intrinsic muscles of the foot in free-standing conditions

Once the baseline activity of these muscle spindles was understood, it was important to assess their function in a physiological role, namely during postural support of the upright free-standing body. To achieve this, single unit muscle spindle activity was recorded in subjects whilst standing on a force platform that measured forces and torques in three orthogonal axes and changes in centre of

pressure. The aim of this study is to determine the relationship between muscle spindle activity and centre of pressure (CoP) in free-standing conditions with and without postural disturbances. By instructing subjects to load and unload their foot the role of spindles in these movements was also examined.

1.5.3 Study 3: Behaviour of cutaneous afferents from the sole of the foot in free-standing conditions

In addition to recording muscle spindle activity it was also recognised that this was the first opportunity to record from cutaneous afferents of the sole of the foot in loaded conditions. While limited by the recording method in identifying the receptive field area and firing thresholds, the role of cutaneous afferents in detecting changes in CoP and contact with surfaces and edges were nevertheless able to be appreciated, although a detailed investigation of this was beyond the scope of this thesis.

2 METHODS

2.1 Data summary

For Study 1, data were collected from 12 healthy subjects (5 women and 7 men; age range: 18 years – 40 years) in 12 experimental sessions. For study 2, data were collected from 11 healthy subjects (3 women and 8 men; age range: 18 – 28 years) in 11 experimental sessions. For study 3, data were collected from 21 healthy subjects (6 women and 15 men; age range: 18 years – 25 years) in 24 experimental sessions. In all studies, all subjects provided written, informed consent. These studies were conducted with the approval of the Human Research Ethics Committee, Western Sydney University, with all procedures conducted in accordance with the principles of the Declaration of Helsinki.

2.2 Subject positioning

The posterior tibial nerve can be recorded with the subject lying prone or supine, in a seated or standing position. Study 1 is recorded with subjects in the supine position while for Studies 2 and 3 recordings were made in seated and standing positions. In the case of supine recordings, it is important that there is space for posterior insertion of the electrode. Subjects were seated in a cushioned chair supporting their legs horizontally. Their feet extended past the edge of the chair and were unsupported, resulting in a natural ankle plantarflexion. The legs were slightly parted and the leg of the recorded nerve was laterally rotated to allow access to the posterior tibial nerve.

Subjects stood on a force plate (Portable Force Platform, AccuGait) which measured Z-axis forces of four quadrants, and X and Y-axis forces between

quadrants. Subjects were asked to stand with both feet parallel and at shoulder-width. The force plate was mounted on a motorized platform (custom-built XY platform, manufactured at Hong Kong University of Science and Technology) to which a safety bar that rose to chest height was mounted, allowing the subjects to support themselves, as well as an accelerometer to measure acceleration of the platform in the X and Y directions. In addition, a motorized tilt table (Athlegen, Australia) was positioned behind the subject to allow them to sit or lie down if needed. Ag-AgCl surface electrodes were attached to the skin overlying the bellies and tendons of the tibialis anterior and soleus muscles of the ipsilateral leg to record electromyographic (EMG) activity. It was sampled at 2 kHz and filtered at 10 Hz-1 kHz. A bipolar goniometer (MLTS700 goniometer, AD Instruments) was attached medially to the foot and leg of the contralateral limb, so that the rotational axis aligned with the medial malleolus, to approximate the ankle angle of the recorded foot. This was done with the assumption of symmetrical changes in ankle angle during postural sway and sinusoidal displacements. The goniometer signal was sampled at 100Hz (DC to 10 Hz bandpass). Figure 2.1 shows the recording arrangement for the standing recordings. A mounted camera (C920 HD Pro Webcam, Logitech) was pointed at the recorded foot with the footage synced to the neural signal so as to provide extra context to assist interpreting gross CoP changes and to detect accessory movements that would not cause notable CoP changes (e.g. toe extension).

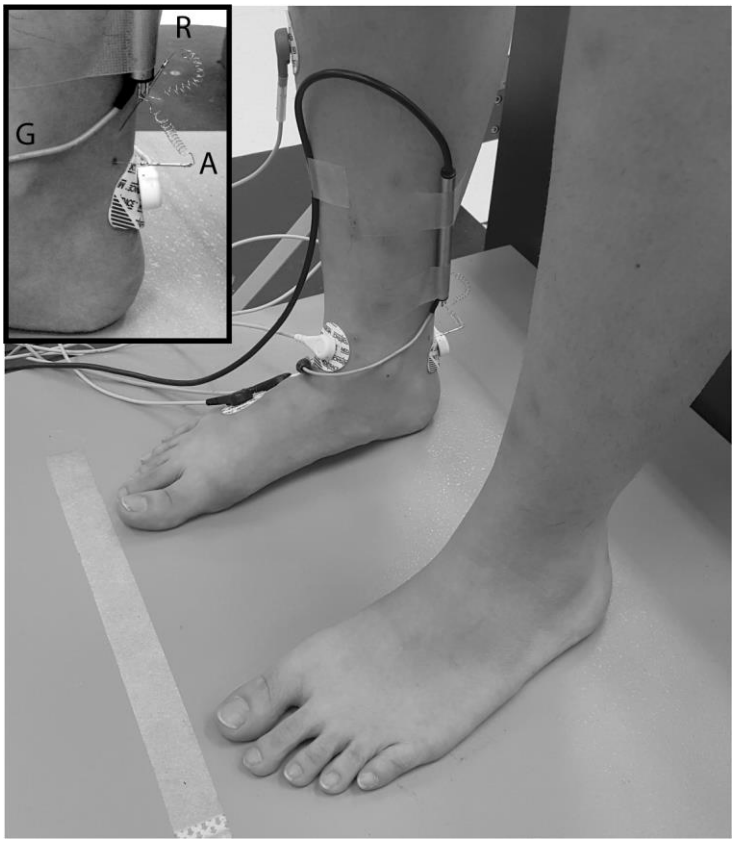

Figure 2.1: *Photograph of the experimental set-up. The inset shows a close-up of the electrodes: A = active microelectrode, R = reference microelectrode, G = ground electrode.*

For both prone and supine positions, the experimenter was comfortably seated, similar to microneurography performed on the common peroneal or median nerves recorded from the same position. However, for both seated and standing positions, the experimenter was required to sit or recline near the subject's feet to search appropriately. In these situations, a cushion and regular adjustment of position were used to prevent muscle strain and numbness of the experimenter: this can be circumvented by having the subject's foot unloaded during the searching process and then lowering the subject to a loaded position for recording.

However, this movement increases the likelihood of electrode instability and should be avoided if possible.

2.3 Intraneural recording of the posterior tibial nerve

The following identification procedure was used for all studies with only the subject's position varying. It is common practice in microneurography to use external electrical stimulation to identify the insertion site for the microelectrode, as this determines the point on the skin with the closest straight-line distance to the nerve. Unfortunately, in the case of the posterior tibial nerve, this direct route would require the electrode to advance through a number of resistant internal structures (i.e., the tendons of the tibialis posterior and flexor digitorum longus and the tibial artery), causing discomfort to the subject and reduction of electrode impedance. In addition, if the electrode has passed through a tendon, any slight movement of the foot or toes will cause the electrode to bend, thus thwarting further attempts to refine electrode placement.

Here, instead of using electrical stimulation, the location of the nerve was determined by anatomic knowledge of its likely position. This was done reliably with the posterior tibial nerve as it is contained in the same fascia sheath as the tibial artery and vein. First, the pulse of the tibial artery was palpated posterior to the medial malleolus to determine the appropriate horizontal plane. The insertion site was identified on this plane medially adjacent to the calcaneal tendon and was marked and sterilized with an alcohol wipe. A 30-mm high-impedance (>1 MΩ) tungsten microelectrode (FHC, Bowdoinham, ME) with a 200-μm diameter and a

2- to 5-μm uninsulated tip was inserted percutaneously at the site, angled anteriorly ~60° to the normal, with an uninsulated reference microelectrode inserted subcutaneously ~2 cm proximal to the recording electrode. The microelectrodes are connected to the input terminals of an isolated headstage (Neuro Amp EX; ADInstruments, Sydney, Australia) via insulated coiled copper wires.

Given that the posterior tibial nerve is typically 20 mm deep to this insertion point, intraneural stimulation is essential for reliable advancement of the microelectrode. Intraneural stimulation through the recording microelectrode, relative to the reference electrode, was applied while the experimenter made adjustments to the angle and depth of the microelectrode so as to approach the nerve. The stimulus involved brief pulses (0.2 ms) of cathodal (depolarizing) stimuli (<1 mA) that were delivered at 1 Hz through the microelectrode connected to a computer-controlled, constant-current isolated stimulator (Stimulus Isolator; ADInstruments); the anode was a Ag-AgCl surface electrode on the skin of the opposite side of the ankle.

The electrode was determined to be closer to the nerve if the current required to activate the nerve was lower, which occurred through advancement of the microelectrode. Successful stimulation of the nerve was determined as either muscle twitch from the supplied muscle, observable by sight or palpation of the associated tendon, or radiating paraesthesiae ("pins and needles") in the cutaneous distribution of the nerve. The subject was given a diagram of the plantar

surface of the foot with which they indicated the area of any paraesthesiae more accurately, removing the temptation to lean forward to point to the area, which would lead to undesirable microelectrode dislodgement. It should be noted that as branches of the medial calcaneal nerve arise proximally to this plane and course posteriorly to the nerve proper, subject reporting of paraesthesia in the sole of the heel was a good indicator that the microelectrode was proceeding along the correct angle. The relatively small diameter of these branches means that they are difficult, but not impossible, to impale. Thus a common pattern of subject reporting during microelectrode advancement was steadily increasing then decreasing sensations in the heel followed shortly by firm muscle twitches and sensations in the anterior or lateral sole as the nerve proper was stimulated.

Stimulus currents were reduced progressively as the response increased in intensity, until they were evoked at currents <0.02 mA, indicating impalement of the nerve fascicle sheath. The stimulating leads were then removed, and the preamplifier and amplifier were switched on, the surface electrode on the skin on the opposite side of the ankle serving as a ground electrode. The isolated headstage (preamplifier) was taped proximally to the recording site and amplified the signal 100×. Further amplification was then performed by the Neuro Amp EX amplifier (total gain 20,000, band pass 0.3–5.0 kHz). The signal was then digitized (LabChart 7, PowerLab 16/35; ADInstruments) and acquired by computer. With the use of both visual and auditory feedback from the intraneural recording, minute adjustments to the position of the microelectrode were made until spontaneous or evoked muscle spindle or cutaneous afferent activity was

encountered. As with other nerves, slight adjustment of the microelectrode position was conducted so as to record spontaneous or evoked neural activity, ideally from a single muscle spindle or cutaneous afferent.

2.4 Characterization of fascicle and afferent type

Characterisation criteria were consistent across all studies. Afferent unit characterization for microneurography requires testing the unit response to controlled stimuli. This requires access to the receptive field and surrounding skin in the case of cutaneous afferents and the ability to manipulate freely muscle tendons and the mobilized joint for muscle spindles, Golgi tendon organs, or joint receptors. These requirements are challenging in the free-standing position, as the sole of the foot is obstructed and flexion of the toes is limited. To overcome this, after achieving reliable afferent activity, the subject was to unload their foot, raising it ~0.05 m above the platform. As movement of the ankle creates instability for the recording, my instructions were specific. The subject first shifted their weight onto the contralateral foot without moving the recorded foot. They then used their ipsilateral internal oblique and contralateral hip abductors to tilt the pelvis so as to raise the ipsilateral leg while stabilizing its hip, knee and ankle joints. Throughout this movement and maintenance of this position, they used both hands for support on a horizontal, insulated metal bar in front of them. It was important that the characterization process was restricted to <2 min at a time to avoid fatiguing the contralateral leg.

In addition to this time limitation during unloaded characterization, it is important to note that as the subject was maintaining an upright posture with the feet relatively immobilized (so as to reduce unnecessary electrode movement), the experimental protocol (including searching and characterization if done in free standing) was limited to 30 minutes. Extended time in this position increased not only fatigue, but risk of lightheadedness and fainting as well. This was mitigated by periodically retracting the microelectrode to a depth of 10 mm, separate from neighbouring tendons, and instructing the subject to rest seated for five minutes as well as to stand up and sit down three times before continuing searching and recording to improve blood circulation to the legs and feet.

A muscle fascicle was identified by the following criteria: *1*) electrical stimulation through the microelectrode induced muscle twitches below a current threshold (0.01–0.02 mA), *2*) percussion or passive stretching of tendons or muscle bellies supplied by the posterior tibial nerve resulted in afferent mechanoreceptor impulses, and *3*) light stroking of the skin did not evoke impulses of tactile afferents. After the fascicle was identified, a single presumed muscle spindle unit was isolated and the associated muscle was noted. Muscle spindle afferents demonstrated a characteristic tonic discharge, of which the mean frequency could be increased by stretching the associated muscle and decreased by passively unloading the muscle. The same criteria used to identify muscle spindles in the intrinsic muscles of the hand were used here (Burke et al. 1988). Briefly, individual digits were subjected to flexion, extension, abduction, adduction, extorsion, and intorsion movements in a systematic manner. Of importance here

is the limitation on toe manipulation that should be observed. As the path of the microelectrode to the nerve is bordered medially by the tendon of flexor digitorum longus and laterally by that of flexor hallucis longus, stretching these tendons can cause movement of the microelectrode, which potentially can result in loss of a recording site. This applies also to active contractions of these muscles. Because of this, it is highly recommended that extension of the toes (particularly the 1st digit) be done carefully or left until the end of the protocol. Additionally, all active contractions should be weak and isometric, with the toes supported against a surface to prevent movement. It was not attempted to determine whether an off-discharge followed an active contraction as it was difficult for the subject to perform certain toe movements selectively (e.g., adduction/abduction of the digits). Furthermore, active flexion of digits, particularly the 1st digit, jeopardized the signal quality due to the proximity of the nerve to the flexor hallucis longus tendon. Twitch tests were not performed as this requires selective stimulation of the innervated muscle through an external electrode or by delivering the stimuli through the recording microelectrode: unfortunately, the fact that the majority of the intrinsic muscles of the foot are deep precluded the former approach, and the lack of a rapid switching relay that prevented the preamplifier blocking during intraneural stimulation prevented the latter. Moreover, given that the presumed muscle spindles responded to passive stretch, they can be ruled out as being Golgi tendon organs.

The criteria used to identify and distinguish cutaneous afferent types were as follows: *1*) the subject reported electrical sensation often described as pins and

needles coincident with electrical stimulation through the microelectrode below a current threshold (0.01–0.2 mA) and *2*) afferent impulses were produced in response to stroking or palpation of the skin. The type of manipulation that elicited a response would determine the mechanoreceptor type: type I units have small receptive fields with well-defined borders [median fast adapting type I (FAI): 38.0 mm^2, slowly adapting type I (SAI): 70.9 mm^2; Kennedy and Inglis 2002]. FAI units respond during dynamic phases of indentation, whereas SAI units respond during static phases. Type II units have larger receptive fields with poorly defined borders (median FAII: 284.2 mm^2, SAII: 127.4 mm^2; Kennedy and Inglis 2002). FAII units are highly sensitive, responding to tapping over areas remote from the receptive field, and SAII units respond to skin stretch and have directional sensitivity (Macefield 1998).

It should be noted that although unloading the subject's foot permitted access to the skin of the foot for unit characterization, there were several limitations. Because of the poor angle, blowing over the receptive field to test FAII response was not possible. Moreover, the restricted time limit for characterization made testing mechanical thresholds with von Frey hairs impractical. Receptive fields were harder to define precisely without a direct line of sight of the sole of the foot.

2.5 Activity characterisation protocols

2.5.1 Seated protocol

This protocol was used for characterisation of identified muscle spindle afferents in the seated position. Following identification of a single muscle spindle afferent,

the metatarsophalangeal and interphalangeal joints were passively manipulated systematically - initially as a slow circumduction of each digit's metatarsophalangeal (MTP) joint was implemented, gradually increasing the radius of rotation. If an afferent response resulted from circumduction of a particular digit, this digit underwent further more specific manipulations. These were – in order of priority – extension, flexion, abduction, adduction, intorsion and extorsion. Each movement was a ramp-and-hold motion to determine minimum and maximum firing rates. Where possible, the proximal interphalangeal joint extension was also tested. Release of the digit was brisk to assess the presence of an off-discharge, an increase in firing due to stretch of the muscle spindle as the muscle returned to its resting length. Following these movements a vibrating probe was applied to the plantar surface or interosseus spaces of the foot over the area of the presumed muscle belly. After a vibration response was tested the subject was asked to weakly extend and flex their digits – an isometric contraction against the experimenter's palm. During these tests the experimenter would also palpate areas of the plantar surface of the foot to check any response to pressure on the muscle belly of the recorded muscle. Where possible, afferents were sorted into primary or secondary endings. Primary units had an irregular spontaneous discharge and/or produced an off-discharge following sudden cessation of a slow ramping contraction. Secondary units had a more regular discharge rate and did not exhibit an off-discharge. For non-spontaneous units the activity was studied during a sustained muscle stretch to achieve a constant response.

2.5.2 Standing protocol

This protocol was used for characterisation of identified muscle spindle and cutaneous afferents in the free standing position. Microneurography recordings depend on the uninsulated electrode tip to remain within a single neuronal axon. The quality of the recording refers to the signal:noise ratio achieved, where an adequate ratio (usually at least more than 3:1) allows for discrimination of the target afferent from the surrounding electrical activity. This is dependent on minimal movement of the electrode and surrounding structures. Free-standing microneurography recordings are hence, by nature, less stable, with recording quality being threatened by both gross and fine changes to the surrounding tissue during postural stabilisation. Acknowledging this limitation, the chosen order of the protocol was to minimize ankle movement until required: *1)* Free unsupported standing; *2)* Free unsupported standing with platform perturbations; *3)* Active manoeuvres; *4)* Transitioning between standing and seated positions. If signal quality diminished between protocols it would either be continued as a multiunit recording or the identification process would be reverted to.

For recordings of non-spontaneous neural activity, at the start and end of each protocol the recording site quality was tested by experimenter manipulation of the identified receptive field. During all recording periods the subject was asked to close their eyes. In free unsupported standing, subjects were asked to stand in comfortable natural stance for a period of at least one minute. Postural perturbations were generated by sinusoidal anteroposterior and mediolateral

movement of the supporting motorized platform. The frequency tested was 0.2 Hz with a maximum acceleration of 5 mG for 50 cycles, delivered via servo-controlled linear actuators. Active manoeuvres requested were leaning anteriorly, posteriorly and laterally as well as raising and lowering the ipsilateral and contralateral feet alternatively. During transition from seated to standing, subjects were asked to use the supporting bar in front of them to take some of their weight and minimize ankle movement.

2.6 Data analysis

Action potentials from single muscle spindle and cutaneous afferents were discriminated using a dual time-amplitide window discriminator (Spike Histogram, LabChart 7; ADInstruments, Sydney, Australia).Discriminator levels of neural activity were adjusted so as to include all positive-going peaks of width <0.5 ms and height >5 μV above the background noise.

For Study 1, the instantaneous frequency of the spindle firing was calculated from the discriminated population, noting the minimum and maximum frequencies achieved. The mean and standard deviation of the frequency were established based on a section of consistent firing of at least 5 seconds. For spontaneously active units this was with no experimenter manipulation and for non-spontaneous units this was during a stimulus of constant pressure over the muscle belly or a constant imposed joint angle. These values were used to calculate the coefficient of variation (CV = σ/μ). Changes in frequency or onset/offset of activity in relation to manipulation of digits were noted.

For Study 2 and Study 3, vertical force data from four quadrants were derived from the supporting force plate (Portable Force Platform, AccuGait, AMTI). This raw data was recorded and used in a formula to provide the centre of pressure (CoP) changes in the x (mediolateral) and y (anteroposterior) directions (Labchart 7, Powerlab 16/35; ADInstruments). Both description of the raw data and the formulae used to calculate the CoP are described in Table 2.1. The CoP data was then smoothed using a Bartlett window of 2 seconds. Patterns between afferent discharge frequency and CoP changes were qualitatively noted and described. This was done with the assistance of the mounted camera footage. Unfortunately, the CoP data did not lend itself to the detection of a sufficient number of cyclic peaks to generate a robust autocorrelation histogram, therefore preventing us for undertaking a crosscorrelogram between the nerve signal and the CoP, necessary for quantitative analysis.

Variable	Formula
CoPx	$\frac{My + 0.05Fx}{-Fz}$
CoPy	$\frac{Mx + 0.05Fy}{Fz}$

Table 2.1: *Formulae for calculating CoP from forces and moments measured with the force plate (Portable Force Platform, AccuGait, AMTI). Eight raw data values were collected representing the vertical (z) forces in each quadrant (Az: left anterior, Bz: right anterior, Cz: left posterior, Dz: right posterior) and the anteroposterior (y) and mediolateral (x) forces between quadrants (i.e. YAC is the anteroposterior force between the left anterior and posterior quadrants). These were used to calculate forces (Fx, Fy, Fz) and moments (Mx, My, Mz) on the x, y and z axes using adjustment coefficients specific to the platform. Finally CoPx and CoPy was calculated from forces and moments.*

3 CHARACTERISATION OF MUSCLE SPINDLES SUPPLYING THE INTRINSIC MUSCLES OF THE FOOT IN UNLOADED CONDITIONS

As mentioned previously, muscle spindle activity has not previously been recorded from the small muscles of the foot. The intrinsic muscles of the foot, like those in the hand are defined by their very small tendons and small actions on a single structure. We know a lot about the behaviour of muscle spindles of the muscles of the hand (Burke et al. 1988, Macefield et al. 1990): they respond in one preferred direction and act about only one joint responding to angular rotation that leads to stretch of the muscle. Given that the foot has the neuromachinery for fine motor control one might expect that the intrinsic muscles of the foot would behave similarly and that muscle spindles acting on the toes act in a manner identical to those seen to those acting on the fingers. Like muscles in the hand they have short tendons but typically when flexing the toes they are flexed in unison. This is in part due to mechanical linkage by the superficial and deep transverse metatarsal ligaments which are attached to the metatarsal heads. Hence, it could be that muscle spindles activity in the foot is sensitive to remote digit movement.

In addition to muscle spindles acting on the toes, the foot is unusual in having muscles that traverse a large area from the heel to the metatarsals (Tosovic et al, 2012), known as the longitudinal arch that is deformed during weight bearing and locomotion. These muscles (abductor hallucis, flexor digitorum brevis and quadratus plantae) are likely to play an important proprioceptive role in standing. Indeed, these muscles have been shown to stabilise the foot architecture against z-axis forces (Kelly et al. 2014) and it is reasonable to assume that spindle afferents provide the substrate for detecting the changes in muscle length produced by these forces.

Ultimately, the goal is to examine their functional role in freestanding. In order the interpret this function it is important that their role in an unloaded state is first characterized, acting as a control condition to when in loaded standing while supported and later free standing. The purpose of this study therefore is to record muscles spindle activity from intrinsic muscles of the foot reporting unit type, firing frequency and response to stretch of the supplied muscle and surrounding structures.

3.1 Unit Summary

Unitary recordings were made from 26 muscle spindle afferents supplying the intrinsic muscles of the foot, obtained with the foot in the position of rest in 12 seated subjects. Firing properties of the afferent sample are reported in Table 3.1. Because the responses are so variable, the unit numbers in Table 3.1 are used to refer to specific units to provide further details on their firing properties. Table 3.2 summarizes the responses of each muscle spindle to movements of the corresponding metatarsophalangeal (MTP) joint.

Unit	Muscle	Sponta-neous	Type	Min Freq (Hz)	Max Freq (Hz)	Mean Freq (Hz)	SD	CV	Stimu-lating Digits
1	AbH	No	II	3.8	15.4	10.6	0.72	0.07	1
2	FHB	Yes	II	3.1	7.4	5.1	0.27	0.05	1
3	AdH	No	Ia	6.4	9.9	-	-	-	1
4	FDB	No	II	4.2	10.3	7.9	0.52	0.07	1
5	FDB	No	Ia	3.9	13.0	-	-	-	1
6	FDB	No	-	6.8	12.7	7.1	0.52	0.07	1
7	Lmb	No	Ia	5.4	43.0	10.5	1.99	0.19	1
8	Lmb	No	-	9.1	18.6	-	-	-	2
9	PlI	No	Ia	8.7	45.2	-	-	-	1
10	PlI	Yes	Ia	4.2	13.0	4.8	0.19	0.04	1
11	PlI	No	II	3.7	5.5	4.8	0.75	0.16	2
12	PlI	Yes	-	5.5	6.3	-	-	-	2
13	DrI	No	II	3.6	21.5	6.3	0.35	0.06	1
14	DrI	No	-	3.5	5.5	5.7	0.21	0.04	2
15	Qud	No	Ia	5.7	77.5	15.0	3.51	0.23	0
16	Qud	Yes	Ia	7.6	62.1	-	-	-	0
17	Qud	No	II	6.2	58.1	12.0	1.35	0.11	0
18	FDM	No	II	6.9	21.1	11.4	1.91	0.17	1
19	FDM	No	-	2.1	8.1	3.0	1.09	0.36	1
20	FDM	No	Ia	4.2	9.9	4.9	0.77	0.16	1
21	FDM	No	Ia	5.5	22.7	13.8	1.59	0.12	1
22	ADM	Yes	Ia	4.9	99.9	7.7	0.74	0.10	1
23	ADM	No	-	3.0	6.0	4.1	0.65	0.16	1
24	ADM	No	-	5.5	6.3	-	-	-	1
25	Und	Yes	-	4.0	5.3	5.1	0.37	0.07	0
26	Und	Yes	-	3.1	6.2	-	-	-	1
			Mean ± SEM	5.1 ± 0.4	23.5 ± 5.0	7.8 ± 0.8			

Table 3.1: *Catalog of recorded units providing for each information about (from left to right) the presumed supplied muscle, whether or not the unit was spontaneous, presumed spindle afferent type, minimum, maximum and mean discharge frequencies, standard deviation and coefficient of variation and number of digits which caused a neural response. Muscle names are abbreviated as follows: abductor hallucis (AbH), flexor hallucis brevis (FHB), adductor hallucis (AdH), flexor digitorum brevis (FDB), lumbrical (Lmb), plantar interosseus (PlI), dorsal interosseus (DrI), quadratus plantae (Qud), flexor digiti minimi brevis (FDM), abductor digiti minimi (ADM) and undecided (Und).*

Digit	Mov	AbH	FHB	AdH	FDB	Lmb	PlI	DrI	Qud	FDM	ADM
1	Ext										
	Flx										
	Add										
	Abd										
	Itor										
	Etor										
2	Ext										
	Flx										
	Add										
	Abd										
	Itor										
	Etor										
3	Ext										
	Flx										
	MAb										
	LAb										
	Itor										
	Etor										
4	Ext										
	Flx										
	Add										
	Abd										
	Itor										
	Etor										
5	Ext										
	Flx										
	Add										
	Abd										
	Itor										
	Etor										
	Stimulated by movement/Increased firing rate										
	Decreased firing rate/Off-discharge on release										
	Stimulated by movement and off-discharge on release										

Table 3.2: *Catalog of afferent responses to different toe movements. Muscle names are abbreviated as follows: abductor hallucis (AbH), flexor hallucis brevis (FHB), adductor hallucis (AdH), flexor digitorum brevis (FDB), lumbrical (Lmb), plantar interosseus (PlI), dorsal interosseus (DrI), quadratus plantae (Qud), flexor digiti minimi brevis (FDM), abductor digiti minimi (ADM) and undecided (Und).*

3.2 Unit Properties

3.2.1 Muscles acting on the big toe

Unitary recordings were made from three muscle spindle afferents supplying the short muscles acting on the big toe. The spindle located in abductor hallucis (unit 1) was silent at rest but was recruited by passive extension and extorsion movements of the 1st MTP joint, firing at ~10 Hz. During a ramp adduction it fired between 3.8 Hz and 15.4 Hz, which varied directly with the excursion angle, as seen in Figure 3.1A. The muscle spindle afferent supplying flexor hallucis brevis (unit 2) was spontaneously active at rest. Its discharge frequency was increased by extension and abduction of the 1st MTP joint; flexion of the same joint decreased the frequency and, at a threshold angle, silenced the unit. The spindle afferent supplying adductor hallucis (unit 3) was spontaneously active, firing at ~8 Hz. It responded only to abduction of the first digit MTP joint, i.e. stretch of the parent muscle.

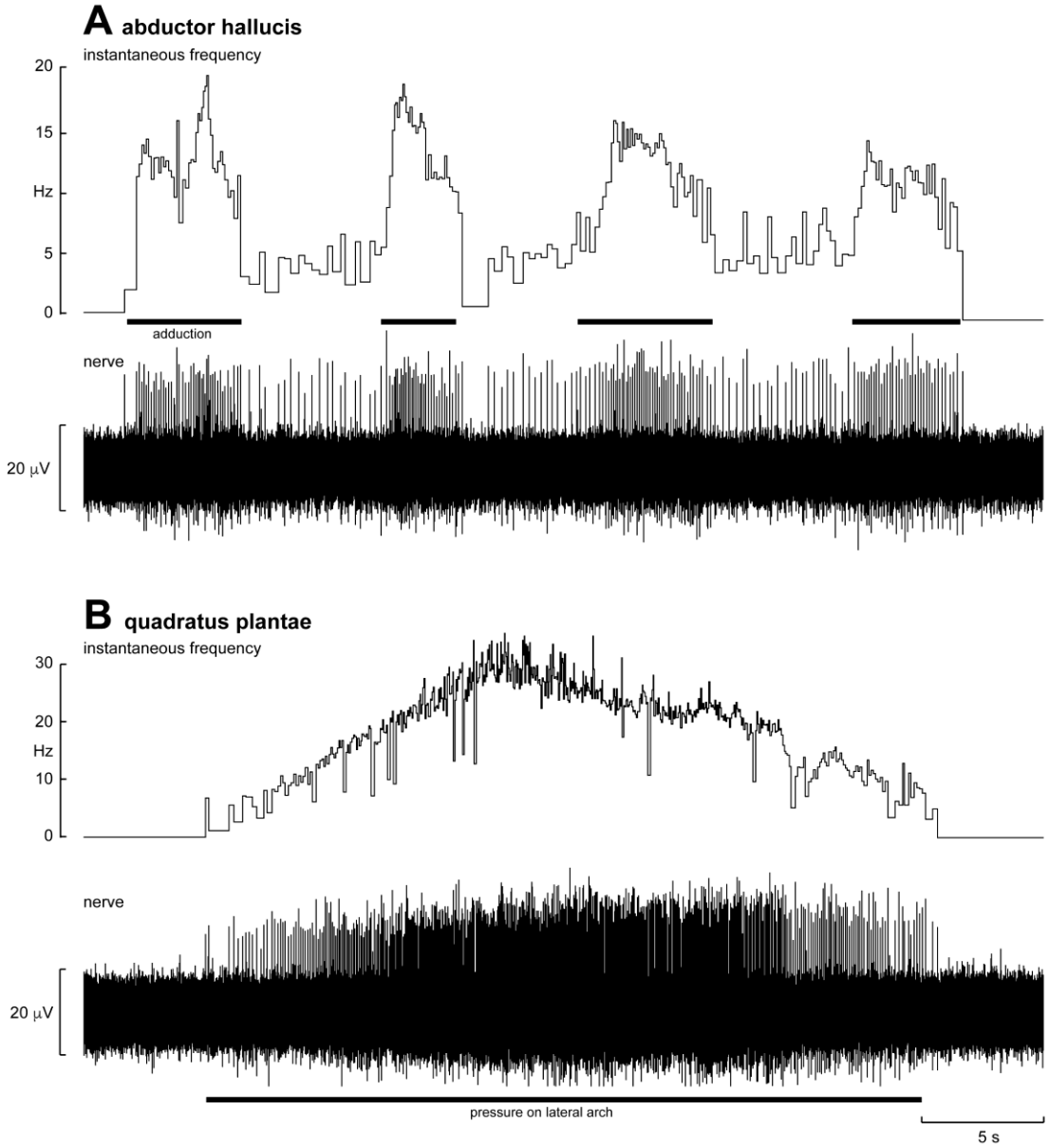

Figure 3.1: *Single-unit recordings from muscle spindles supplying the abductor hallucis muscle (**A**) and quadratus plantae muscle (**B**). Both units were silent at rest and were stimulated by passive manipulations made by the experimenter. In **A**, the experimenter adducted the first digit alternating between maximum stretch (indicated by black bars) and stretch at the activation threshold. In **B**, the experimenter applied a slowly increasing pressure over the belly of the quadratus plantae muscle. When the maximum frequency was achieved the pressure was slowly decreased and finally removed.*

3.2.2 Flexor Digitorum Brevis

There were three recordings made from spindles supplying flexor digitorum brevis, none of which were spontaneously active. They were all activated by passive extension of the associated MTP joint (units 4 and 5: 2nd MTP; unit 6: 4th MTP). Unit 5 was a primary unit that produced an off-discharge when MTP joint flexion was released. For unit 4, extension of the interphalangeal joint while the MTP joint was extended would further increase the mean discharge frequency, from 6.0 to 9.9 Hz. It also had an off-discharge following release of abduction and responded to palpation of the plantar surface of the second metatarsal.

3.2.3 Lumbricals

There were two recordings made from spindles supplying lumbrical muscles, neither of which were spontaneously active. Unit 8, presumed to supply the 3rd lumbrical, responded strongly to extension of the 4th MTP and vibration applied to the 4th metatarsal, and weakly to extension of the 3rd MTP. Unit 7 (2nd lumbrical) responded to passive extension, flexion and lateral abduction of the 3rd MTP, firing briefly during the movement and during release of the digit. In addition, it responded to vibration of the plantar surface of the 3rd metatarsal.

3.2.4 Plantar Interosseous

There were four recordings made from spindles supplying the plantar interosseous muscles. Two of these units were spontaneously active while two were not. Unit 10 (1st plantar interosseous) had a unique pattern of decreasing

discharge variability during sustained extension, flexion, medial abduction and lateral abduction after a transient increase in frequency, as seen for extension in Figure 3.2A. If the movement was brisk it would produce higher frequencies without a decrease in variability (seen during flexion in Figure 3.2A). On release, there was an off-discharge and an increase in variability to its resting state. Medial abduction generated the greatest off-discharge, while lateral abduction had the weakest. In addition, this unit responded to vibration applied to the plantar surface of the 3rd metatarsal. Unit 9 (2nd plantar interosseous) responded only to abduction – and more weakly extension – of the 4th MTP joint as well as vibration between the 3rd and 4th metatarsals on the plantar surface. Units 11 and 12 (3rd plantar interossei) responded to abduction and extension of the 5th MTP but also weakly to extension of the 4th MTP.

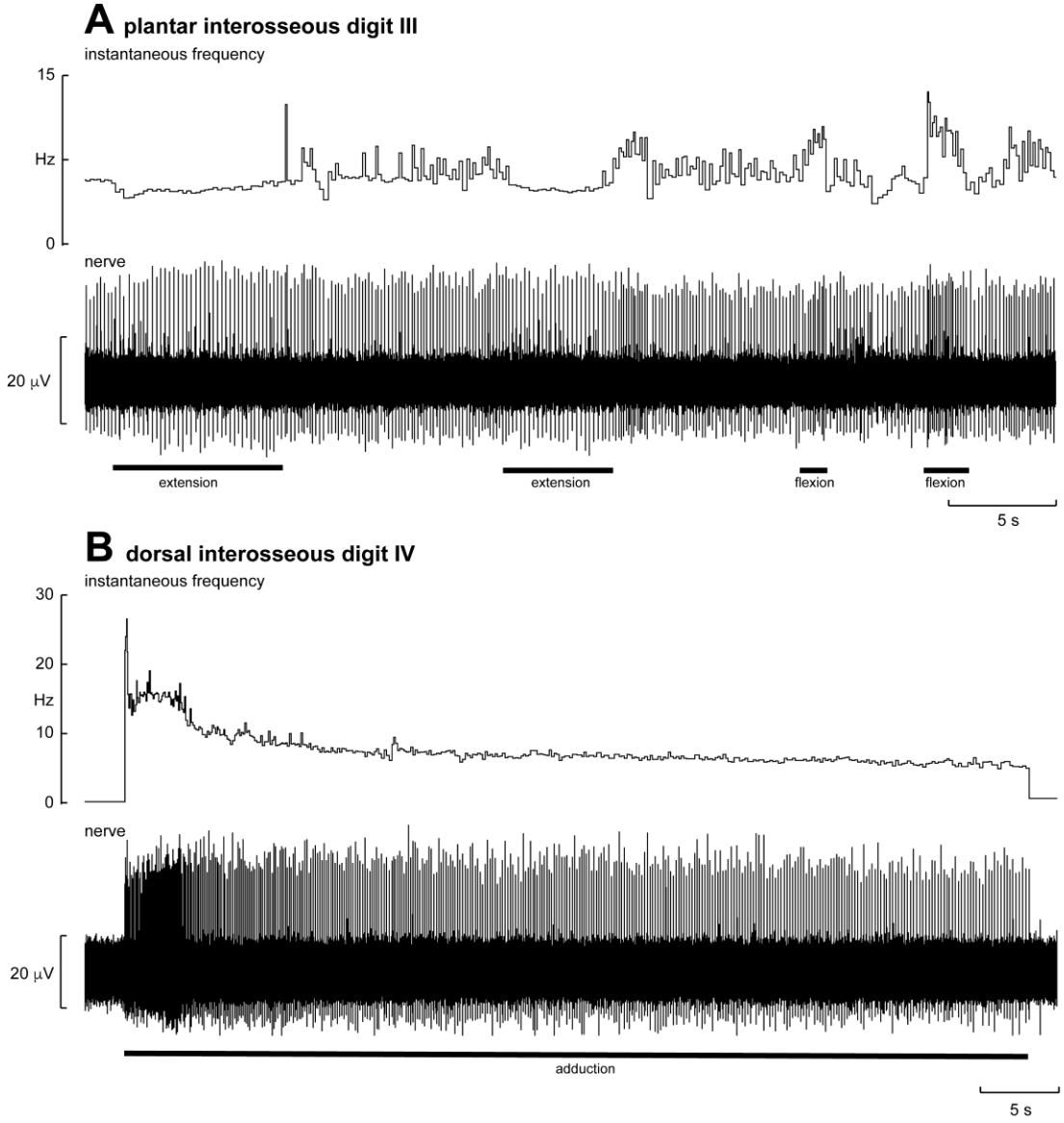

Figure 3.2: *Single-unit recordings from muscle spindles supplying the 1st plantar interosseus muscle (**A**) and the 4th dorsal interosseus muscle (**B**). In A, the 3rd digit is passively flexed and extended. In B, the 4th digit is passively adducted. The black bars below each trace indicate the period and type of movement. Note that the adduction movement was held at a constant angle throughout the stimulation.*

3.2.5 Dorsal Interosseous

There were two recordings made from spindles supplying dorsal interosseous muscles, neither of which were spontaneously active. Unit 13 (4th dorsal interosseous) responded strongly to adduction of the 4th MTP and weakly to extension. Figure 3.2B demonstrates this strong response to adduction, which gradually plateaued to a constant frequency until the digit was released. Unit 14 (1st dorsal interosseous) responded strongly to palpation in the dorsal interosseous space between the 1st and 2nd digit in addition to abduction of the 1st and 2nd digit.

3.2.6 Quadratus Plantae

There were three recordings made from spindles supplying the quadratus plantae muscle. One was spontaneously active and two were not. All responded to palpation of the posterior lateral arch anterior to the heel with no response from any passive toe movements. Figure 3.1B shows unit 17 responding to slowly increasing and decreasing ramping pressure over the supplied muscle's belly. Unit 16 fired erratically and was silenced by palpation. On release of the pressure it fired several brief bursts as the deformed skin and underlying muscle returned to its resting position.

3.2.7 Flexor Digiti Minimi Brevis

There were four recordings made from spindles supplying the flexor digit minimi brevis muscle, none of which were spontaneously active. They all responded to

extension of the 5th MTP. Units 19, 20 and 21 fired an off-discharge after flexion. Unit 19 had a slow, erratic discharge during extension independent of the degree of stretch. It also responded strongly to vibration on the plantar surface of the 5th metatarsal and weakly to 5th MTP abduction. In addition to the responses previously listed, unit 21 was activated by palpation of aforementioned plantar surface.

3.2.8 Abductor Digiti Minimi

There were three recordings made muscle spindle afferents from abductor digiti minimi. Unit 22 was spontaneously active at rest, increasing its firing during extension of the 5th MTP joint and palpation of the posterior lateral arch. It was also silenced by abduction and produced an off-discharge when returning to the neutral position. Units 23 and 24 were not spontaneously active at rest, but responded to adduction of the 5th MTP and pressure on the lateral 5th metatarsal.

3.2.9 Unidentified

Two muscle afferent recordings could not be identified, as they had no reliable response to toe movements or palpation, perhaps because sufficient muscle stretch could not be adequately provided. They were, however, both spontaneously active, providing further evidence for spontaneous muscle spindles in the intrinsic muscles of the foot. Unit 25 had a very regular firing rate, while unit 26 fired more erratically but would decrease its variability when contact was made with the 4th digit.

3.3 General features of muscles spindles of the intrinsic muscles of the foot

Here recordings have been collected from 26 individual muscles spindles supplying all of the muscle groups included in the intrinsic muscles of the foot. They share similar properties to those found in the long muscles of the leg and forearm as well as those in the intrinsic muscles of the hand. As seen in Table 3.2 most units act similarly to what we would expect from what we know about muscle spindles in the hand and more proximal leg muscles, being stimulated or increasing a tonically active firing rate when a digit was moved in a way that would stretch the supplied muscle or being silenced when the reverse movement was performed or the muscle was compressed. However, the characteristics of each response – discharge frequency, variability and dynamic sensitivity – were often unique, even between units sharing a supplied muscle. One explanation for this is the likelihood that different muscle spindles can provide length information of different axes within a single muscle belly, according to their orientation in parallel to the extrafusal muscle fibres. That said, the variability in results speaks to the functional complexity of muscle spindles in the intrinsic foot muscles.

A common finding was the low proportion of spontaneously firing units with the toes in their naturally resting joint angles. Of the 26 units recorded, only seven units (27%) maintained a constant firing rate with no active movement or external contact with the foot. Interestingly, when 13 spindles were recorded from the intrinsic hand muscles four of these (30%) were spontaneously active (Burke et al. 1988); a similar result to the present study. When Vallbo (1974b)

investigated the ante-brachial finger flexors, which are long muscles with long tendons, it was noted that 39% of units (primary: 30%; secondary: 55%) were active with the metacarpophalangeal joints at a resting state of 140° flexion. For the pre-tibial flexors, which are also long muscles with long tendons, 62% of units were spontaneously active while the ankle was plantarflexed at 25° (primary: 76%; secondary: 83%) (Burke et al. 1979b). The differences between these muscle groups suggest that muscle spindles important for proprioception will have a stretch threshold below the innervated muscle's resting length. Conversely, for muscle groups where detecting changes to the stable state or assisting fine motor movement is important, such as those located in the short muscles in the hand, it is more likely to be silent at rest.

Recordings from muscle spindles of the ante-brachial finger flexors have shown the mean steady state discharge frequency of primary units to be around 6 Hz and around 9 Hz for secondary units with the metacarpophalangeal joints at 180° (Vallbo, 1974b). From that study it was also determined these steady state discharge rates were a simple function of the joint angle. In the pretibial flexors, mean resting discharge in primary units is 9.6 Hz and 10.5 Hz in secondary units (Macefield et al. 2003). In the only previous recordings of intrinsic muscle spindles of the hand, discharge frequency was not quantitatively assessed (Burke et al. 1988). In the current study, there was no clear pattern across all units, primary and secondary, in the intrinsic foot muscle spindle recordings. Of the spontaneous units, unit 22 (abductor digiti minimi) was the only unit that had a similar resting discharge of 7.7 Hz. Unit 16 (quadratus plantae) and unit 26 (undecided) fired

erratically but with different patterns and units 2 (flexor hallucis brevis), 10 (plantar interosseus) and 25 (unknown muscle) fired at around 5 Hz with low variability. As the mean discharge rates calculated for the non-spontaneous units were achieved at a particular joint angle they are not representative of a resting state, which should be considered 0 Hz with the foot and toes in their natural position of rest. The differences between the spontaneous primary firing rates of abductor digiti minimi and plantar interosseus could be attributed to the difference in resting tension of the muscle.

This work can be used as a baseline for further study with a larger sample population to gain a better perspective on the variability of units located within the same muscle belly as the present research would not be sufficient to capture that.

4 BEHAVIOUR OF MUSCLE SPINDLES SUPPLYING THE INTRINSIC MUSCLES OF THE FOOT IN FREE-STANDING CONDITIONS

In the previous chapter, muscle spindles of the intrinsic foot muscles in the unloaded condition were characterised. It was documented that the majority of the unloaded units are silent at rest, but each had the capacity to encode passive movements at one or more joints. In addition, it was noted that there was no consistent discharge frequency between the spontaneous units, likely owing to their differing resting lengths. This now provides the control data necessary to investigate the effect of loading and free standing on their firing characteristics. Of particular interest is whether or not they will be spontaneously active when free standing, what their mean firing rate and discharge variability is when in a relatively stable free-standing state and whether or not changes in firing frequency correlate with changes in centre of pressure (CoP) that would indicate a capacity to encode corresponding deformation of the sole of the foot. As with the unloaded state, a large variety of responses that are dependent on the supplied muscle belly's anatomy and its involvement in a particular manoeuvre can be expected.

4.1 Unit Summary

Stable recordings from 12 muscle spindle afferents were obtained during free, unsupported standing, six of which were spontaneously active. While two units were initially identified as the subject sat on the tilt table, with the leg pendant and the foot unloaded, the majority of the recordings were obtained while the subject was already standing. Of the two units recorded before standing was initiated, one – located in flexor digitorum brevis – was spontaneously active at rest and the other – located in flexor hallucis brevis – was silent. There were insufficient data

collected to make confident assertions about the spindle response to loading. In the one recording obtained in the seated position and followed as the subject stood, the spindle was recruited momentarily as the weight on the foot increased, suggesting that the associated foot deformation met the activation threshold of the spindle. It seems likely that for those muscle spindles that are spontaneously active in the unloaded condition that we would see modulation of their firing rate during the transition from seated to standing.

Of the 10 units recorded in the standing position, four were located in flexor digitorum brevis, two in flexor digiti minimi brevis, one in adductor hallucis and another in flexor hallucis brevis; the parent muscle of two muscle spindle endings could not be determined.

4.2 General features in loaded conditions

Figure 4.1 shows a recording from a muscle spindle ending located in flexor hallucis brevis. The muscle spindle was silent at rest (unloaded condition), and when the subject is resting his foot on the force plate (loaded condition), but it can be seen that it is recruited not in response to transition to standing, represented by the change in the force trace (measuring vertical force), but for a 15 second period after during which the subject was adjusting their stance. Interestingly, it would appear that its discharge is related to the postural adjustments required of standing, as indicated by the EMG recorded over tibialis anterior and soleus; the unit did not maintain an ongoing discharge during standing. Note also the very

small changes in ankle angle during the transition from the loaded to standing position.

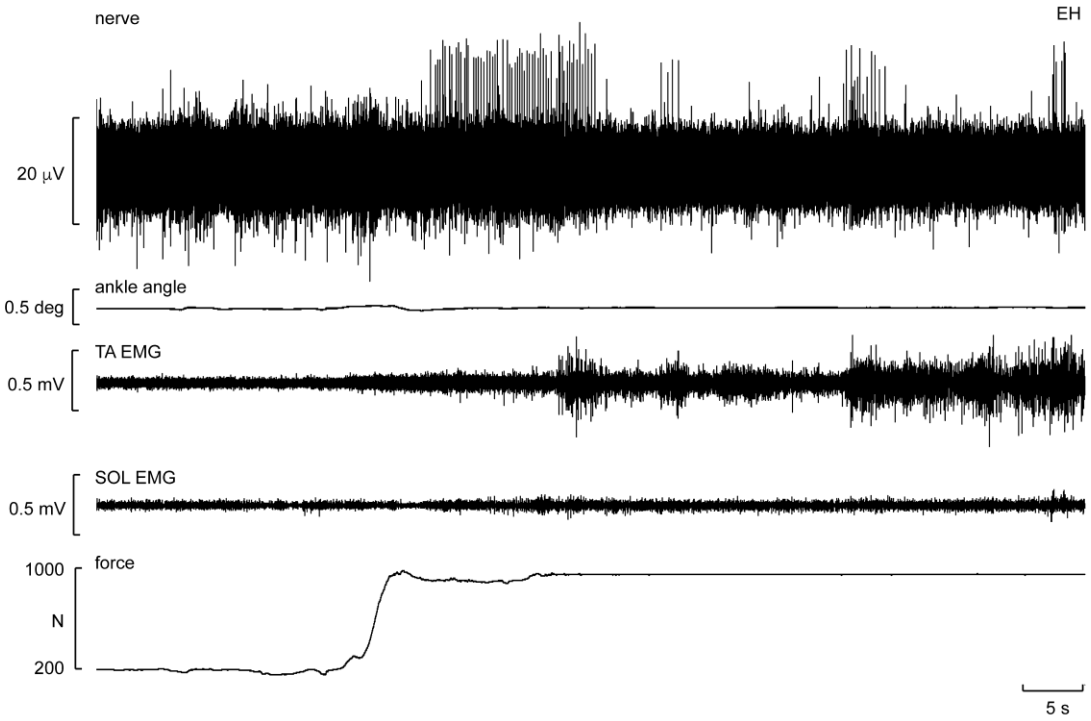

Figure 4.1: *Single-unit recording from a muscle spindle supplying the flexor hallucis brevis muscle. The ending was silent at rest and is shown during the transition from the loaded (foot resting on force place) to the standing condition. deg, Degrees; EH subject identifier; EMG, electromyography; SOL, soleus; TA, tibialis anterior*

A recording of a muscle spindle in the same muscle in a different subject is shown in Figure 4.2, this time in free, unsupported standing. This ending was also silent at rest, and could be activated by weak voluntary active plantarflexion of the big toe. It would appear that spontaneous activation of this spindle is indeed due to slight postural adjustments (note the slight changes in ankle angle) that lead to activation of the muscle as a compensatory mechanism. It can also be seen that in the freestanding condition, as indicated by the increase in EMG of the tibialis

anterior and soleus muscles, this muscle spindle shows as increase in activity. Although EMG activity could not be recorded from this muscle, or any intrinsic muscle of the foot, it is likely that there was also an increase in EMG of this muscle and hence an increase in fusimotor drive; this may be due to compensatory reactions during postural sway.

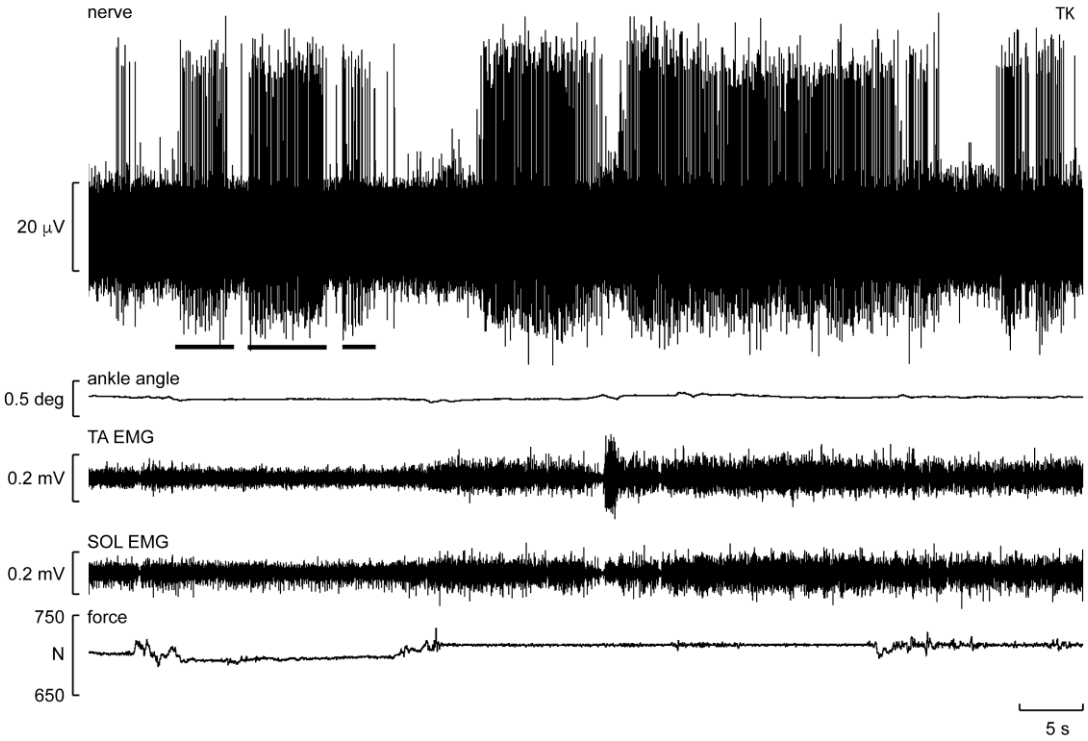

Figure 4.2: *Single-unit recording from a muscle spindle supplying the flexor hallucis brevis muscle during free standing. Three horizontal bars indicate periods in which the subject was asked to perform weak active flexion of the big toe. deg, degrees; EMG, electromyography; SOL, soleus; TA, tibialis anterior; TK, subject identifier*

Of the 12 muscle spindles recorded, the firing of eight covaried with changes in the centre of pressure (CoP), with five endings responding to postural sway in the anteroposterior axis, one responding to side-to-side sway (mediolateral motion) and two responded to sway along both axes. An example of a unitary recording

obtained from a secondary muscle spindle afferent located in flexor digitorum brevis during free standing is shown in Figure 4.3. This unit presented a spontaneous background discharge, shown in unsupported free standing with the eyes closed (Figure 4.3A). In Figure 4.3B, when the subject was exposed to anteroposterior movement of the supporting platform (note the acceleration signal in the third trace) there was an increase in the magnitude of sway. The postural perturbations increased the variation in CoP on the y-axis, with a five-fold increase in range, which accentuated the frequency response of the spindle ending. The maximum modulation of the spindle's firing rate was ~5 Hz. While this may seem low, it is worth noting that this corresponds to ~45% of the ending's mean frequency. It is also apparent that there is no clear correlation between spindle frequency and the ankle angle, despite the latter varying somewhat with CoP as the body rotates about the ankle.

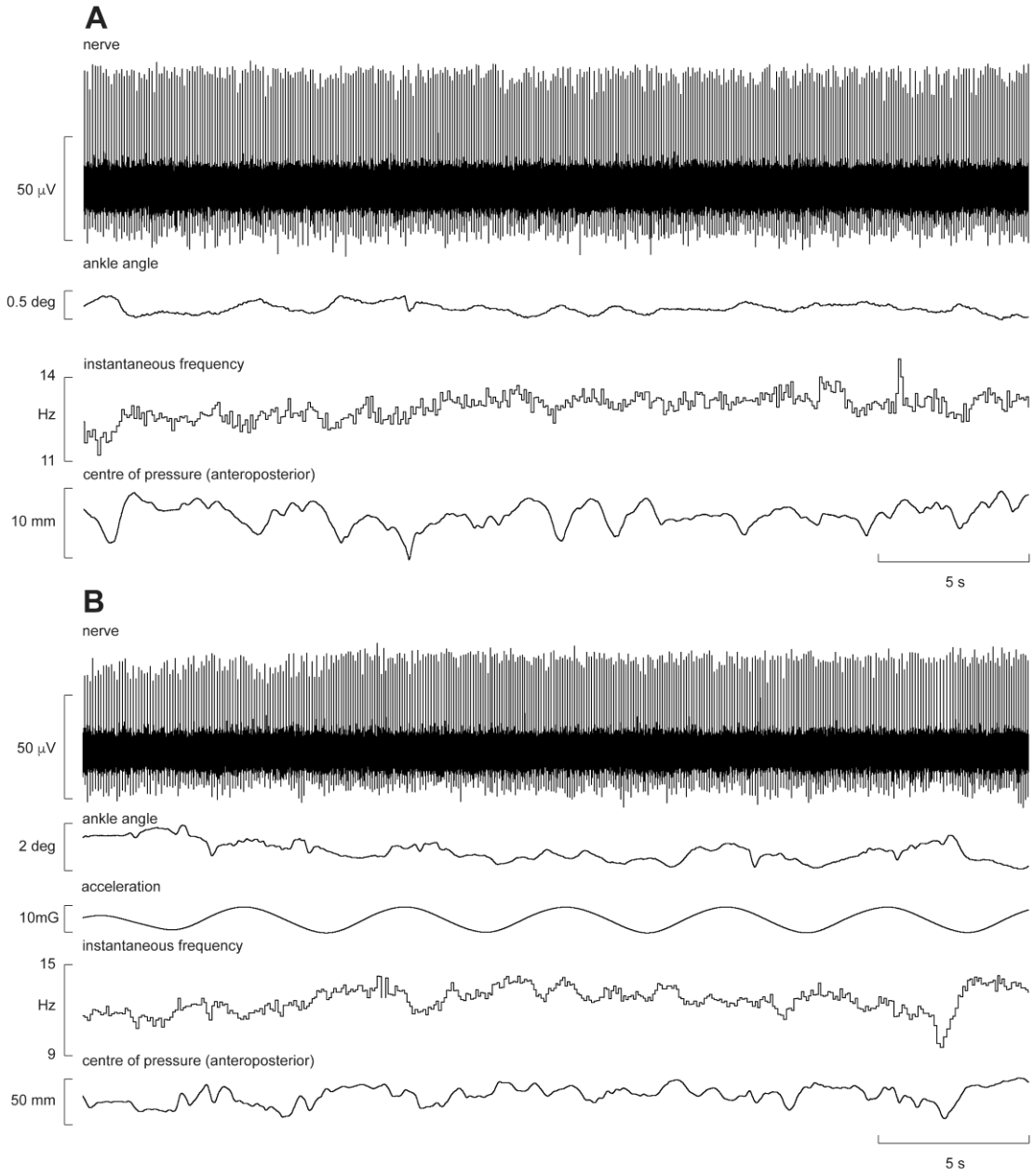

Figure 4.3: *Single-unit recording from a spontaneously active muscle spindle secondary ending located in flexor digitorum brevis during unsupported free-standing. In* ***A****, the subject is standing with eyes closed. In* ***B****, the platform on which the subject is standing is being moved sinusoidally along the anteroposterior axis.*

The mean frequency of this muscle spindle is shown superimposed on changes in CoP during free standing with the eyes closed (Figure 4.4A) and during postural

perturbations (Figure 4.4B). It can be seen that the fluctuation in spindle firing rate covaried with fluctuations in CoP. Mean discharge frequency and the anteroposterior CoP were smoothed using a Bartlett window of 2 seconds. Data from two other spindles are shown in Figure 4.4C and D. Again, we see modulation of the spindle response by changes in CoP, with most turning points and occasionally the magnitude of the traces correlating. From the CoP scales we can see that these changes are detected over shifts as small as 1 mm.

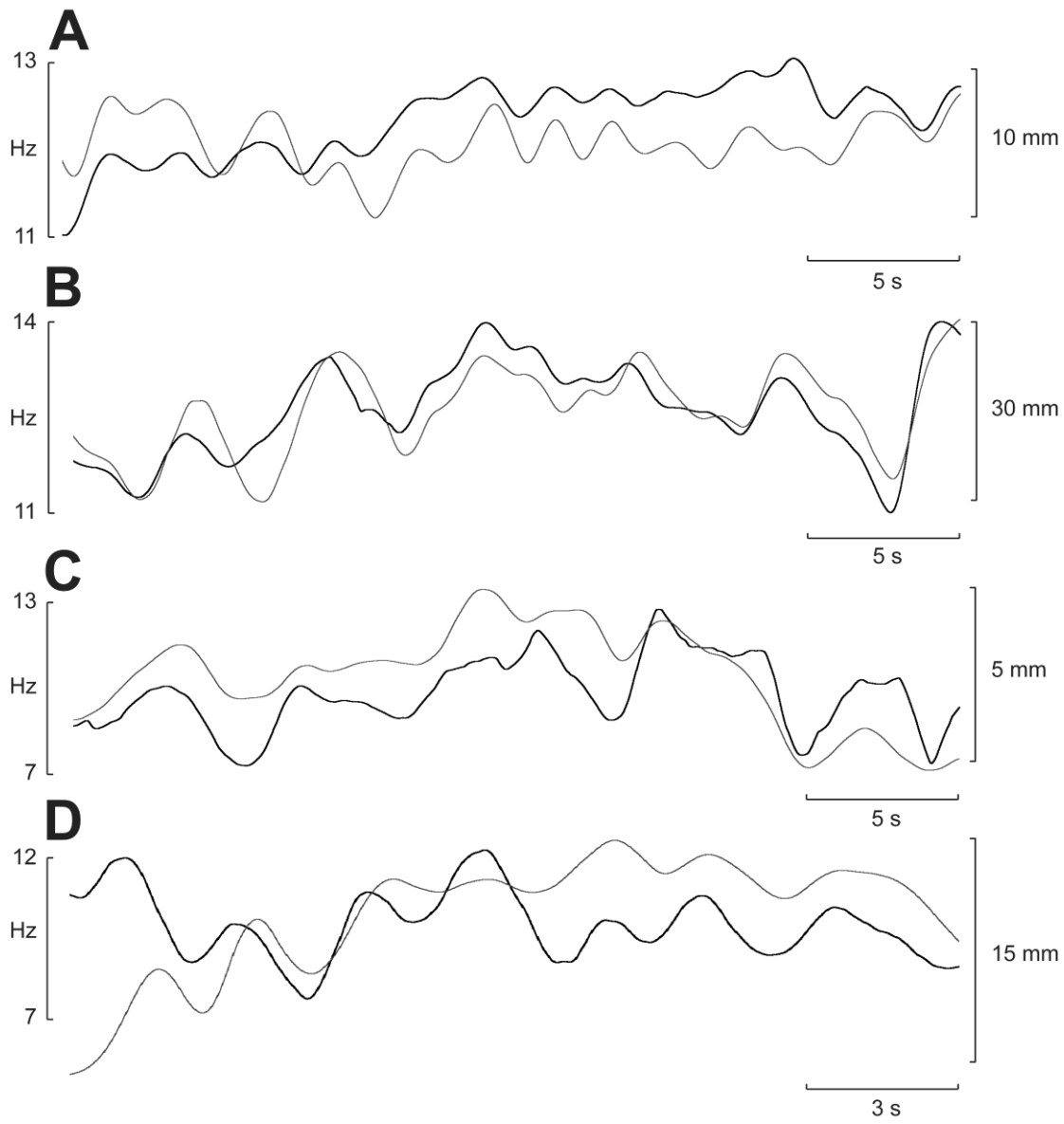

Figure 4.4: *shows the spontaneous fluctuations in mean frequency of three muscle spindles (thick lines) superimposed onto corresponding changes in CoP (anteroposterior). CoP, smoothed using a Bartlett window of 2 seconds (thin lines). Panels* ***A*** *and* ***B*** *correspond to the same recording shown in Figure 1.3A and 1.3B. Data from another two units recorded from flexor digitorum brevis muscle (****C****) and flexor hallucis brevis muscle (****D****) are also shown.*

Finally, while four muscle spindles were not tonically active during standing, they still responded to transient changes in posture, evidently encoding the resultant changes in muscle length. An example of a muscle spindle ending, located in flexor hallucis brevis, responding to incidental postural adjustments can be seen in Figure 4.5. Note the very small changes in CoP and ankle angle. We can also see preceding tibialis anterior EMG activity, likely responsible for the subsequent anterior CoP shifts, alternating with spindle activation. Given that this pattern begins with a corrective activation to posterior sway it suggests that spindle activity of the flexor hallucis brevis does not reflect TA muscle activity but rather the resultant muscle stretch associated with the anterior-directed changes in CoP. In other words, the change in firing likely reflects a compensatory contraction of the intrinsic foot muscles for stabilisation.

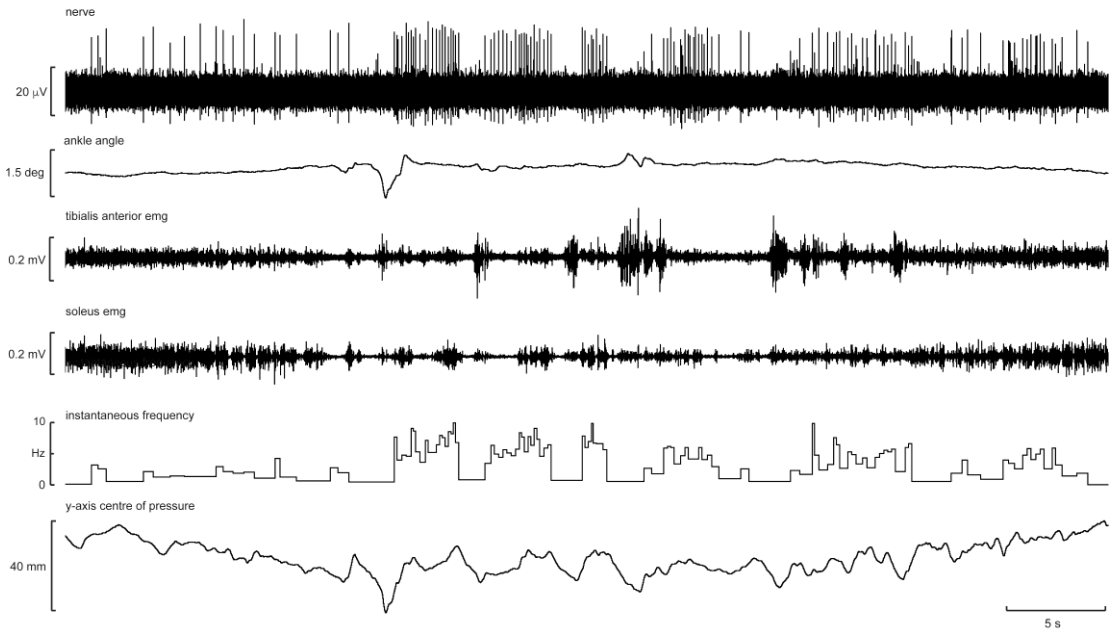

Figure 4.5: *Single-unit recording from a muscle spindle supplying the flexor hallucis brevis muscle during free standing. Note the covariation of firing with spontaneous fluctuations in CoP during postural sway.*

While the current sample size limits statistical analysis of the behaviour of these units, the data presented clearly demonstrate the role of muscle spindles in reporting on changes of CoP during free standing. It appears that this role is shared by both spontaneously active units and those that are silent at rest. In addition, they appear to activate during transition from seated to standing position. However, it remains to be determined if this is due to the muscle spindles detecting stretch of muscles as a consequence of the mechanical changes produced by this movement or the compensatory muscle activity (i.e. a fusimotor-driven response). It is expected that these response will vary, depending largely on the position of the parent muscle belly as well as the alignment of the muscle spindle within the surrounding extrafusal muscle. Further larger studies will be required to better characterise the role of each individual muscle groups' spindles, ideally with concurrently recorded EMG activity, with intramuscular wire electrodes from these small intrinsic muscles of the foot.

5 BEHAVIOUR OF CUTANEOUS AFFERENTS FROM THE SOLE IN FREE-STANDING CONDITIONS

Based on single-unit microneurographic recordings previously made from the tibial and sural nerves in supine subjects, we have a good understanding of the quantity, density, distribution, receptive field area and mechanical threshold of cutaneous afferents supplying the sole of the foot (Trullson 2001, Fallon et al. 2005, Strzalkowski et al. 2018). There is a significant discrepancy in the unit density of type I afferents innervated by the tibial nerve. These units have a higher density in the toes, lateral arch and metatarsals. As these regions provide the base of support during free standing it would be reasonable to suggest that cutaneous afferents play a significant role in edge detection, which would add a unique access to the neural substrate for postural maintenance from what muscle spindles would contribute. We have seen that removing cutaneous feedback has impacts on balance and gait (Meyer et al. 2004; Nurse and Nigg 1999; Perry et al. 2000). While these previous microneurography studies have provided a rich data set for cutaneous afferents of the foot's sole, they are limited by having been undertaken with the participant laying prone. As such, any proposed mechanisms by which cutaneous afferents from the plantar surface of the foot contribute to the control of upright posture in unsupported standing, in the absence of data, remain speculative. Here, a sample of single-unit and multiunit recordings of cutaneous afferents in the foot via microneurography of the posterior tibial nerve is presented. Given that there are four classes of low-threshold mechanoreceptor in human glabrous skin, including that of the sole, the current study does not provide an exhaustive assessment of their firing properties during standing. Nevertheless, it does uncover some features that I suggest are important.

5.1 Multiunit Recordings

Because of the difficulty in securing stable recordings from individual tactile afferents in free-standing conditions, much of the analysis was limited to multi-unit recordings, obtained from 28 sites within cutaneous fascicles of the posterior tibial nerve. These recordings were evaluated qualitatively to provide information on the population behaviour of tactile afferents in the sole of the foot, particularly with respect to their capacity to encode loading forces and CoP changes. As a population, cutaneous afferents in the sole of the foot responded with a large burst of impulses on initial contact with the force plate by the participant's foot. A secondary burst on loading and a burst on unloading was also apparent, i.e. the population of afferents responded to the dynamic changes in skin deformation associated with loading and unloading.

5.1.1 Afferents located on the toes

Fourteen of the multiunit recordings had receptive fields over the part of one or more digits; 8 on individual digits, 6 on multiple digits. Only one site presented spontaneous activity. The multi-unit activity of four sites demonstrated clear correlation with CoP changes in freestanding. In all cases this was during anterior shifts in CoP and for one recording, activation would only occur at the extreme limits of this movement. This includes the single spontaneous recording that would increase in activity during pressure shifts. There was a latency of ~10ms between the nerve signal and the pressure signal which could represent deformation of the skin occurring prior to pressure applying to the platform. Four of these recordings were not modulated by changes in CoP in freestanding.

However, they did activate briefly (~500ms) when contact of the receptive field was either made or broken with the platform. This would occur during unsupported freestanding as the lateral digits briefly extended spontaneously as well as when the participant was asked to shift their CoP anteriorly so that the tips of their toes would contact the platform. Four recordings had activations during freestanding that was not distinctly correlated with CoP changes or toe movement. Two recordings were completely silent during freestanding and sinusoidal platform movement but responded to passive pressure over their receptive field by the experimenter.

Three recordings had receptive fields located between adjacent digits. None were spontaneously active in freestanding. Two recordings, both between digits 4 and 5, were activated by anterior CoP changes. The third recording, between digits 2 and 3, did not respond to CoP changes, nor loading or unloading of the foot. However, it was activated during active flexion and extension of the toes, as documented by asking the participant to voluntarily flex or extend the toes. This could potentially represent activation due to the sheer forces between toes.

One recording had a receptive field over the distal 4^{th} metatarsal. It was spontaneously active during standing and would increase its activity with anterior CoP changes and occasionally with lateral CoP changes. This likely reflects increases in pressure over the receptive field.

5.1.2 Afferents located on the medial arch

Four recordings had receptive fields over the medial arch, with some extending to the medial heel. There was an inconsistent pattern of activity, but none were spontaneously active in freestanding. Two recordings behaved similarly to those located on the toes, with non-specific activations that would occasionally correlate with anterior CoP changes. The receptive field of another multi-unit site did not contact the platform and it was silent throughout freestanding. Finally, one recording was activated while the contralateral foot was actively adjusted. This could represent a response to deformation of the arch as weight increases over the ipsilateral foot.

5.1.3 Afferents located on the lateral arch

Four multi-unit recordings had receptive fields over the lateral arch, two anterior over the 5^{th} metatarsal and two posterior over the cuboid bone. The posterior recordings did not present spontaneous activity but were both activated by anterior and lateral CoP changes. Neither of the anterior recordings were in contact with the platform and did not exhibit any activity during freestanding, with or without imposed platform movement.

5.1.4 Afferents located on the central arch and heel

One recording had a receptive field on the central arch anterior to the heel. It was not spontaneously active but was activated by the posterior extremes of CoP shifts. Finally, one recording was recorded from the calcaneal branch of the posterior tibial nerve and had a receptive field over the lateral heel. It was silent

at rest but activated by overt foot movement. This activity was not correlated with any measured changes in CoP.

5.2 Unitary Recordings

In addition to these multi-unit recordings, unitary recordings were made from 15 cutaneous afferents supplying the sole of the foot. Afferents were identified according to criteria used to classify tactile afferents in the hand (Johansson & Vallbo, 1979). Five of these were fast-adapting type 1 (FAI) and five were slow-adapting type 1 (SAI). Five recordings were made of slow adapting type 2 (SAII) units and I have yet to make a recording from fast-adapting type 2 (FAII). It is believed that this is to be largely the result of their lower distribution as shown in previous studies. Moreover, given that FAII afferents respond to very brisk events, it is unlikely that they would contribute meaningful information on slow changes in posture. Nevertheless, like those in the hand, we can predict that those in the sole of the foot would respond to contact and release events between the skin and the supporting surface. Due to the small sample size of collected units the conclusions that one can draw from observations made based on unit type are limited. Indeed, I would need a very large sample of unitary recordings from each receptor type (~20 from each class), covering the different compartments of the foot. It is likely that the role of a particular mechanoreceptor is dependent on both its morphological type and the location of its receptive field.

5.2.1 Slowly adapting type I units

Of the five single SAI units recorded each had varying levels of activity in free standing. All units were on the digits; two on the third digit, two on the fourth and one on the first. Both units on the third digit were spontaneously active in freestanding firing at a frequency of ~8 Hz and could increase to 30 Hz if passively manipulated. However, there was no clear modulation with CoP changes. One unit of the fourth digit would intermittently activate in a pattern not related to CoP changes while the other was more silent but would clearly module with maximal anterior CoP shifts (Figure 5.1). The unit on the first digit was silent in freestanding with a few intermittent bursts not correlated with measured CoP changes.

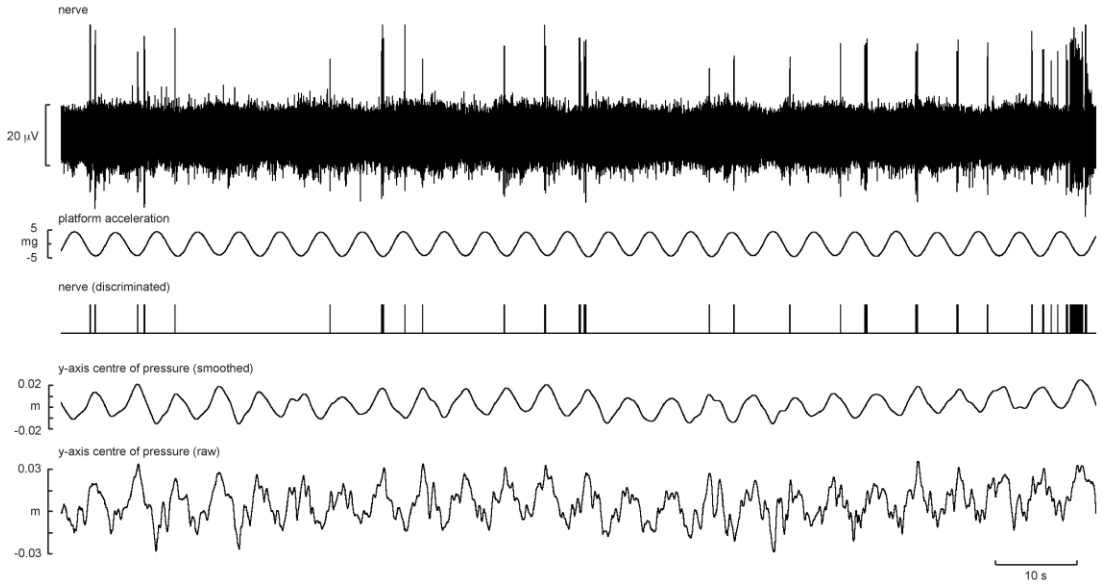

Figure 5.1: *Nerve recording from an SAI afferent supplying the distal plantar surface of the 1st digit in freestanding with anteroposterior platform movement. Also shown (from top to bottom) is the acceleration of the platform (milliG), discriminated spikes, and smoothed and raw trace of anteroposterior CoP changes (metres).*

Figure 5.1 displays a recording from a single SAI unit innervating the distal plantar surface of the fourth toe. It was recorded while the subject was in an unsupported, freestanding position with perturbations applied by a moving platform (acceleration indicated by the second channel). By comparing the smoothed trace of CoP changes on the anteroposterior (y) axis to the platform acceleration it can be seen that the platform contributed to similar, regular perturbations. SAI unit action potentials clearly correlate with these peaks in negative acceleration and anterior centre of CoP. This demonstrates that these units' sensitivity to pressure may be used in a freestanding context to indicate movement of the foot in a direction specific to the location of the unit. It should be noted the unit is also seen to fire at other stages of the platform's cycle including once at the extreme positive acceleration. Independent toe movement still providing pressure on the unit, shown by the brief anterior shift in CoP at this point, can explain this.

5.2.2 Slowly adapting type II units

The five SAII units recorded also responded variably, but an interesting pattern of behaviour was apparent. Like the muscle spindle endings reported in the previous chapter, spontaneously active SAII afferents demonstrated a clear modulation with changes in centre of pressure during freestanding. Figure 5.2 is a recording from a single SAII unit supplying the plantar surface overlying the medial three metatarsals. Again, the platform motion can be seen as an effective method of producing predictable shifts in centre of pressure as the body sways with the sinusoidal linear acceleration. By comparing the smoothed trace of CoP to the firing rate of the SAII unit, it is clear that the unit exhibited regular sinusoidal

changes in instantaneous frequency between roughly 10 and 16 Hz. As SAII afferents are known to be sensitive to skin stretch it might be doing this by encoding the sheer forces on the skin associated with the platform motion. Importantly, changes in afferent frequency were seen to precede CoP changes by ~100ms. This could represent signalling the deformation of the skin occurring prior to gross pressure change and is a testament to both the sensitivity of these afferents to changes in their mechanical environment. While further recordings of this phenomenon will strengthen this argument, it can nevertheless be seen that clear and informative single-unit recordings are possible by recording from the posterior tibial nerve in the freestanding position, despite considerable subject movement. Another unit with a receptive field that could not be adequately defined responded similarly with clear anteroposterior CoP changes. One unit on the medial arch showed modulation to mediolateral movement, increasing frequency during lateral CoP changes at a higher mean frequency (~24 Hz). Another unit with an unknown receptive field had continuous firing with no changes with CoP, and one over the 4th of 5th metatarsals had no spontaneous activity but was activated by pressure and loading of the foot. This spectrum of activity and sensitivity to postural changes suggests that the functional benefit of an individual unit is dependent on the location of its receptive field, the orientation of its fibres and its activation thresholds. Indeed the powerful encoding seen by some units is likely a product of incidental overlap with the CoP parameters measured while the data encoded from other units may serve a different role.

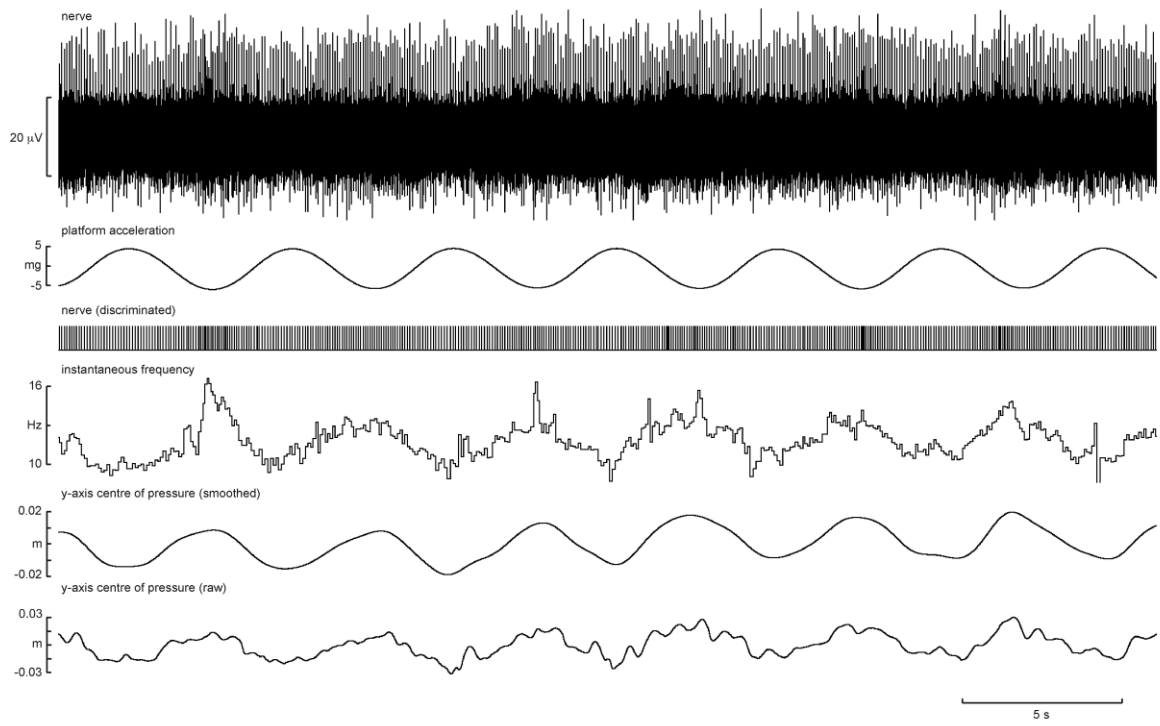

Figure 5.2: *Nerve recording from an SAII afferent supplying the skin over the 2nd and 3rd metatarsals in freestanding with anteroposterior platform movement. Also shown (from top to bottom) is the acceleration of the platform, discriminated spikes, instantaneous frequency of the discriminated spikes and smoothed and raw trace of anteroposterior CoP changes.*

5.2.3 Fast adapting type I units

Two of the FAI units recorded demonstrated no activity during unsupported free standing. Both of their receptive fields were on the lateral sides of digits. In contrast one FAI on the base of the 5th digit was activated during platform-based postural sway.

Three of the five single FAI units recorded were completely silent in standing. In all case these units had receptive fields over the lateral or medial edge of different digits which did not contact the platform. Two other units, located on the pad of the fifth digit and the base of the fifth metatarsal respectively, did activate briefly during free-standing however these activations did not appear to correlate with CoP on the axes measured. As FAI units are sensitive to force velocity it is expected that there is little activity during zero or constant forces on their receptive fields.

The multiunit data dominated by FAI units (as indicated by their response to initiation and release of pressure) was seen to also be highly sensitive to loading and unloading of the foot. These examples are illustrated here. Figure 5.3 is an oligounitary recording dominated by two to three FAI afferents with receptive fields over the distal 4th metatarsal, recorded from a free standing subject. The activity at the beginning of the recording is the afferent response to the experimenter lightly stroking the receptive field while the foot is unloaded. The subject then loads, unloads and loads the foot again, the degree of unloading shown by the CoP trace. Afferent responses can be seen at each of these dynamic events. Notably the afferent response is very brief compared to the loading/unloading processes and occurs at the beginning of loading and end of unloading, corresponding to when skin contact is made or broken with the platform.

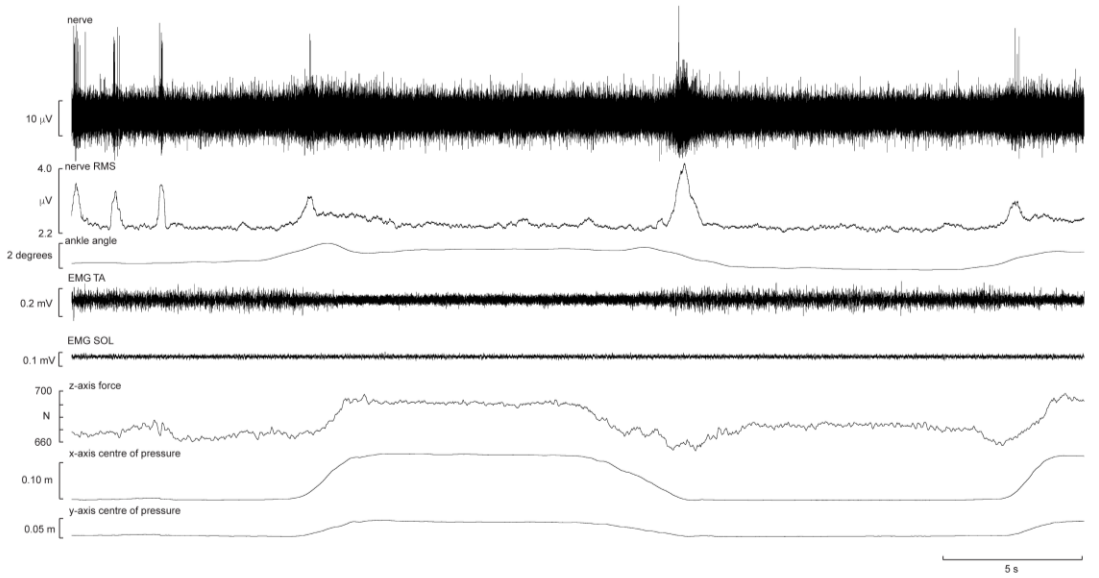

Figure 5.3: *Oligounitary cutaneous recording responding to pressure on the distal 4th metatarsal recording in freestanding with the ipsilateral foot being slowly loaded, unloaded and loaded again. Also shown (from top to bottom) is the RMS of the nerve signal, ankle angle, tibialis anterior and soleus EMG, z-axis forces and CoP changes recorded along the x and y axes.*

Figure 5.4 is another multiunit recording of largely FAI afferents with their receptive field over the base of the 5th metatarsal in a different free standing subject. In this instance the subject is asked to slowly load their ipsilateral foot, resulting in a change in centre or pressure on the x-axis (mediolateral). Here, distinct bursts of neural activity can be seen not just when skin contact is made with the platform but also during the final stage of loading, presumably when the underlying bone applies pressure on the skin. This suggests that the activity of some units depends on a complex response to deformation of the skin rather than just superficial contact.

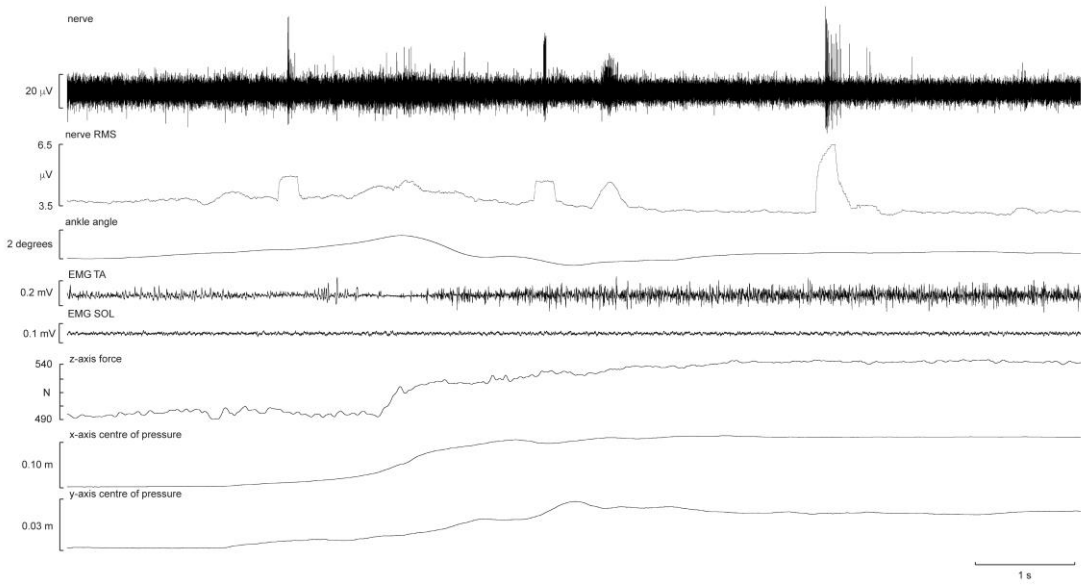

Figure 5.4: *Multiunit cutaneous recording responding to pressure on the base of the 5th metatarsal during slow loading of the ipsilateral foot. Also shown (from top to bottom) is the RMS of the nerve signal, ankle angle, tibialis anterior and soleus EMG, z-axis forces and CoP changes recorded along the x and y axes.*

The number of single unit recordings was low but an examination of the firing characteristics of individual units was beyond the scope of this thesis. Such an analysis would require a recording of at least 20 units per unit type over each distribution area across the foot (toes, medial and lateral arches, central arch and heel). However, conclusions can be made about the overall pattern of population response from the small sample of single units obtained and the largely qualitative data obtained from multiunit recordings. I have shown that cutaneous afferents in the sole of the foot can encode mechanical transients associated with free standing and some can encode changes in CoP associated with postural sway. This then leads to the suggestion that tactile afferents from the sole of the foot may well

provide useful proprioceptive information that the central nervous system uses in parallel with inputs from the muscle spindles of the intrinsic foot muscles.

6 GENERAL DISCUSSION

The primary aim of this thesis was to record from and to interpret the firing properties of muscle spindles located in the intrinsic foot muscles. This aim arose initially from a desire to extend the work of my Bachelor of Medical Research (Knellwolf et al. 2016). In that study I investigated whether or not modulation of the vestibular system using galvanic vestibular stimulation (GVS) would affect the discharge variability of muscle spindles in the pretibial flexors while the subject was in a "near-vertical" position, presuming that pathways between the vestibular system and the fusimotor system would be engaged only with the postural threat of this positioning. Following the negative results of that study I investigated a method to record from muscle spindles relevant for postural control in unsupported free standing. Recordings have been made successfully from the pretibial flexors via the common peroneal nerve but I wanted to prioritise recording stability to potentially allow spindle recordings during gross movements such as the transition between seated and standing. I theorised that the posterior tibial nerve – supplying the cutaneous afferents of plantar surface of the foot and the muscle spindles of the small intrinsic muscles of the foot – remained relatively stable during the transition from seated to standing, compared to recordings from the common peroneal nerve, and would also be generally more stable during unsupported free standing.

Through a combination of reviewing the literature of the anatomical area, performing an ankle dissection on a cadaver and testing the method on my supervisors and I, a method for performing microneurography recordings on the posterior tibial nerve was developed. This method was summarized in a

publication (Knellwolf et al. 2018). In ***Study 1*** I recorded from muscle spindles of the intrinsic foot muscles in subjects in a seated position with their legs raised and feet unsupported. The aim of this study was to investigate the behaviour of these muscle spindles in a controlled context, assessing their responses to palpation and joint movements and compare them to those in the long muscles as well as the intrinsic muscles of the hand. In ***Study 2***, recordings of spindles of the intrinsic foot muscles were made in free standing subjects, this time assessing their responses to postural changes and determining the presence of modulation by changes in changes in CoP. Whilst recording from the posterior tibial nerve, it was inevitable that I collected a non-negligible amount of cutaneous afferent activity. ***Study 3*** summarises these data and my interpretation of its role in posture maintenance.

I should point out that these were not trivial experiments: compared to recordings from other peripheral nerves, recordings from the posterior tibial nerve are difficult to perform, not the least being the depth of the nerve and the need to maintain stable recordings during the transition from seated (unloaded) to loading and to unsupported freestanding. Nevertheless, I believe this body of work has provided a significant contribution to the literature, as attested to by an editorial in The Journal of Neurophysiology, in which the author states that *"these recent studies provide an integral step toward fully characterizing the responses of afferent fibers in standing and ultimately toward restoring these senses to populations with neurological injury or disease"* (Petersen 2019).

6.1 Behaviour of spindle afferents in unloaded conditions and during free unsupported standing

I have for the first time recorded from muscle spindles of the intrinsic muscles of the foot via the posterior tibial nerve. Using these data, the responses of muscle afferents in all intrinsic foot muscle groups, including their range of discharge frequencies, discharge variability and associated joint movements was documented. From this, it was seen that they behave similarly to muscle spindles studied in long muscles of the leg and forearm and the intrinsic muscles of the hand which, like those of the foot, have very short tendons. Having a short or negligible tendon means that in-series compliance of the muscle-tendon unit is reduced, potentially allowing a more faithful transfer of mechanical events by stretch receptors in the muscles.

As the present study did not attempt to target specific muscles within the foot, the convenience sample sizes within individual muscles were relatively poor, with at most four recordings being made from an individual muscle. This challenge is inherent in the task of attempting to characterise the behaviour of muscle spindles in ten different muscle groups, which likely have distinct as well overlapping responses to movement of the toes or conformational changes in the foot associated with loading by the entire body weight (strictly, approximately half of the body weight per foot). Future studies will need to acquire greater sample sizes within individual muscle groups (~20 for each), which will be the next step in characterising the discharge frequencies and variability of these units in freestanding and during disturbances to posture.

I saw a low proportion of spontaneously firing units (27%) in muscle spindles in the foot, which is similar to the proportion seen in the intrinsic hand muscles (Burke et al. 1988) but significantly lower than those of the pretibial flexors (Burke et al. 1979b). This could represent the differences in incidental resting positions of the joints in these experiments, with ankle plantarflexion in the supine position increasing resting stretch of pretibial flexors compared to the resting toe flexion which would decrease stretch of muscles across these joints. It may also suggest that long muscles of the leg have intrinsically lower thresholds than short muscles of the hands and feet as the proprioceptive feedback that improves posture maintenance is more valuable for muscles acting across the ankle joint than it is for foot stabilisation or object manipulation. Moreover, as noted above, the small muscles of the hand and foot, unlike the extrinsic muscles acting on the wrist or ankle joints, or the fingers or toes, have very short tendons; the consequent reduction in in-series compliance provided by long tendons would mean that there is no slack to be taken up, thereby contributing to their restign discharge properties.

With regards to discharge frequency, there was no clear pattern in firing rates across units and so it is not possible to compare the current data with previous data obtained from extrinsic muscles of the hand (Vallbo 1974b) or ankle p (Macefield et al. 2003). Doing so would require standardization of a resting joint angle for each joint to be monitored with individual goniometers. Differences seen were likely attributed to the difference in resting tension of the muscle.

6.2 Behaviour of spindle afferents during free unsupported standing

Stable recordings were obtained from 12 muscle spindle endings during unsupported standing. I expected the proportion of muscle spindles presenting a spontaneous discharge to be higher in the standing position, from both the mechanical perspective of the muscles being stretched by arch deformation and the functional perspective that the body would then receive information of loading forces within the foot. Indeed, this prediction was borne out. Six (50%) of the spindle endings were spontaneously active in standing and the discharge of each of these showed covariation with changes in CoP. This is likely a result of the increased stretch, due to loading of the foot exceeding the activation threshold of spindles in the unloaded condition. Of the remaining spindles that were not tonically active, they all were recruited during postural perturbations, evidently responding to transient changes in muscle length, two of which these changes were associated with CoP changes along the recorded axes. These findings suggest that muscles spindles in the intrinsic muscles of the foot form part of the neural substrate for control of upright stance, by way of providing proprioceptive feedback of muscle length and, presumably, the shape of the foot.

Deformation of the arch has been shown to be a function of weight on the knee when seated and of ankle angle in standing (Wright et al. 2012). This suggests that muscles with origins and insertions across the arch, such as abductor hallucis, flexor digitorum brevis and quadratus plantae would experience greater stretch during standing; muscle spindles within these muscles may provide information on the position of the body over the base of support. EMG recorded from some

these muscles increases in single leg stance compared to double leg stance (Kelly et al. 2012), and with loaded weight up to 150% body weight – the point at which significant lateral arch deformation plateaus. Additionally, electrical stimulation of these muscles was able to counter the deformation (Kelly et al. 2014). This shows the importance of these muscles in maintaining the shape of the foot, allowing elastic absorption of ground forces.

EMG responses in tibialis anterior and gastrocnemius have been reported during manipulations of the digits and metatarsals, arguing that sensory input from the foot can trigger responses in muscles acting about the ankle (Wright et al. 2012). These results would suggest that muscle spindles in the intrinsic muscles of the foot may be involved in driving these compensatory motor responses. However, I have also seen examples of these spindles playing a simple reactive role to CoP changes instigated by long leg muscle activity. At this point, while the relationship of muscle afferent activity and changes in CoP (likely influencing changes in supplied muscle length) is clear, the causal relationship is yet to be determined. As noted above, future studies in which EMG is recorded from individual intrinsic foot muscles would allow us to correlate their discharge with muscle activity, and thereby differentiate between changes in spindle firing as a consequence of an increase in fusimotor drive and those that are the consequence of postural adjustments. Of course, it is routine to record EMG from the long muscles from which muscle spindles are being recorded, and this can be done with surface electrodes. For the small intrinsic muscles this is more difficult, requiring as it

does invasive intramuscular wires to be inserted into those many small muscles which cannot be accessed via surface electrodes. This was not attempted in the current set of studies as it would add an additional level of complexity to that already faced by developing this new recording methodology of recording from the posterior tibial nerve.

Finally, we know that the sensorimotor control of the small muscles of the foot is similar to the of the hand: recording somatosensory evoked potentials (SSEPs) from the scalp while delivering weak electrical stimuli through a microelectrode inserted into the motor point of abductor hallucis reveals that muscle spindle afferents from the intrinsic muscles of the foot enjoy a rapid sensory transmission to the cortex and a rapid corticospinal motor projection (Macefield et al. 1989).

6.3 Behaviour of cutaneous afferents during unsupported standing

While a detailed consideration of the roles of cutaneous afferents from the sole of the foot is beyond the scope of this thesis, I nevertheless obtained stable recordings in a small number of experiments. Most of these were multiunit recordings which, although they provide limited information content, do give an overall population response of mechanoreceptors in the sole of the foot to loading and unloading, and to the effects of postural sway. Though the multi-unit data was hard to discriminate into an individual unit types, a common pattern seen was a mix of FAI and SAI behaviour in which onset and offset of force would cause a peak in amplitude from the nerve signal, as seen in FAI units, while the baseline nerve

activity would increase for the duration of the force as seen with SAI units. Size of the fascicular innervation territory recorded was likely a function of the number of single units recorded by the electrode.

A useful result of the collation of data is elucidating the emerging patterns of activity that occurred in afferent groups with receptive fields in particular regions of the foot. This is understandable as much of their activity can be predicted from what is known about the afferent responses to punctate stimulation as seen in characterisation (Strzalkowski et al 2018). Unlike in muscle spindles found in the long muscles of the pretibial flexors, whose orientation and points of attachment are largely uniform, the characteristic responses of skin afferents to a set stimulus is more variable. This is expected considering the increased complexity of skin deformations experienced during free-standing, locomotion and unloaded active movements (Smith et al. 2019). As such, when assessing the function of a receptor to detect the narrow criteria of encoding specific axes of CoP change in relaxed free-standing; its response to that stimulus is dependent not only on its intrinsic characteristics but also its location, depth and orientation in the skin. Hence, while some units did not respond in this experiment series they likely serve another similar function which can be hypothesised from the responding units.

With regards to afferents innervating the toes, activity would often only occur during toe movement either during behavioural movement or as a response to postural change. For example, rapid posterior change in CoP due to anterior acceleration would lead to a reflex extension of the toes. At points where contact

was broken or made with the platform, multi-unit activity was seen, which is characteristic of FAI unit encoding of dynamic changes. In addition, at extremes of anterior CoP change when the tip of a digit was pressed into the platform as part of a stabilising flexion, these units might fire. However, many afferents innervating the toes were either silent during freestanding or their activity was not correlated with changes in CoP, likely owing to their location.

It would make sense given the increased distribution of cutaneous afferents on the toes (Strzalkowski et al. 2018) would reflect an increased prioritisation of the area for detecting postural disturbances. We have seen the toes flex and extend to effect postural stabilisation applying pressure to the ground (Tortolero et al. 2008). Minute focal stimulation of the toes has been shown to result in significant balance changes (Viseux et al. 2018) reinforcing the sensitivity of this area. Despite this evidence, the data collected in the present report suggests that cutaneous mechanoreceptors in the toes are largely not engaged by the task of free unsupported standing or even during postural disturbance. If they do activate it may happen in response to corrective toe movements, rather than informing them. There are two caveats to this interpretation. Firstly, many of the units recorded supplied skin that did not directly contact the ground at rest and would not be expected to be involved in the previously described studies. Secondly, the limited activity seen in a unitary recording is naturally compensated for by the increased density of units in the toes and they may indeed provide a powerful afferent signal when a population response is considered.

A multi-unit recording over the ball of the foot responded to both anterior and lateral motion and was spontaneously active during freestanding. I have also shown single SAII units being modulated by one axis of CoP change. Together, SAI units encoding the constant vertical deformation due to body weight, and SAII units encoding stretch due to sheer forces, would provide a neural substrate for both senses of weight and CoP changes. Similarly, units of the medial arch have been seen to respond to both anteroposterior CoP changes and increases in vertical forces as documented during unloading of the contralateral foot. In this case it is likely that cutaneous stretch detectors (SAII units) would play a greater role than force detectors (SAI units) due to the reduced contact with the ground and increased deformation of the arch relative to the heel or lateral arch (Smith et al. 2019)

Units located in the lateral arch appeared to subserve a combination of roles. SAI and SAII units could detect vertical deformation and stretch similar to that seen in the ball of the foot, and FAI units on the lateral foot above the ground would respond when making contact during lateral CoP changes. This indicates this area would be important for both continuous monitoring of weight and CoP and for edge detection. One unit was recorded from the calcaneal nerve supplying the skin over the sole of the heel. As expected, this unit activated more prominently during posterior motion.

With regards to detecting CoP change a common trend noted was that the change in afferent frequency preceded the stimulating change in CoP often by ~100ms.

This difference could be a measure of the deformation of the skin preceding the gross adjustment of the foot rather than the skin deforming after the pressure change has taken place. If this is the case it highlights the sensitivity of these units and usefulness for the role of postural maintenance, where rapid detection is vital for a rapid response to destabilisation events.

6.4 Limitations

Given the complexity of the foot and mechanical constraints, these observations are largely qualitative. For most units there was no measurement of the joint angles: the toes are small and are in close apposition to each other. Nevertheless, these studies have the advantage of specifically identifying the supplied muscles using a greater variety of movements. However, without specific angle data, a quantitative analysis of position sensitivity as a function of change in impulses per second per degree is unable to be provided, which would provide a valuable comparison with the data previously obtained for the long muscles acting on the hand (Vallbo 1974b). The superficial and deep transverse metatarsal ligaments are narrow bands that connect the plantar surfaces of the metatarsal heads. Given the mechanical coupling in the foot, it is understandable that some spindles or cutaneous afferents responded to movements of more than one digit, as movement of an adjacent digit could cause deformation of the muscle belly or movement of the associated digit (and overlying skin). For example, one plantar interosseous spindle responded to extension of both the 4th and 5th digits in addition to abduction of the 5th digit. This pattern is understandable with short muscles having their origins and insertions in close proximity.

It should be noted that although unloading the subject's foot permitted access to the skin of the foot for unit characterization, there were several limitations. Because of the poor angle, blowing over the receptive field to test FAII response was not possible. Moreover, the restricted time limit for characterization made testing mechanical thresholds with von Frey hairs impractical. Receptive fields were harder to define precisely without a direct line of sight of the sole of the foot.

In the free-standing studies, besides limitations on access to receptive field and toe movement that have been described, an unexpected complication of vertical positioning was encountered; that being the tendency for subjects to experience a presyncopal episode. This would manifest as a gradual onset of nausea, lightheadedness, blurred vision and eventually loss of postural tone. During the consenting process, subjects were informed of the possibility of these symptoms and would inform experimenters when they occurred. This would prompt retraction of the recording electrode to a depth of ~10mm, having the subject sit down on the table positioned behind them, followed by lying down as they were moved into supine position. They would appear lethargic with reduced alertness and would have a pallid complexion. The subject recovered spontaneously in 5-10 minutes. At this point the search and recording process was restarted. In most cases this event would occur once in a procedure but occasionally would occur several times with a single subject, prompting cessation of the recording process and supportive care.

Based on what is known about the environmental stressors experienced at the time of onset to this presyncopal episode, I propose a number of mechanisms for this phenomenon. Firstly the presence of prodromal symptoms with a situational stimulus suggests a vasovagal aetiology over a cardiac one. Orthostatic stress produced by prolonged standing is a classic precipitating factor for vasovagal syncope (Hainsworth 2004). This is paired with relative immobilisation – as instructed for the purpose of a stable nerve recording – reduces voluntary contraction of lower limb muscles, an important driver of venous return in normal standing (Mazayshvili 2018). It would make sense that a confluence of these factors would predispose subjects to vasovagal syncope. In addition this response has also occurred immediately following insertion of the electrode prior to prolonged standing. In this instance it may reflect the fear-induced vasovagal response as occurs when blood is taken (Öst et al. 1984).

This phenomenon on several occasions lead to ending the experiment procedure or withdrawing the electrode leading to incomplete data collection and is an inherent challenge to free-standing microneurography studies of the foot. I approached reducing the incidence with a number of methods. Firstly, subjects were asked to eat and drink appropriately prior attending the experiment as to ensure adequate baseline glucose and blood volume. Secondly, the subject was encouraged to frequently flex and extend their contralateral toes to improve venous return. Finally, upright searching was limited to 30 minutes after which the electrode would be withdrawn to a point clear of tendons and neurovascular structures and the subject was asked sit for five minutes then to sit and stand 3 times to allow for gross muscular contraction and improved venous return. I

believe these measures significantly mitigated the risk of vasovagal episodes associated with prolonged standing. However the point of onset, symptoms and efficacy of mitigating factors was not quantitatively studied in this series of experiments and further investigation would benefit the application of this method for future studies.

Unfortunately, EMG activity was not recorded from these muscles. The accuracy of surface electrodes for the small deep muscles would be questionable as it was difficult for the subjects to volitionally contract individual muscles independently, though we know in some humans (such as those who can learn to paint with their foot following loss of upper limb function) this is possible. Moreover, as noted above, we know that muscle spindle afferents in the intrinsic muscles of the foot have a rapid transmission to the somatosensory cortex, arguing that the sensorimotor control of the foot is similar to that of the hand (Macefield & Gandevia 1992). While EMG recordings have been made from some accessible intrinsic foot muscles (e.g. abductor hallucis, abductor digiti minimi) using intra-muscular electrodes (Kelly et al. 2012), I could not justify performing invasive intramuscular recordings and the inherent risk of causing a compartment syndrome, given the confined spaces within the complex tissue layers of the foot. Moreover, I would only know the correct muscle to record from after testing passive movements and performing palpation of the muscle, the latter being particularly difficult for some of the muscles, so it would require intramuscular wire electrodes within all intrinsic muscles. Most EMG recordings from the intrinsic muscles of the hand are likewise limited to accessible muscles, such as

the first dorsal interosseous, abductor digiti minimi or abductor pollicis brevis (Elek & Dengler 1995; Howell et al. 1995; Zijdewind et al. 1999; Hu et al. 2014).

Finally, I do not believe this sample of muscle afferents recorded in the unloaded condition included Golgi tendon organs; we know that human tendon organ afferents do not respond to passive muscle stretch when the parent muscle is relaxed, though do respond to vibration when the muscle is contracting (Fallon & Macefield 2007). Given this, it is possible that some of the afferents that were recorded in standing subjects may have been tendon organ afferents, and in the absence of intramuscular EMG data we will not know. Nevertheless, I think it unlikely, given that all presumed muscle spindle afferents, with two exceptions, could be identified by palpation of the muscle belly or stretch of the parent muscle.

6.5 Future Directions

So, what does this mean? Are cutaneous afferents from the sole of the foot, or muscle spindle afferents in the intrinsic muscles of the foot more important in postural control? We know from the effects of anesthetising the foot, by occluding blood supply below the ankle increases postural sway by 17% (Fitzpatrick et al. 1994). But what this doesn't tell us is the relative contributions of cutaneous and muscle afferents in the foot to maintaining posture. Selective anaesthesia of the cutaneous afferents of the sole of the foot has demonstrated minor increases in average CoP velocity (~11%) (Meyer et al. 2004). However, these authors argue that this indicates that muscle afferents play a larger role in posture maintenance

as greater postural sway (40-60%) is seen in diabetic neuropathy where both muscle and cutaneous afferents are affected (Boucher et al. 1995, Simoneau et al. 1994).

These observations cannot differentiate between the relative roles of muscle spindles and cutaneous afferents: clearly both can encode various aspects of upright stance and its perturbation. However, it can be argued that much of the information provided by tactile afferents, with the potential exception of SAII afferents, appears to be incidental (e.g. making and breaking contact with the supporting surface during behavioural or reflex toe movements). We know within the somatosensory system there a lot of redundancy. Although there are many sensory channels that feed information into the CNS, it unlikely that all of them are used. However, if one channel is blocked it is possible for the other modalities to substitute their role. By understanding these modalities from direct microneurographic recordings we can build the foundation for prosthetic substitution of such senses in the future.

A role for these data in an applied research setting would be in developing prostheses for improving postural stability. By improving this ability in populations with impaired peripheral feedback, this technology may reduce incidence of falls and fall-related injuries. There is currently a wealth research of non-invasive methods for improving posture maintenance (Ma et al. 2016). The common strategy used has been firstly accurately collecting information of postural disturbances and then providing visual (Halická et al. 2014) or auditory

(Dozza et al. 2007) feedback to the subject to inform top-down compensatory behaviour or electrotactile (Nataraj et al. 2013) or vibrotactile (Ma et al. 2015) feedback that involves more streamlined proprioceptive pathways. These balance data may be collected through use of external sensors – force plates measuring CoP (Vuillerme et al. 2007) and motion capture systems measuring centre of mass (Geiger et al. 2001) – that while providing comprehensive data are spatially confined to an experimental setting. Wearable systems collecting inertial motion data (through accelerometers or gyroscopes) (Wall and Kentala 2010) or pressure data (through force sensors attached to the plantar foot surface; Ma et al. 2015) have been developed to allow a portable alternative that allows a wider range of applicable scenarios including activities of daily living.

With regards to feedback strategies, while audio and visual feedback systems have been shown to improve postural control in populations with proprioceptive deficits, they utilise sensory pathways that are not optimised for processing the complex postural data detailed in this report. Vibrotactile feedback strategies (Ma et al. 2015; Koehler-McNicholas et al. 2019) seem more promising in some cases stimulating cutaneous afferents within the same dermatome of the sensory-deficient region. In this way, they provide more native feedback which benefits from central pattern generators in the central nervous system already trained for receiving these afferent inputs (Guertin 2013). By directly recording the afferent signals of both muscle and cutaneous receptors, this body of work represents the next step in understanding how our bodies interpret postural change as well as opens a new avenue for direct substitution of this signal.

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8 APPENDICES

Appendix 1 is an article produced during my Bachelor of Medical Research in which I investigated the response of muscle spindles supplying the tibial flexors of the leg to galvanic vestibular stimulation in near-vertical position.

Appendix 2 is an article describing the effects of random- vs. constant-amplitude sinusoidal linear acceleration on skin sympathetic nerve activity, in which I contributed to the data collection.

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The vestibular system does not modulate fusimotor drive to muscle spindles in relaxed leg muscles of subjects in a near-vertical position

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Knellwolf TP, Hammam E, Macefield VG. The vestibular system does not modulate fusimotor drive to muscle spindles in relaxed leg muscles of subjects in a near-vertical position. *J Neurophysiol* 115: 2529–2535, 2016. First published March 2, 2016; doi:10.1152/jn.01125.2015.—It has been shown that sinusoidal galvanic vestibular stimulation (sGVS) has no effect on the firing of spontaneously active muscle spindles in either relaxed or voluntarily contracting human leg muscles. However, all previous studies have been conducted on subjects in a seated position. Given that independent vestibular control of muscle spindle firing would be more valuable during postural threat, we tested the hypothesis that this modulation would become apparent for subjects in a near-vertical position. Unitary recordings were made from 18 muscle spindle afferents via tungsten microelectrodes inserted percutaneously into the common peroneal nerve of awake human subjects laying supine on a motorized tilt table. All recorded spindle afferents were spontaneously active at rest, and each increased its firing rate during a weak static contraction. Sinusoidal bipolar binaural galvanic vestibular stimulation (±2 mA, 100 cycles) was applied to the mastoid processes at 0.8 Hz. This continuous stimulation produced a sustained illusion of "rocking in a boat" or "swinging in a hammock." The subject was then moved into a near-vertical position (75°), and the stimulation repeated. Despite robust vestibular illusions, none of the fusimotor-driven spindles exhibited phase-locked modulation of firing during sinusoidal GVS in either position. We conclude that this dynamic vestibular stimulus was insufficient to modulate the firing of fusimotor neurons in the near-vertical position. However, this does not mean that the vestibular system cannot modulate the sensitivity of muscle spindles via fusimotor neurons in free unsupported standing, when reliance on proprioceptive feedback is higher.

gamma motoneurones; vestibular; postural control

THE ABILITY OF THE BODY to determine spatial orientation and manage postural and locomotive control is largely dependent on the vestibular apparatus, comprising the semicircular canals (responsive to rotational acceleration) and otoliths (responsive to horizontal and vertical linear acceleration; Lackner and DiZio 2005). Muscle spindles are specialized stretch receptors that lie parallel to skeletal muscle and provide proprioceptive information regarding limb, neck, and torso position in space. This sensory input is integrated with vestibular signals, allowing the body to perform complex, dynamic equilibrium tasks (Pozzo et al. 1995). Muscle spindles are innervated by static and dynamic γ-motoneurons (fusimotor neurons) that, through their actions on intrafusal muscle fibers, can change their sensitivity to muscle stretch. Although direct recordings from γ-motoneurons are technically difficult in humans, changes in fusimotor drive can be reliably inferred from changes in the firing of muscle spindle afferents, from which single-unit recordings can readily be obtained in awake humans via an intraneural microelectrode inserted percutaneously into a peripheral nerve (microneurography). Because many muscle spindles are active at rest, owing to the prevailing degree of muscle stretch in the receptor-bearing muscle, a fall in firing rate during a voluntary contraction is interpreted as unloading of the spindle by shortening of the extrafusal muscle. Conversely, if the firing rate is maintained or increases, or a muscle spindle is recruited, during a voluntary contraction, it can be concluded that fusimotor neurons have been activated.

It is generally accepted that, in humans, skeletomotor (α) and fusimotor (γ) neurons are coactivated during voluntary contractions, with little evidence of independent control of the two motor systems. However, it is known in the cat that the fusimotor neurons can change the sensitivity of the muscle spindles independently, improving their signaling capacity when walking along a narrow branch, for example. Moreover, monosynaptic and polysynaptic connections exist between the vestibular and fusimotor systems (Pompeiano 1972; Pompeiano et al. 1966), and electrical stimulation of the vestibular nuclei in the cat has been shown to lower recruitment thresholds for γ-motoneuron activation (Diete-Spiff et al. 1967).

In humans, galvanic vestibular stimulation (GVS), a means of selectively changing the spontaneous discharge of afferents originating in the vestibular apparatus, has been used extensively to study the contributions of the vestibular system to posture and locomotion. For instance, it has been shown to lower the voluntary recruitment threshold of α-motoneurons to the legs (Fitzpatrick and Day 2004; Wardman and Fitzpatrick 2002). We have used sinusoidal GVS (sGVS) to determine whether the vestibular system can change the sensitivity of muscle spindles but found no evidence of such modulation of spontaneously active muscle spindles in relaxed leg muscles (Bent et al. 2007). Given that there is negligible fusimotor drive to relaxed muscles in humans (Burke et al. 1979; Macefield 2012), this could be explained by the possibility that any vestibular modulation of γ-motoneurons would not be observed if the fusimotor neurons themselves are not already active. In a subsequent study, we showed that there was no vestibular modulation of muscle spindles in active leg muscles, in which subjects were asked to perform a weak voluntary contraction to engage the fusimotor neurons to the receptor-bearing muscles (Bent et al. 2013). However, because both of these studies were conducted in seated subjects, it is possible

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that the absence of any vestibular modulation was related to this particular experimental condition.

Here, we asked the question, would the vestibular apparatus be able to induce modulation of fusimotor neurons, as assessed by changes in muscle spindle stretch sensitivity, in the upright condition, when task-dependent changes in segmental and supraspinal circuitry may be expected to occur? Indeed, changes in posture have been shown to modulate muscle spindle sensitivity from stimulation of mechanoreceptors in the foot (Aniss et al. 1990). Teleologically, this makes sense: changes in posture would reduce stability and require a more accurate feedback on limb positioning. From this concept of task dependency, we posit that the physiological changes associated with adoption of the upright stance bring about an upregulation of vestibular modulation of fusimotor drive while recognizing that an increase in vestibular modulation of α-motoneurons only occurs when postural activity is coupled to body sway (Fitzpatrick et al. 1994; Luu et al. 2012). In the current study, we tested the hypothesis that the vestibular system can increase fusimotor activity independent of any changes in skeletomotor activity. To achieve this, we recorded from single muscle spindle afferents in human subjects in two postural conditions: supine and during upright tilt to a near-vertical position.

METHODS

Data were collected from 10 healthy subjects (4 women and 6 men; age range 18–28 yr) over 14 experimental sessions. All subjects provided written, informed consent. The study was conducted with the approval of the Human Research Ethics Committee, Western Sydney University, with all procedures conducted in accordance with the principles of the Declaration of Helsinki.

Experimental protocol. Throughout the experiment, participants were in 2 positions: originally supine and then near-vertical (75° from supine). In the supine position, participants lay on a motorized tilt table (Athlegen) with both feet against a rigid footplate at the end of the table. A vacuum pillow (Germa, Kristianstad, Sweden), placed just proximal to the knees, was used to elevate the knee to permit access to the common peroneal nerve. In the near-vertical position, the ankle joints remained at the same angle as in the supine position (~10° knee flexion, ~5° ankle dorsiflexion). Electromyographic (EMG) activity was recorded from the muscles of the anterior compartment of the leg from which muscle spindle activity was recorded as well as the plantarflexors to ensure that the muscles were quiescent in the supine condition and active in the upright position. Disposable Ag/AgCl electrodes were placed over the muscle belly and tendon of tibialis anterior and soleus. EMG activity was amplified, filtered (bandwidth 10 Hz to 1 kHz, 50-Hz notch), and digitized at 2 kHz (LabChart 7, PowerLab 16/35; ADInstruments, Sydney, Australia).

Transdermal electrical stimulation, delivered through a 1-mm probe via an optically isolated constant-current source (0.2 ms, 1–10 mA, 1 Hz; ML180; ADInstruments), was used initially to locate the common peroneal nerve at the fibular head. An insulated tungsten microelectrode (FHC) was then inserted percutaneously, and a low-impedance reference electrode was inserted subdermally ~1 cm away. Electrical stimuli (0.2 ms, 0.1–1 mA, 1 Hz) were delivered through the microelectrode as it was advanced toward the nerve. Muscle twitches evoked at currents <0.02 mA indicated that the microelectrode tip had penetrated a muscle fascicle. Neural activity was amplified (gain 20,000, band pass 0.3–5.0 kHz) using an isolated amplifier (Neuro Amp EX; ADInstruments) and digitized at 10 kHz (LabChart 7, PowerLab 16/35; ADInstruments). A muscle fascicle was identified by the following criteria: *1*) electrical stimulation through the microelectrode induced muscle twitches below a threshold of 0.02 mA, *2*) percussion or passive stretch of tendons or bellies of muscles supplied by the common peroneal nerve resulted in muscle afferent mechanoreceptor impulses, and *3*) light stroking of the skin did not evoke impulses of tactile afferents. After the fascicle was identified, a single, spontaneously active, muscle spindle unit was isolated. Muscle spindle afferents demonstrated a characteristic tonic discharge, of which the mean frequency could be increased by stretching the parent muscle and decreased by passively unloading the muscle. Spindle afferents were provisionally classified as primary endings according to their high dynamic sensitivity to stretch, typically followed by a pause in firing, and a more variable discharge; secondary endings possessed low dynamic sensitivity and low discharge variability.

Following spindle identification, sinusoidal galvanic vestibular stimulation (sGVS) was applied via surface electrodes over the mastoid processes behind the ears in a bipolar binaural configuration. Stimulation (100 cycles, ±2 mA, 0.8 Hz) was delivered using an optically isolated current stimulator (A395; World Precision Instruments). During stimulation, the subject's head was positioned neutrally with respect to the body, supported on a headrest of the table with the nose midline. The control voltage was recorded with the nerve and EMG signals. Subjects were asked to close their eyes before and during the stimulation, which was delivered at unexpected times, and to report on any sensations at the conclusion of the recording. Following a recording in the supine position, the subject was moved into the near-vertical position via the motorized tilt table. Unfortunately, some muscle spindle afferent unitary recordings were lost during this movement, owing to stiffening of the subject's legs in the upright position. For those recordings that remained stable, sGVS was then repeated. Occasionally, a new muscle spindle afferent was isolated in the near-vertical position.

Data analysis. Action potentials from a single muscle spindle afferent and the positive peaks of the sinusoidal stimulus were discriminated using software (Spike Histogram, LabChart 7; ADInstruments). Discriminator levels of neural activity were adjusted so as to include all positive-going peaks of width <0.5 ms and height >5 μV above the background noise. Instantaneous frequency of the discriminated spindle firing was also calculated. The mean and standard deviation of the frequency were established and used to calculate the coefficient of variation (CV = μ/σ), used as a measure of discharge variability. To determine the existence of a temporal correlation between the sinusoidal vestibular input and the muscle spindle firing, the same software was used to construct cross-correlation histograms between the positive peaks of the sinusoidal GVS and the spindle afferent spikes as well as autocorrelation histograms of the GVS data (50-ms bins). The period over which the histograms were calculated was equivalent to eight cycles of GVS. The histogram data were exported as text to a statistical and graphic analysis program (Prism 6 for Mac OS X v6.0g; GraphPad Software), and a smoothed polynomial function was generated. Fourth-order smoothed polynomials (twelve neighbors) were used to fit curves to the cross-correlation histograms. Vestibular modulation was quantified by measuring the difference in the number of spikes on the smoothed curve at the peak of the modulation and at the trough. These were then converted into a measure of modulation by employing the following formula: modulation index (%) = [(peak − trough)/peak] × 100. One-tailed *t*-tests or Mann-Whitney tests were used to determine whether mean firing rate, discharge variability, and modulation index were significantly higher in the upright position; $P < 0.05$ was considered statistically significant.

RESULTS

Recordings were obtained from eighteen single muscle spindle afferents across fourteen experiments. Five of the endings were located in tibialis anterior (TA), two in extensor hallucis

longus (EHL), six in extensor digitorum longus (EDL), and five in the peronei muscles. From these spindles, thirteen recordings were made in the horizontal position, and nine were made in the near-vertical position. All of the afferents were spontaneously active at rest and increased their mean firing rate during passive stretch or a weak voluntary contraction of the receptor-bearing muscle. Based on high variability of resting discharge and high dynamic sensitivity to stretch, nine afferents were classified as primary endings; nine afferents with low discharge variability and low dynamic stretch sensitivity were classified as secondary endings.

Sinusoidal galvanic vestibular stimulation (GVS) was delivered twice, once in the supine position and again once the subject had been moved to the upright position. All subjects reported strong illusions of movement. The perceived movement matched the frequency of the GVS and was described as the perception of either "swinging in a hammock" or "being pushed gently on alternating sides of the head." In a few subjects, nausea and/or light-headedness was reported in the near-vertical position, although this occurred with and without GVS.

Experimental records from one subject in the supine position are shown in Fig. 1. This spindle primary ending, located in tibialis anterior, showed a fairly regular spontaneous discharge that was initially increased at the onset of sGVS. It can be seen that this transient increase in discharge rate and variability disappeared after ~10 s, the spindle resuming its regular spontaneous discharge at the same mean frequency as before the stimulation. Although there was some low-level EMG in soleus, there was no detectable activity in the receptor-bearing muscle. Figure 2 shows this same recording following passive tilting of the subject to a near-vertical position. It should be pointed out that there was no overt change in muscle length because the feet were firmly placed in contact with the footplate in the supine position, so as to allow the subject to bear load on the feet when tilted upright. Clearly, EMG increased in the leg muscles, but this EMG did not infiltrate the nerve signal. It is apparent that discharge variability was higher in the upright position than in the supine position (cf. Fig. 2); this high variability continued as sGVS was applied but was not maintained for the duration of stimulation. This behavior was seen only in this particular muscle spindle ending; no other spindle afferents exhibited an increase in mean firing rate and such an overt increase in discharge variability during sGVS.

Figures 3 and 4 provide examples of cross-correlation histograms computed for 2 muscle spindle endings recorded in the near-vertical position. Mean modulation indices for the horizontal and near-vertical positions from the sample of muscle spindles are shown in Fig. 5. There was no significant modulation in spindle firing between the 2 states ($P = 0.26$, 1-tailed Mann-Whitney test).

As noted in the Introduction, the presence of fusimotor activity as a result of α-γ coactivation can be demonstrated by changes in mean frequency and discharge variability. Mean data are shown in Fig. 6. It can be seen in Fig. 6*A* that the mean discharge frequency did not change significantly between the 2 positions. Importantly, despite the EMG evidence of skeletomotor activity in the upright position, mean firing rate did not decrease; there was no unloading of the spindles, and mean firing rate was evidently maintained by the ongoing fusimotor drive. This is supported by the significant increase in resting discharge variability ($P = 0.0179$, 1-tailed Mann-Whitney test) in the upright condition (Fig. 6*B*).

DISCUSSION

Using sinusoidal GVS, we have shown that there is no significant vestibular modulation of fusimotor-driven muscle

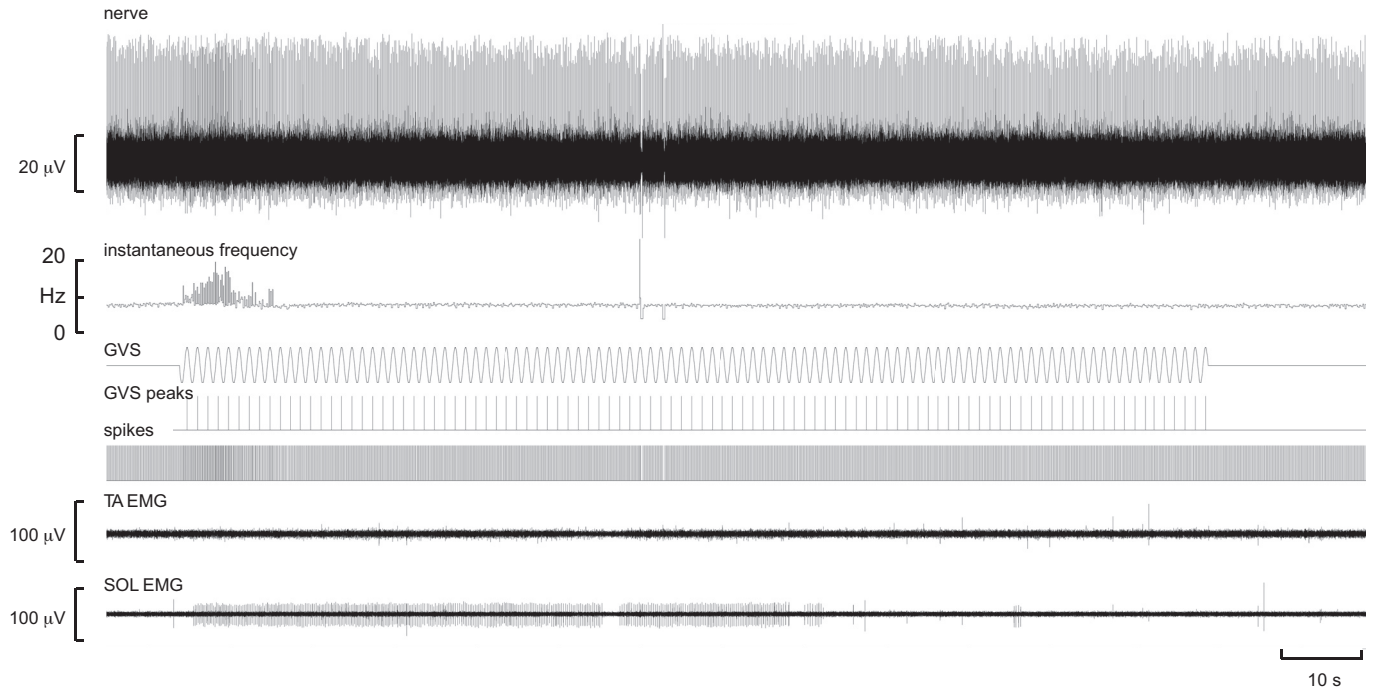

Fig. 1. Single-unit recording of muscle spindle activity in supine position. Individual spikes were extracted from the raw nerve data and illustrated as standard pulses (spikes). The positive peaks of sinusoidal galvanic vestibular stimulation (GVS) were similarly discriminated and are displayed as standard pulses (GVS peaks). These timing events were used to generate cross-correlation and autocorrelation histograms. TA, tibialis anterior; SOL, soleus.

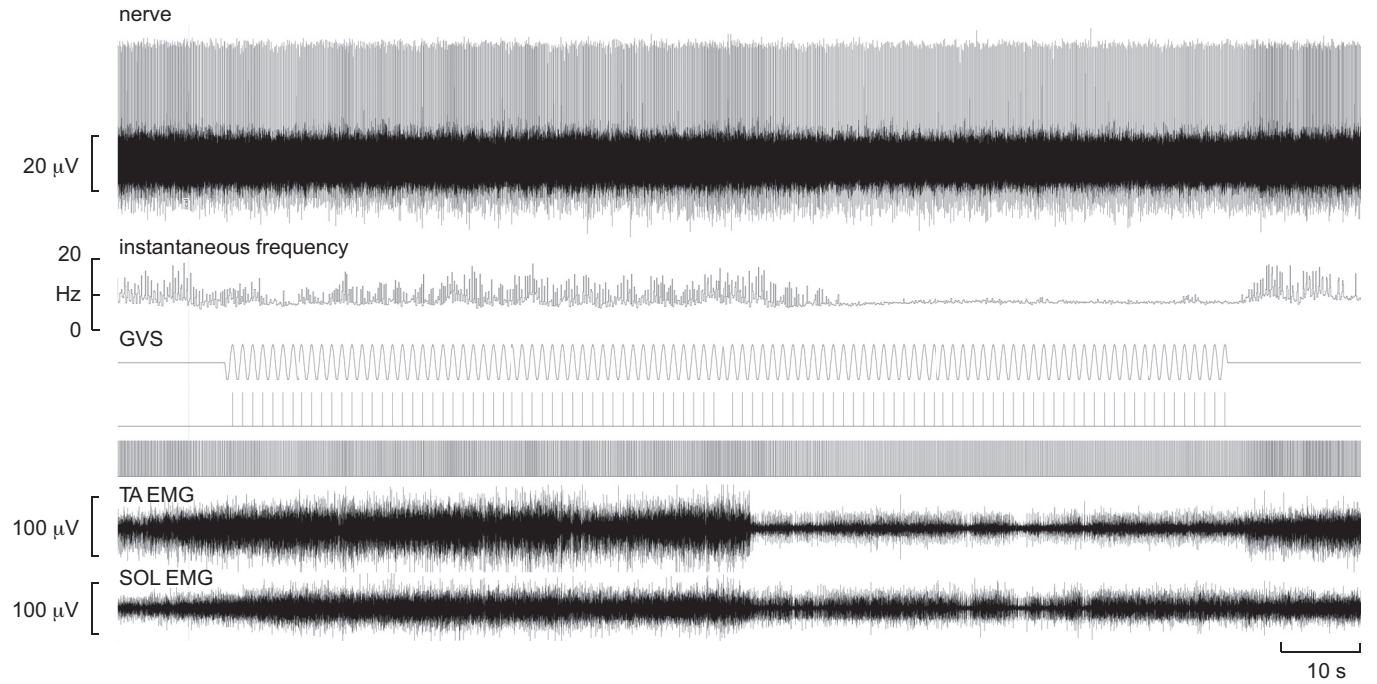

Fig. 2. Single-unit recording of muscle spindle afferent in near-vertical position. Data obtained from the same subject as Fig. 1.

spindles in a near-vertical, supported position. As outlined in the Introduction, previous studies from this laboratory have failed to find evidence of vestibular modulation of muscle spindles in leg muscles while relaxed (Bent et al. 2007) or during volitionally generated static contractions (Bent et al. 2013). These results contradict assumptions made from the relationship between the vestibular and proprioceptive systems as examined in the cat.

Previous physiological evidence and teleological consideration of the two systems each leads to the assumption of significant physiological interdependence. Both play important roles in creating a meaningful and continuous mental representation of the orientation of the body in space. Muscle spindles are considered the most important source of proprioceptive information (Burke et al. 1988; Proske and Gandevia 2009), primarily in determining limb, neck, and torso position with respect to the body. The vestibular system integrates this proprioceptive information with inputs of head position with respect to gravity (Lackner and DiZio 2005), completing the mental image that plays a vital role in posture and locomotion. We recently showed that sinusoidal stretch of muscle spindles in the neck muscles caused a pronounced sinusoidal modulation of muscle sympathetic nerve activity in supine subjects, presumably via the vestibular nuclei (Bolton et al. 2014).

Based on the above, it is reasonable to posit that the vestibular system would possess some capacity to modulate the sensitivity of muscle spindles independently, namely through the fusimotor system. This suggestion is supported by previous evidence in the cat. The interaction between somatosensory and vestibular input in the vestibular nuclei, motoneurons, and spinal interneurons has been well-documented (Anastasopoulos and Mergner 1982; Pompeiano and Brodal 1957; Rubin et al. 1977; Wilson 1988; Wilson et al. 1966). In the cat hindlimb, monosynaptic and polysynaptic connections have been established from vestibular nuclei to α-motoneurons (Grillner et al. 1970; Lund and Pompeiano 1965; Orlovsky 1972) and γ-motoneurons (Grillner et al. 1969; Pompeiano 1972; Pompeiano et al. 1966). Diete-Spiff and colleagues (1967) demonstrated an increase in muscle spindle discharge in the cat hindlimb after stimulation of the lateral, medial, and inferior vestibular nuclei as well as during direct stimulation the VIIIth cranial nerve.

Despite the obvious links established in animal studies, there has yet to be any convincing and reproducible human evidence that can identify independent modulation of spindle gain via the fusimotor system. Reflex connections of human fusimotor neurons with cutaneous and muscle afferents (Aniss et al. 1990; Gandevia et al. 1994) as well as visual inputs (Jones et al. 2001) have indicated the potential substrate for such independent control to exist. However, studies in relaxed (Bent et al. 2007; Gandevia et al. 1994; Ribot-Ciscar et al. 2000) and active (Aniss et al. 1990; Bent et al. 2013; Jones et al. 2001; Nafati et al. 2004) muscles have yet to demonstrate independent fusimotor changes in response to vestibular perturbations.

The concept of task dependency for independent control has been a primary driver in the direction of our research. Aniss and colleagues (1990) conducted a study investigating the influence of low-threshold cutaneous and muscle afferents from mechanoreceptors in the sole and dorsum of the foot on fusimotor activation of spindles supplying the pretibial muscles. Although no activation was demonstrated in sitting, for both quiescent muscles and during voluntary contraction, it was clearly seen during contractions in response to overt postural sway in unsupported standing. In addition, it has been shown that the gain for vestibular-evoked EMG responses is modified according to need for vestibular information, such as in free, unsupported standing (Fitzpatrick et al. 1994; Luu et al. 2012). This led to the proposal that the postural standing task is necessary to establish the reflex pathways. From this evidence, we posit the physiological change that allows these pathways is an upregulation of vestibular activity as required of

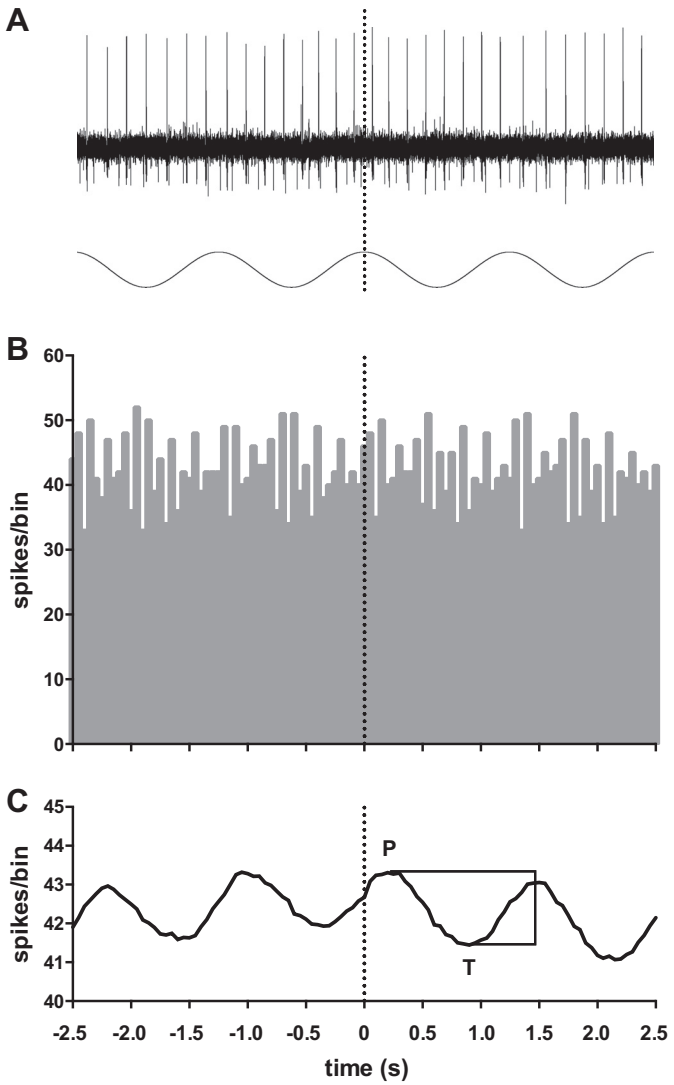

Fig. 3. Cross-correlation histogram between single spindle afferent and GVS in near-vertical position. *A*: sample of the spike train used to calculate the cross-correlation histogram in *B*; the 0.8-Hz sine wave represents the sGVS. *C*: smoothed data (4th-order polynomial) used to determine the modulation indices from the peak (P) and trough (T); modulation index = [(peak − trough)/peak] × 100.

the task. It is likely that vestibulospinal pathways were engaged in the near-vertical position, but we cannot be certain. Nevertheless, we know that discharge variability increased significantly in this position, consistent with an increase in fusimotor drive to the receptor-bearing muscle.

However, we have in the present study demonstrated that no independent modulation of the fusimotor system occurs via the vestibular apparatus in the near-vertical position. One possible explanation for this is that whereas the subject's position during stimulation is designed to simulate a standing position, the subject's center of gravity remains over the table, thus distributing a portion of his or her weight against a stable surface rather than his or her legs. In addition, there was no attempt to introduce overt postural sway. These limited conditions may reduce the need for precise monitoring of muscle length and position in space that could necessitate independent modulation of the fusimotor system. With these considerations, it remains to be seen whether significant modulation would be detected in freestanding subjects, particularly with postural perturbations. If this were the case, our present study would provide evidence for the task dependency of these pathways, as it is clear that they are nonexistent while the subject is stable regardless of being in the supine or near-vertical position.

Methodological considerations. There have been very few human studies that have obtained single-unit recordings from individual muscle spindle afferents in freestanding conditions (Aniss et al. 1990; Burke and Eklund 1977; Inamura et al. 1993). The microneurographic technique depends on the precise insertion of the microelectrode so as to impale the myelin sheath of a single myelinated sensory axon (10–12 μm in diameter) and to maintain this intraneural recording site during the entire recording process. This, in turn, requires that the subject maintain a static position throughout the experimental protocol, so as not to dislodge the electrode, and that EMG signals from the leg muscles do not swamp the nerve signal.

The use of a tilt table in our study was to mitigate risk of losing the recording site during transition between the two

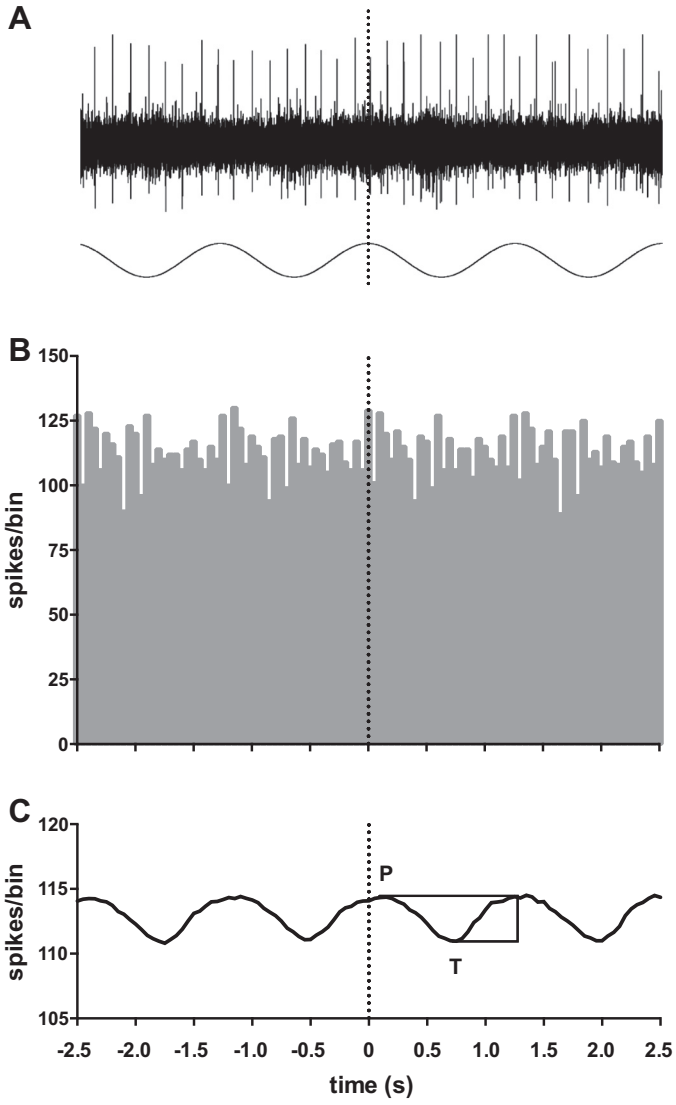

Fig. 4. Cross-correlation histogram between single spindle afferent and GVS in near-vertical position. Same format as Fig. 3; data from a different muscle spindle.

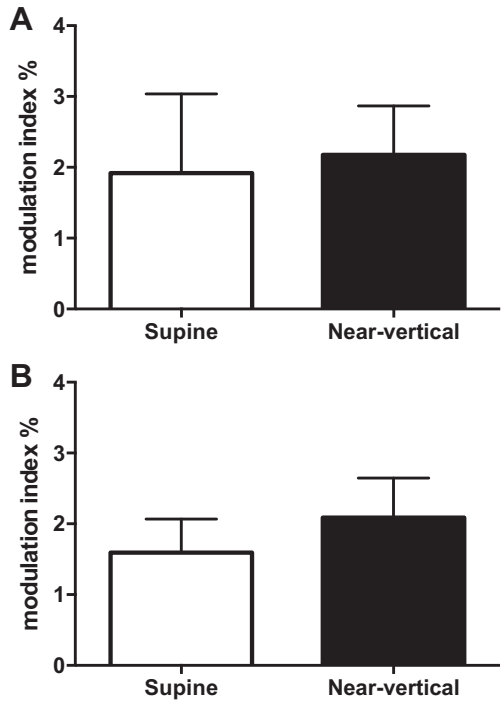

Fig. 5. Vestibular modulation indices of muscle spindle afferents during GVS in supine and near-vertical positions. Paired (*A*) and unpaired (*B*) data are shown. Modulation index = [(peak − trough)/peak] × 100. There was no significant difference between the supine and near-vertical positions.

positions. Despite this, many recordings were obtained in the supine position and subsequently lost in the near-vertical position due to deterioration or abolishment of recording quality: recordings were obtained from thirteen units in the supine position but only nine in the near-vertical position. One contributing factor to the instability is the recording site. The common peroneal nerve, although very accessible, is mobile and susceptible to stretch during changes in posture.

Because of the difficulty in establishing and maintaining a reliable and suitable recording, our experimental protocol was time-limited and focused on the primary comparison of supine and near-vertical recordings during sinusoidal GVS and thus wanting of further investigation. Unlike similar studies investigating the effects of GVS, we did not include a sham GVS period. This was not seen as relevant as previous studies (Bent et al. 2007, 2013) have demonstrated that no modulation occurs with sham sGVS, let alone during actual sGVS. We also only delivered sGVS at one frequency, 0.8 Hz. This frequency was selected as previous studies have shown it to represent realistic vestibular input encountered during sway in quiet stance. However, it would be of interest to test different frequencies to eliminate the possibility that the vestibular pathways to the fusimotor system are more susceptible to significantly higher or lower frequencies. For example, marked vestibular modulation of muscle (Hammam et al. 2011) and skin (Hammam et al. 2012) sympathetic nerve activity has been demonstrated at frequencies of 0.08–0.18 Hz as well as at frequencies up to 2 Hz (Grewal et al. 2009; James et al. 2010). It may also be possible that the vestibular system would respond to higher frequencies, matching average step rate or some other balance-dependent task. Inclusion of a wider range of frequencies in future studies with a more stable recording procedure should be considered. Despite this, we do believe that tilting subjects to a near-vertical position in the present study did adequately engage the fusimotor system: as noted above, the increase in discharge variability in this position is consistent with an increase in fusimotor drive to the muscle.

Implications. In terms of determining the nature of the relationship between the vestibular system and the fusimotor system, the present research presents two potential implications. First, it is possible that there is no physiological need for the vestibular system to change the sensitivity of spindles in standing independently. Aside from spindles, it is known that certain myelinated cutaneous afferents provide proprioceptive information about joint movements (Aimonetti et al. 2007; Burke et al. 1988; Hulliger et al. 1979), albeit to a smaller extent. This has been shown to occur in the absence of changes in muscle or joint afferent input (Edin and Johansson 1995). Considering the range of potential inputs for measuring and calibrating posture and comparing the capacity of the muscle spindles and vestibular system to detect these changes, it is possible that there is no need for further tuning of the fusimotor system for normal, postural, and locomotive tasks, which does not discount the contributions of muscle spindles from the leg muscles in postural control. Alternatively, it may be that the

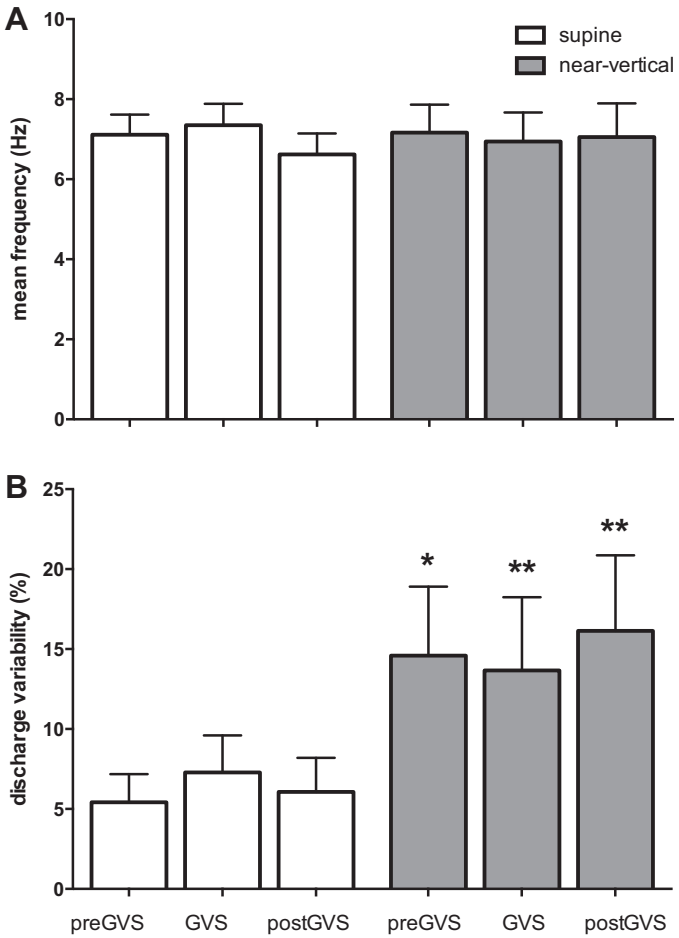

Fig. 6. Mean frequency (*A*) and discharge variability (*B*) of spindle firing rate during sGVS. Discharge variability (coefficient of variation) = mean/SD × 100. Firing rate was calculated before GVS (preGVS), during the initial 10 s of GVS (GVS), and after GVS (postGVS) in both supine and near-vertical positions. *$P < 0.05$; **$P < 0.02$.

current and previous studies did not adequately engage the vestibular system to a degree that necessitates independent modulation of spindles. This implies that we would see modulation in more demanding postural or locomotive tasks such as free, unsupported standing with and without perturbations to the upright posture.

Conclusions. We have shown that there is no significant vestibular modulation of muscle spindle activity in the pretibial flexors in humans tilted into a near-vertical position despite evidence that the fusimotor system is engaged in this position.

GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

E.H. and V.G.M. conception and design of research; T.P.K., E.H., and V.G.M. performed experiments; T.P.K. analyzed data; T.P.K., E.H., and V.G.M. interpreted results of experiments; T.P.K. prepared figures; T.P.K. drafted manuscript; T.P.K., E.H., and V.G.M. edited and revised manuscript; T.P.K., E.H., and V.G.M. approved final version of manuscript.

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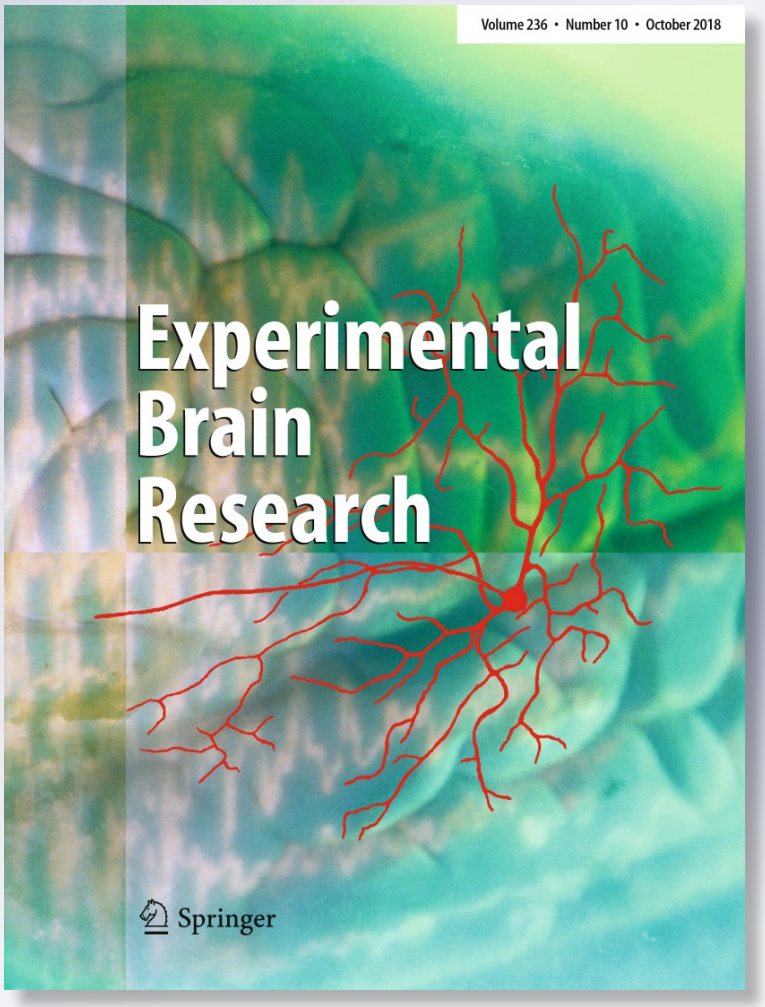

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RESEARCH ARTICLE

Random-amplitude sinusoidal linear acceleration causes greater vestibular modulation of skin sympathetic nerve activity than constant-amplitude acceleration

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Abstract

We tested the hypothesis that random variations in the magnitude of sinusoidal linear acceleration cause greater modulation of skin sympathetic nerve activity (SSNA), but not muscle sympathetic nerve activity (MSNA), than sinusoidal stimuli of the same frequency but constant amplitude. Subjects ($n = 22$) were seated in a sealed room mounted on a linear motor that could deliver peak sinusoidal accelerations of 30 mG in the antero-posterior direction. Subjects sat on a padded chair with their neck and head supported vertically, thereby minimizing somatosensory cues, facing the direction of motion in the anterior direction. Each block of sinusoidal motion was delivered at 0.2 Hz, either with a constant-amplitude (root mean square 14 mG) or randomly fluctuating amplitudes of the same mean amplitude. MSNA (n = 12) and SSNA ($n = 10$) were recorded via tungsten microelectrodes inserted into muscle or cutaneous fascicles of the common peroneal nerve. Cross-correlation analysis was used to measure the magnitude of vestibular modulation. The modulation index for SSNA was significantly higher during delivery of random vs constant-amplitude acceleration (31.4 ± 1.9 vs 24.5 ± 2.5%), but there was no significant difference in the modulation indices for MSNA (28.8 ± 2.9 vs 33.4 ± 4.1%). We conclude that the pattern of vestibular stimulation affects the magnitude of modulation of sympathetic outflow to skin but not to muscle. Presumably, this is related to the subperceptual development of nausea, which is known to be associated with greater vestibular modulation of SSNA but not MSNA.

Keywords MSNA · Otolithic organs · SSNA · Sympathetic · Vestibular

Introduction

The capacity of the vestibular apparatus—comprising the bilateral otolithic organs (utricle and saccule) and semicircular canals—to modulate the sympathetic nervous system is well-established from neuroanatomical and neurophysiological studies in experimental animals (for review see Yates et al. 2014; McCall et al. 2017). Various non-invasive methods—caloric stimulation, head-down neck flexion, off-vertical axis rotation, galvanic stimulation and linear acceleration have been used to demonstrate similarly robust vestibular modulation of sympathetic outflow to muscle and skin in humans, with the conclusion that it is the otolithic organs, rather than the semicircular canals, that are responsible for the generation of vestibulosympathetic reflexes (for review see Carter and Ray 2008; Yates et al. 2014; Hammam and Macefield 2017).

Most recently, our laboratory has used low-amplitude, low-frequency sinusoidal linear acceleration as a means to activate the utricle or saccule in humans physiologically: slow sinusoidal acceleration (4 mG, 0.08 Hz) causes a marked modulation of muscle sympathetic nerve activity (MSNA) and skin sympathetic nerve activity (SSNA), either with the body seated and head vertical—which favors stimulation of the utricle (Grewal et al. 2012; Hammam et al.

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2013)—or with the body and head supine, which favors stimulation of the saccule (Hammam et al. 2014a; Bolton et al. 2016). Modulation of MSNA is apparent at accelerations as low as 1.25 mG when delivered at 0.2 Hz, which is well below the threshold for detection of the sinusoidal motion (~ 6.5 mG at 0.2 Hz), and shows a linear increase in the magnitude of modulation up to 30 mG (Hammam et al. 2014b). This emphasizes not just how sensitive the vestibular afferents are to slow accelerations but also that vestibulosympathetic reflexes have a very low threshold for initiation.

However, what we do not know is whether the pattern of vestibular stimulation affects the magnitude of sympathetic modulation: given that perceptions of motion can lead to motion sickness in certain individuals during sinusoidal galvanic vestibular stimulation (sGVS) when delivered at low frequencies (0.08–0.18 Hz), one might expect that random variations in the intensity of vestibular stimulation will lead to greater perceptions of motion sickness. And, given that those subjects who report nausea during sGVS show a greater vestibular modulation of SSNA (Hammam et al. 2012) but not MSNA (Klingberg et al. 2015), one might expect that random fluctuations in acceleration will lead to increases in vestibular modulation of SSNA but not of MSNA. Here we tested the hypothesis that random variations in the magnitude of sinusoidal linear acceleration cause greater modulation of SSNA than sinusoidal stimuli of the same frequency but constant amplitude. Given that occupants of tall buildings may experience nausea when the building sways in the wind (Lamb and Kwok 2017), and that such building motion typically features random fluctuations in amplitude at the resonant frequency of the building, we obtained a set of random-amplitude linear accelerations from a model of a tall building exposed to gusts of airflow in a wind tunnel. These were delivered to the seated subject at the same frequency (0.08 Hz) and mean acceleration (root mean square 14 mG) as a set of constant-amplitude stimuli. This amplitude was chosen as it is above perceptual threshold for detection of motion and detection of direction of motion (Hammam et al. 2014b).

Methods

The study was conducted at the Hong Kong University of Science and Technology. Experiments were performed on 22 subjects (11 male, 11 female; 18–23 years); all participants were Chinese. Muscle sympathetic nerve activity (MSNA) was recorded in 12 subjects and skin sympathetic nerve activity (SSNA) in 10. All participants provided informed written consent to the experiments, which were approved by the Human Research Ethics Committees of the Hong Kong University of Science and Technology and Western Sydney University. As per recommended protocol when recording sympathetic activity, participants were asked to avoid any caffeinated beverages and to abstain from smoking. During the study, subjects were seated in a padded armchair with their feet on the floor and the head supported by a padded and enclosed frame that kept the head and neck in a vertical position. The head was then stabilized with a Velcro strap to avoid rotations and other movements, so linear acceleration primarily activated the utricular hair cells in the vestibular apparatus. The chair was placed against the rear wall of a 4×3 m room. The room was built on a motion simulator platform that contained two sets of linear motors capable of accelerating the room in the horizontal plane with a maximum amplitude of 30 mG (0.3 ms^{-2}). The room contained no windows, so there were no visual cues of movement. Subjects were asked to relax during the recordings with eyes closed. They were provided with earplugs and were exposed to white noise through headphones to minimize any auditory cues to motion.

Two sets of sinusoidal linear acceleration, each lasting 31 min, were applied at a time unknown to the subject. Both sets were delivered at 0.08 Hz and comprised 100 cycles: in one (constant amplitude) the amplitude was kept at 14 mG, in the other it varied randomly with a mean RMS amplitude of 14 mG. The latter series was obtained from the behavior of a model tall building in a wind tunnel; the amplitude was scaled to match the standard deviation of the target RMS amplitude (14 mG). The two patterns of stimuli are illustrated in Fig. 1. A two-minute rest interval was placed between the two sets of stimuli and the order of presentation was randomized using a set of random numbers. Between trials, subjects were asked to open their eyes and report any perceptions of motion, nausea or any other discomfort. Nausea/discomfort levels were measured using a potentiometer signal (scale 0–10) whereby zero indicates no discomfort and ten being unbearable nausea and vomiting.

Microneurography was performed on the left leg, which was supported horizontally. Sympathetic nerve activity either to muscle vascular bed (MSNA) or to skin (SSNA) was recorded from fascicles of the left common peroneal nerve via a tungsten microelectrode (FHC, Bowdoin, ME, USA) inserted percutaneously at the fibular head; an uninsulated reference microelectrode was inserted subdermally ~ 1 cm away. Intraneural stimulation (0.01–1.0 mA, 1 Hz, 0.2 ms pulses), delivered to the microelectrode via an isolated stimulator (Stimulus Isolator, ADInstruments, Sydney, Australia) was used to guide the microelectrode tip into a muscle or cutaneous fascicle of the nerve. A muscle fascicle was identified as such when electrical stimulation at 20 µA through the microelectrode induced muscle twitches in the fascicular innervation territory without radiating paraesthesiae. The microelectrode was manually advanced until spontaneous bursts of MSNA with a clear cardiac rhythmicity were encountered, and a sustained increase in burst

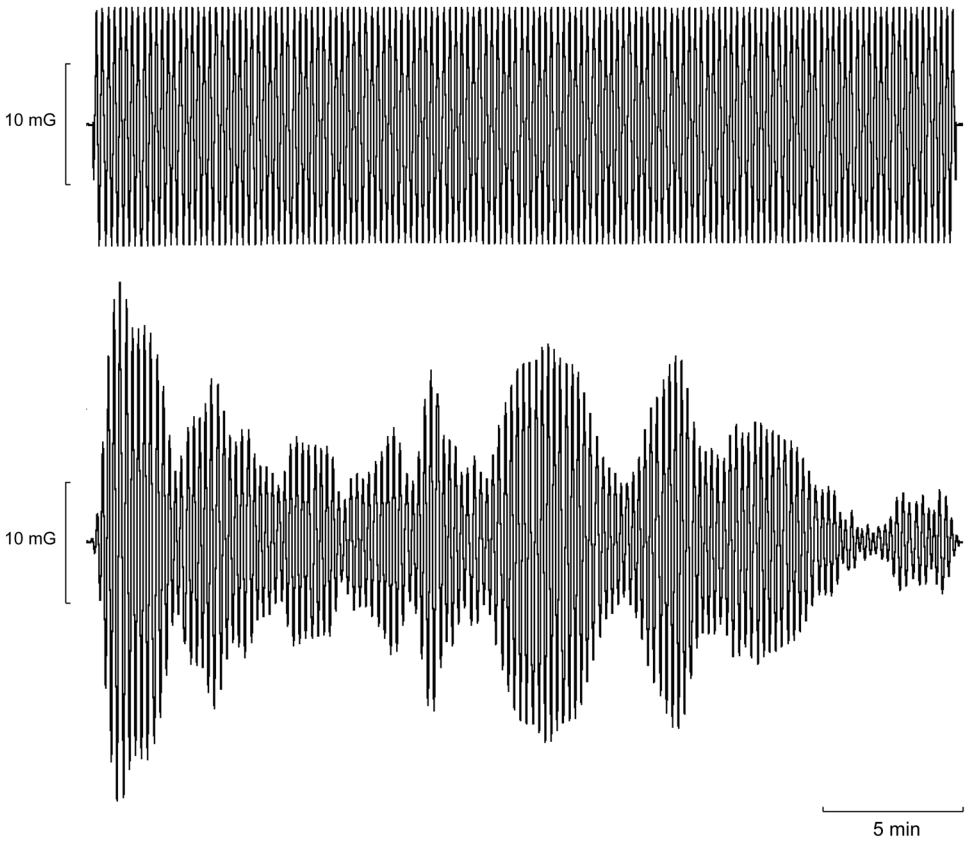

Fig. 1 Samples of the constant-amplitude (top) and random-amplitude (bottom) sets of sinusoidal linear accelerations, both delivered at 0.08 Hz and with a RMS amplitude of 14 mG. The signals were obtained from the accelerometer located inside the moving room

amplitude and frequency during an inspiratory-capacity apnoea (Macefield and Wallin 1995). The absence of muscle twitches yet the presence of radiating parasethesiae at 20 µA, coupled with afferent responses to stroking the innervation territory, indicated that the microelectrode had entered a cutaneous fascicle. The microelectrode was manually advanced until spontaneous bursts of skin sympathetic nerve activity (SSNA) were encountered, identified by the following features: (1) a burst could be evoked by a brisk sniff and, with the subject's eyes closed, an arousal burst could be evoked by an unexpected tap on the nose or a loud shout (Delius et al. 1972), (2) the bursts were typically longer than those comprising MSNA, (3), unlike MSNA, there was no sustained increase in burst amplitude and frequency during an inspiratory-capacity apnoea (Macefield and Wallin 1995) and (4) cardiac modulation of SSNA was weak (Macefield and Wallin 1999).

Neural activity was amplified (gain 20,000, bandpass 0.3–5.0 kHz; NeuroAmp EX, ADInstruments, Sydney, Australia) and stored on a computer (10 kHz sampling) using a computer-based data acquisition and analysis system (PowerLab 16SP hardware and LabChart 7 software; ADInstruments, Sydney, Australia). Other measurements included ECG (0.3–1.0 kHz), recorded with Ag–AgCl surface electrodes on the chest and sampled at 2 kHz, respiration (DC-100 Hz), recorded via a piezoelectric transducer (Pneumotrace, UFI, Morro Bay CA, USA) wrapped around the chest, and non-invasive continuous blood pressure recorded from a finger (Portapres, Finapres Medical Systems, The Netherlands). Acceleration was measured using a high-sensitivity accelerometer, with a threshold of < 10 µG (QA650, Honeywell, USA), fixed to the floor of the room.

Sympathetic nerve activity was displayed as an RMS-processed (root mean square, moving average time-constant 200 ms) signal. As described previously, the primary analysis was conducted on the raw, negative-going, sympathetic spikes (Bent et al. 2006). Negative-going spikes in the neurogram (with a half-width of 0.2–0.5 ms), R-waves of the ECG and the positive peaks of the accelerometer signals were detected using window discriminator software (Spike Histogram for Macintosh v2.2, ADInstruments, Sydney, Australia). Auto-correlation histograms were constructed from the R-waves of the ECG and from the positive peaks of the accelerometer signals in the Y direction to provide timing signals for the cardiac and movement cycles (see Fig. 1). Cross-correlation histograms (50 ms bins) were constructed between MSNA or SSNA and the cardiac timing signals to calculate cardiac modulation of MSNA or SSNA. For MSNA, spike discriminator levels were adjusted so that negative-going spikes exhibited a robust cardiac modulation, as revealed by a strong cross-correlation between the neural activity and the ECG (50 ms bins); the same discriminator

settings were used for construction of cross-correlograms between MSNA and the positive peaks of the accelerometer signals to calculate vestibular modulation of MSNA. Similar thresholds were used for SSNA, although the cardiac modulation was weaker. The histogram data were exported as text to a statistical and graphical analysis program (Prism 6 for Macintosh v6.0, GraphPad Software, USA) to fit the data to a smoothed polynomial: lower-order polynomials were used to fit curves to the slower vestibular cross-correlograms, while higher-order polynomials were required to fit curves to the cardiac cross-correlograms. Quantification of vestibular modulation of MSNA or SSNA was performed by measuring the difference in the mean number of spikes (calculated from the 100 cycles of stimulation) on the smoothed curve at the peak of the modulation and at the trough. The same approach was used to calculate cardiac modulation of MSNA or SSNA. For each MSNA or SSNA recording the vestibular or cardiac modulation indices were expressed as a percentage by employing the following formula: [(peak-trough)/peak] × 100. All data were normally distributed (D'Agostino-Pearson Omnibus normality test). Two-tailed paired *t* tests and repeated-measures analysis of variance (ANOVA) were used to determine if there were significant differences ($P < 0.05$).

Results

No subjects reported discomfort or nausea during the experiment, either with constant-amplitude or random-amplitude sinusoidal motion. Experimental records from one subject exposed to random-amplitude acceleration are shown in Fig. 1. Muscle sympathetic nerve activity (MSNA) was recorded in this subject, and the negative-going sympathetic spikes have been discriminated and represented as standard pulses (spikes) for constructing cross-correlation histograms between the positive peaks of the acceleration signal or the R-waves of the ECG.

MSNA

Vestibular cross-correlation histograms during constant- and random-amplitude motion are shown for one subject in Fig. 2. As we have reported previously (Hammam et al.

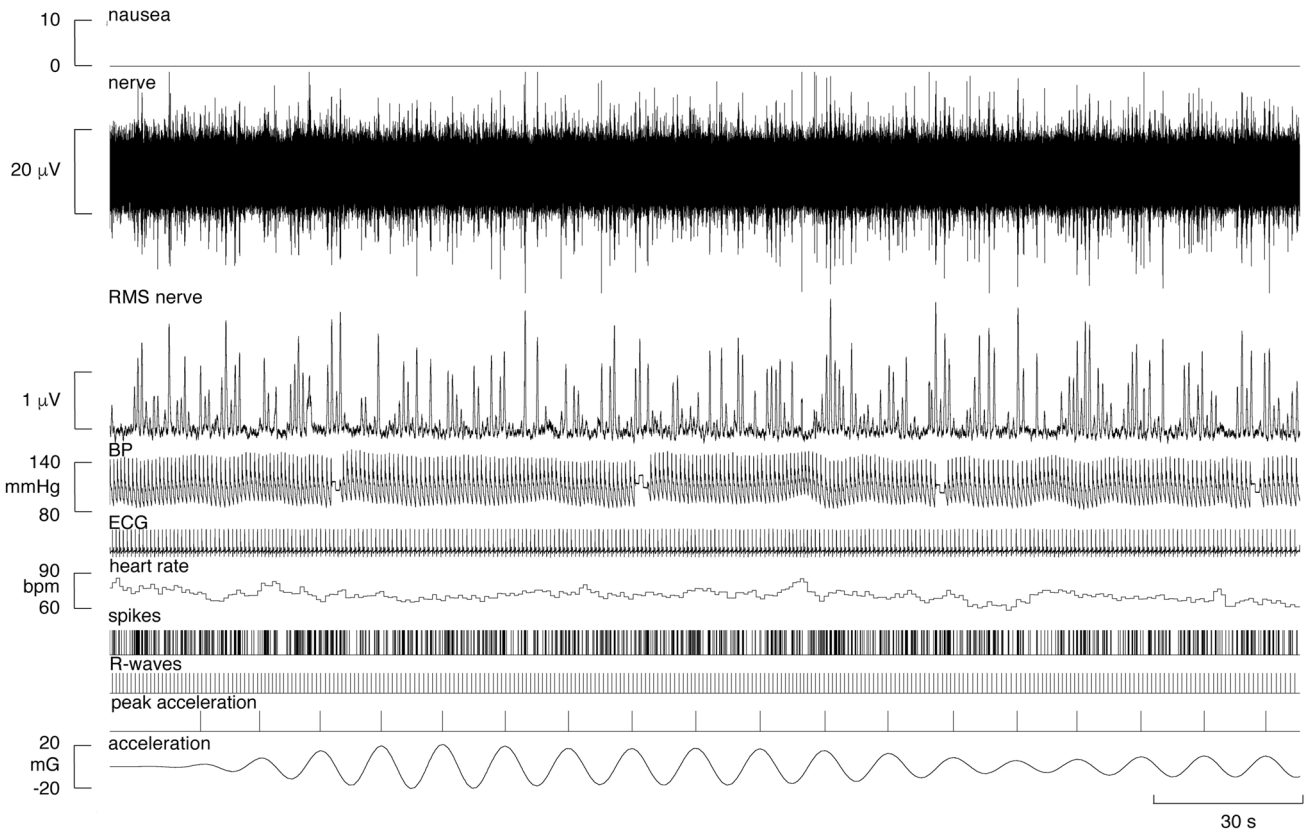

Fig. 2 Multi-unit recording of muscle sympathetic nerve activity during random-amplitude sinusoidal linear acceleration at 0.08 Hz. Below the nerve recording is a root mean square (RMS) processed version of the signal. Negative-going sympathetic spikes are shown as standard pulses below (spikes); these were used to generate the cross-correlation histograms between the vestibular (peak acceleration) or cardiac (R-waves) signals

2013), two peaks of modulation are observed during sinusoidal linear acceleration at 0.08 Hz: one peak related to the positive (forwards) phase of acceleration and one peak related to the negative (backwards) phase of acceleration. It can be seen that the vestibular modulation of MSNA was lower during random-amplitude motion in this subject (Fig. 3).

Mean (± SD) data for the vestibular and cardiac modulation of MSNA are presented in Fig. 4. Data from the individual subjects are shown in Table 1; it can be seen that 7 of the 12 subjects showed a decrease in modulation index during random-amplitude stimulation. Although the mean amplitude of vestibular modulation appeared lower during random-amplitude motion, there was no significant difference in vestibular modulation indices during constant-amplitude (33.4 ± 14.3%) or random-amplitude (28.8 ± 10.0%) motion ($p = 0.50$, paired t test). As expected, cardiac modulation of MSNA was much higher than vestibular modulation of MSNA. Moreover, there were no differences in cardiac modulation across conditions, either at rest (86.4 ± 1.7%) or during constant-amplitude (86.1 ± 3.0%) or random-amplitude (83.0 ± 4.7%) motion ($p = 0.51$, repeated-measures ANOVA).

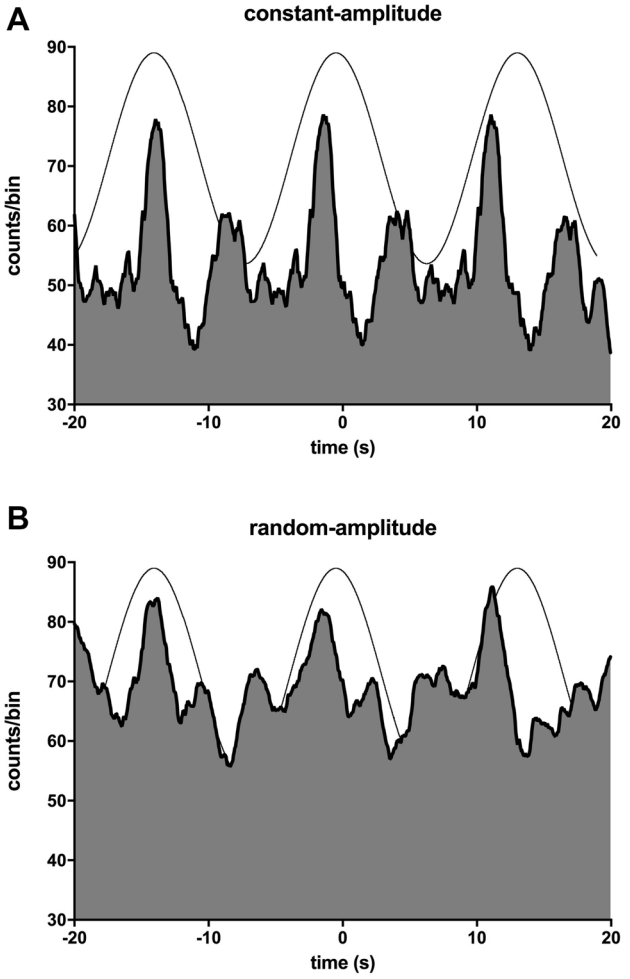

Fig. 3 Cross-correlation histogram between MSNA and acceleration during constant-amplitude (**a**) and random-amplitude (**b**) acceleration in the antero-posterior direction for one subject. The histograms have been fitted with a smoothed polynomial. The superimposed sinusoid schematically represents the acceleration profile of the platform: motion in the forward direction is indicated by the positive phase of the sinusoid, which includes the period of acceleration before the peak and deceleration after the peak. Note that the modulation of MSNA is lower during random-amplitude motion

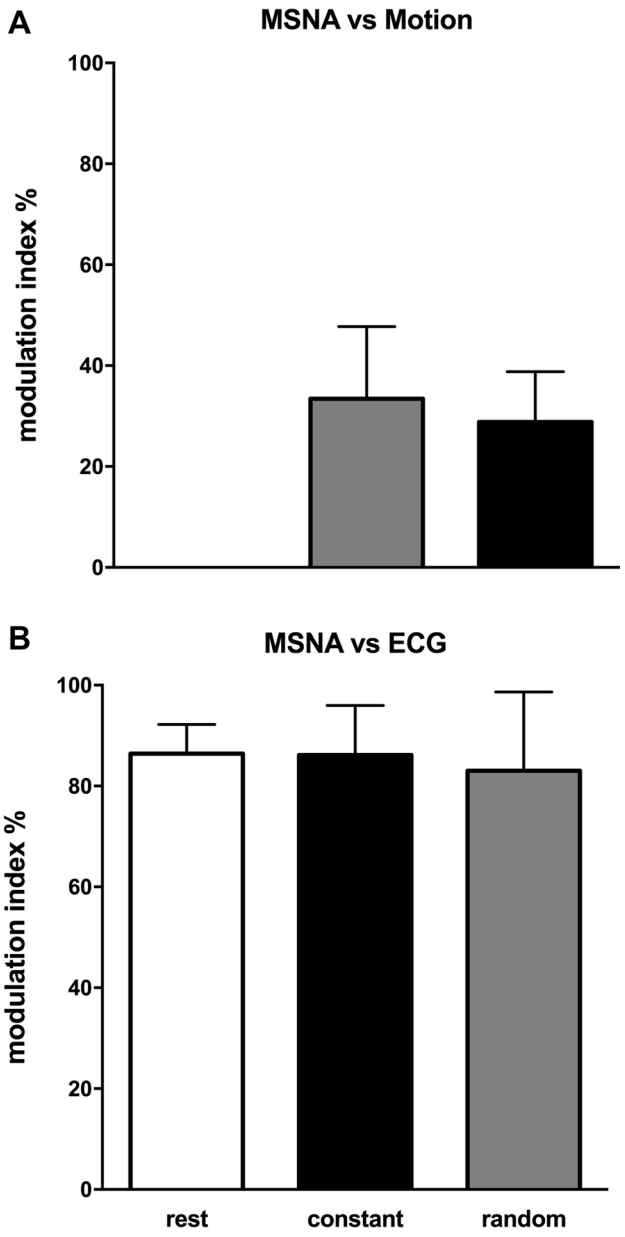

Fig. 4 Mean vestibular (**a**) and cardiac (**b**) modulation indices of MSNA at rest and during constant-amplitude and random-amplitude acceleration amplitude (vestibular modulation = 0 in the absence of a sinusoidal vestibular input). Mean ± SD data from 12 subjects

Table 1 Vestibular modulation indices for MSNA and SSNA during constant-amplitude and random-amplitude linear sinusoidal acceleration

MSNA constant-amplitude	MSNA random-amplitude	SSNA constant-amplitude	SSNA random-amplitude
30.1	23.9	27.4	38.7
49.7	31.0	38.5	32.4
49.0	21.2	15.9	36.8
27.6	17.6	31.6	38.2
29.5	23.1	15.5	31.4
40.6	18.8	28.4	25.0
62.7	28.2	16.6	22.0
19.5	19.8	20.5	23.8
32.9	47.3	31.35	35.1
21.4	38.4	19.3	30.5
15.4	33.4		
22.8	43.3		
33.4 ± 14.3	28.8 ± 10.0	24.5 ± 8.0	31.4 ± 6.1

Each row represents mean data from one subject; note that different subjects were used for recording MSNA and SSNA. Mean ± SD are provided in the bottom row

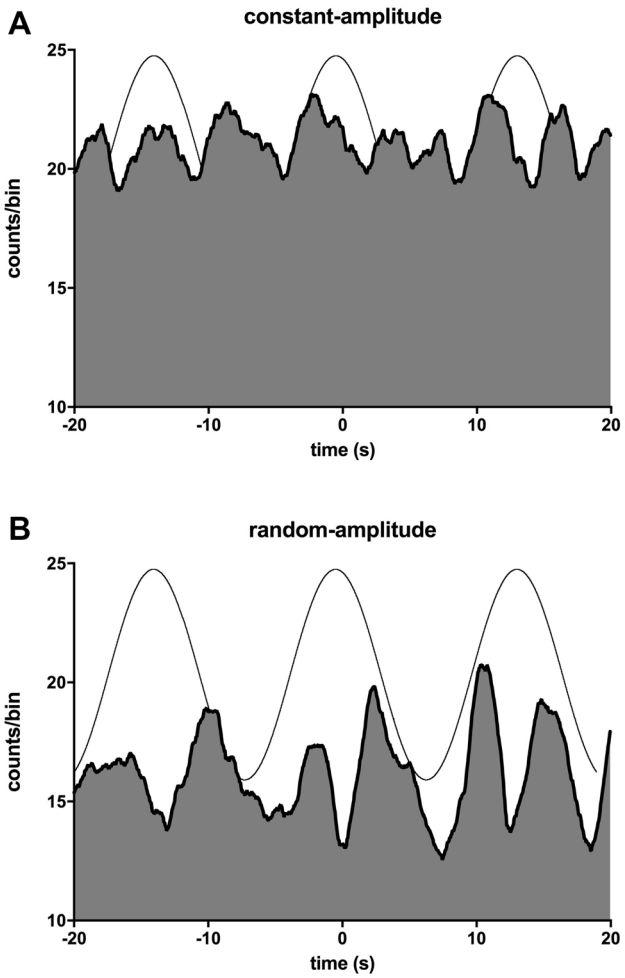

Fig. 5 Cross-correlation histogram between SSNA and acceleration during constant-amplitude (**a**) and random-amplitude (**b**) acceleration in the antero-posterior direction for one subject. The histograms have been fitted with a smoothed polynomial. The superimposed sinusoid schematically represents the acceleration profile of the platform: motion in the forward direction is indicated by the positive phase of the sinusoid, which includes the period of acceleration before the peak and deceleration after the peak. Note that the modulation of SSNA is higher during random-amplitude motion

SSNA

Vestibular cross-correlation histograms during constant- and random-amplitude motion are shown for one subject in Fig. 5. Again, two peaks of modulation can be seen, and it is clear that vestibular modulation of SSNA was higher during random-amplitude motion.

Mean data for the vestibular and cardiac modulation of SSNA are presented in Fig. 6. Data from the individual subjects are shown in Table 1; it can be seen that 8 of the 10 subjects showed an increase in modulation index during random-amplitude stimulation. On average, vestibular modulation of SSNA was lower during constant-amplitude (24.5 ± 8.0%) than during random-amplitude (31.4 ± 6.1%) motion ($p = 0.02$, paired t test). Cardiac modulation of SSNA was much smaller than the cardiac modulation of MSNA and, as with MSNA, there were no significant differences in cardiac modulation across conditions, either at rest (35.9 ± 3.6%) or during constant-amplitude (38.0 ± 3.4%) or random-amplitude (35.6 ± 5.5%) motion ($p = 0.71$, repeated-measures ANOVA).

Discussion

We have shown that randomly varying acceleration has no effect on the magnitude of vestibular modulation of MSNA, but does cause greater vestibular modulation of SSNA. There was no effect on cardiac modulation of either MSNA or SSNA. We had previously shown that low-frequency sinusoidal galvanic vestibular stimulation (0.08–0.18 Hz, 2 mA), delivered binaurally, caused greater modulation of SSNA, but not of MSNA, in those subjects who reported nausea (Hammam et al. 2012; Klingberg et al. 2015). Actually, there was a trend for the vestibular modulation of MSNA to be lower in those who experienced nausea, though this failed to reach significance (Klingberg et al. 2015); a similar trend was seen with MSNA in the current study, vestibular modulation of which tended to be lower during random-amplitude stimulation. However, we found that, unlike galvanic stimulation, sinusoidal linear acceleration did not cause nausea, or at least none was reported.

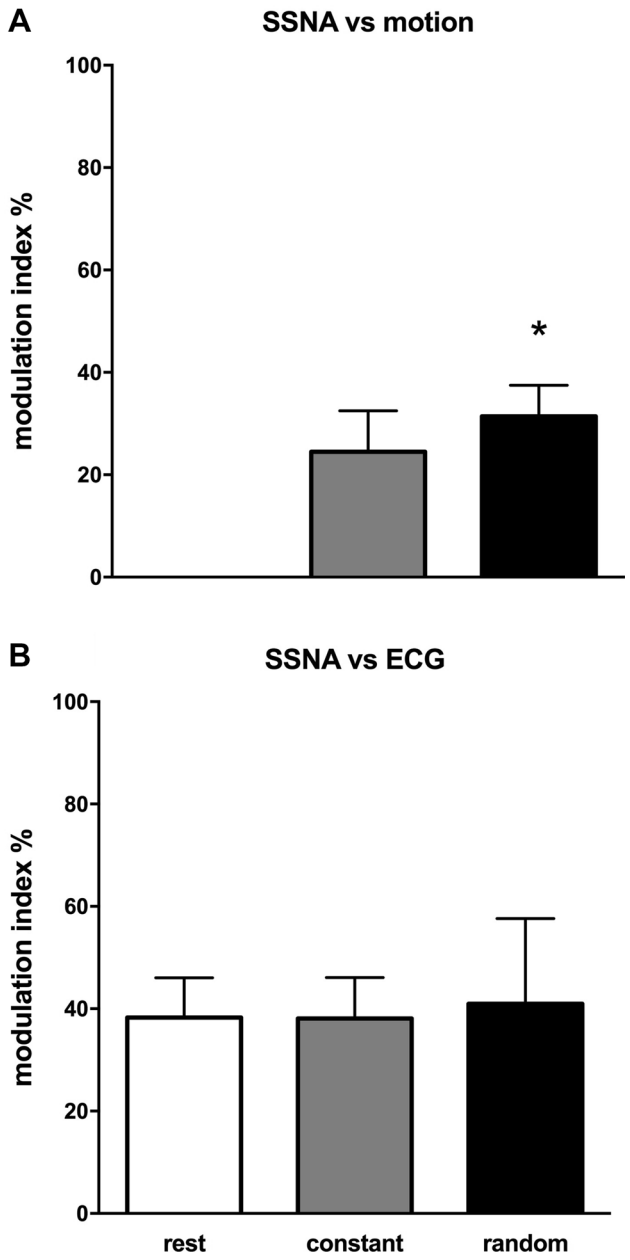

Fig. 6 Mean vestibular (**a**) and cardiac (**b**) modulation indices of SSNA at rest and during constant-amplitude and random-amplitude acceleration amplitude (vestibular modulation = 0 in the absence of a sinusoidal vestibular input). Mean ± SD data from 10 subjects

Given that we had previously shown that vestibular modulation of MSNA is apparent during sinusoidal linear acceleration at amplitudes that subjects cannot even perceive (Hammam et al. 2014b), it is possible that we are seeing physiological evidence of incipient nausea in the increased modulation of SSNA during random-amplitude motion. Indeed, everyone who has experienced motion sickness at some time in their life will know empirically that symptoms of motion sickness may start to appear well before one is aware of feeling sick. In other words, overt sickness takes time to develop, and it is our contention that the physiological signs appear at very low, subperceptual, accelerations.

In our earlier work on the use of sinusoidal linear acceleration, we used a fixed acceleration of 4 mG and a fixed frequency of 0.08 Hz. This allowed us to see two peaks of modulation of MSNA and SSNA, with one peak we argued being related to motion of the vestibular hairs in one direction and the other to movement in the other direction. This was apparent whether we were preferentially stimulating the utricle in seated subjects (Grewal et al. 2012; Hammam et al. 2013) or the saccule in supine subjects (Hammam et al. 2014a; Bolton et al. 2016). In a later study, which like the current one was conducted on the much larger motorized platform in Hong Kong, we could deliver accelerations up to 30 mG. In that study, we demonstrated a linear increase in the modulation of MSNA with acceleration amplitudes ranging from 1.25 to 30 mG (Hammam et al. 2014b). We did not record SSNA in that study, so we do not know whether there is also a linear relationship between acceleration amplitude and the modulation of SSNA.

Interestingly, during delivery of low-frequency sinusoidal GVS subjects invariably report perceptions of "rocking in a boat" or "swinging from side to side in a hammock" (Bent et al. 2006; Grewal et al. 2009). In other words, unlike the sinusoidal linear acceleration we employed here and in our other studies, the perception of motion produced by sinusoidal GVS is not linear: in addition to the perception of side-to-side motion there is an up-and-down component. Moreover, there is never a perception of sinusoidal rotation in the vertical plane. Accordingly, it may be that motion sickness is more likely to occur when more than one axis of motion is involved during actual movement—or with the perceived motion during sinusoidal GVS. Nevertheless, the fact that random-amplitude sinusoidal linear acceleration caused greater modulation of SSNA than constant-amplitude acceleration supports our contention that this is a physiological sign of subperceptual nausea.

Conclusions

We conclude that the pattern of vestibular stimulation affects the magnitude of modulation of sympathetic outflow to skin but not to muscle. Presumably, this is related to the subperceptual development of nausea, which we have previously shown to be associated with greater vestibular modulation of SSNA but not of MSNA. As noted above, not all subjects reported nausea during sinusoidal GVS, yet the mean modulation of SSNA was significantly higher in those that did (Hammam et al. 2012; Klingberg et al. 2015). It is possible that continuing the random-amplitude acceleration beyond the duration studied here would lead to the development of

overt nausea in at least some of the subjects; future studies will be required to examine this, but we do know that random-amplitude wind-induced building motion does lead to nausea being reported by some of the occupants (Lamb and Kwok 2017). Nevertheless, our work emphasizes the importance of vestibulosympathetic reflexes in humans as well as the differential control of sympathetic outflow to muscle and skin.

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