Muscle spindle responses to horizontal support surface perturbation in the anesthetized cat: insights into the role of autogenic feedback in whole body postural control

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Honeycutt CF, Nardelli P, Cope TC, Nichols TR. Muscle spindle responses to horizontal support surface perturbation in the anesthetized cat: insights into the role of autogenic feedback in whole body postural control. J Neurophysiol 108: 1253–1261, 2012. First published June 6, 2012; doi:10.1152/jn.00929.2011.—Intact cats and humans respond to support surface perturbations with broadly tuned, directionally sensitive muscle activation. These muscle responses are further sensitive to initial stance widths (distance between feet) and perturbation velocity. The sensory origins driving these responses are not known, and conflicting hypotheses are prevalent in the literature. We hypothesize that the direction-, stance-width-, and velocity-sensitive muscle response during support surface perturbations is driven largely by rapid autogenic proprioceptive pathways. The primary objective of this study was to obtain direct evidence for our hypothesis by establishing that muscle spindle receptors in the intact limb can provide appropriate information to drive the muscle response to whole body postural perturbations. Our second objective was to determine if spindle recordings from the intact limb generate the heightened sensitivity to small perturbations that has been reported in isolated muscle experiments. Maintenance of this heightened sensitivity would indicate that muscle spindles are highly proficient at detecting even small disturbances, suggesting they can provide efficient feedback about changing postural conditions. We performed intraaxonal recordings from muscle spindles in anesthetized cats during horizontal, hindlimb perturbations. We indeed found that muscle spindle afferents in the intact limb generate broadly tuned but directionally sensitive activation patterns. These afferents were also sensitive to initial stance widths and perturbation velocities. Finally, we found that afferents in the intact limb have heightened sensitivity to small perturbations. We conclude that muscle spindle afferents provide an array of important information about biomechanics and perturbation characteristics highlighting their potential importance in generating appropriate muscular response during a postural disturbance.

posture; proprioception

INTACT CATS AND HUMANS RESPOND to support surface perturbations with broadly tuned, directionally sensitive muscular activation (Henry et al. 1998; Macpherson 1988). The directionality of these tuning curves is robust, appearing impervious to alterations in stance configuration (Torres-Oviedo et al. 2006) and perturbation parameters such as velocity (Diener et al. 1988). Conversely, the magnitude of the muscular response increases at shorter stance widths (forelimb to hindlimb) (Henry et al. 2001; Torres-Oviedo et al. 2006) and increasing perturbation velocity (Diener et al. 1988). The sensory origins of these responses are not known, and conflicting hypotheses are prevalent in the literature. It has been suggested that lower limb proprioception does not play a major role in triggering the muscular response during balance corrections (Bloom et al. 2000). It has also been suggested that cutaneous receptors in the footpads could provide the needed directional information (Ting and Macpherson 2004). Recent evidence from our laboratory, however, does not support either of these hypotheses (Honeycutt et al. 2009b; Honeycutt and Nichols 2010).

Rather, we hypothesize that the directionally sensitive muscular response to support surface perturbations, as a component of the global postural response, is driven largely by rapid autogenic proprioceptive pathways (Honeycutt et al. 2009a; Honeycutt and Nichols 2010; Nichols et al. 1999). Mammalian muscle spindles have been the subject of extensive research over the last century due to their considerable role in proprioceptive regulation of the motor system (Matthews 1991; Prochazka et al. 1989; Stein et al. 2004). Most of the physiological studies of muscle spindles have focused on their signaling properties in relation to the parameters and history dependence of muscular length change (Haftel et al. 2004; Hasan and Houk 1975; Houk et al. 1981b; Nichols et al. 1999; Prochazka et al. 1989). By virtue of the anatomy of the musculoskeletal system, integrated feedback from muscle spindles gives exquisitely detailed information concerning limb and joint movement configuration. In isolated muscle, spindle receptors also show sensitivity to initial starting lengths and velocities (Houk et al. 1981b) remarkably similar to those responses seen in whole muscle responses during whole body postural perturbations from different initial starting stance widths (Henry et al. 2001; Torres-Oviedo et al. 2006) and perturbation velocities (Diener et al. 1988). Finally, the high conduction velocity of these afferents indicate that muscle spindle receptors are tailor-made to provide the fast, relevant information needed for corrective responses during postural disturbance. Despite these observations, the role of muscle spindles has been minimized in the literature (Bloom et al. 2000) likely due to the paucity of data about the response properties of these afferents during three-dimensional perturbations of the intact limb.

Therefore, the primary objective of this study is to obtain direct evidence for our hypothesis by establishing that muscle spindle receptors in the intact limb can provide appropriate information to drive the muscle response to whole body pos-
tural perturbations. Our second objective is to determine if spindle recordings from the intact limb generate the heightened sensitivity to small perturbations that has been reported in isolated muscle experiments. If this sensitivity is maintained, it would indicate that muscle spindles are efficient at detecting even small disturbances in posture, making them suitable to drive the quick muscular response to these disturbances. A final objective was to observe the response characteristics of muscle spindle afferents outside the sagittal plane. It has been shown that several muscles of the hindlimb can exert important nonsagittal plane actions (Lawrence et al. 1993). If these afferents are sensitive outside the sagittal plan, it could indicate that muscle spindle afferents provide important feedback about the nonsagittal actions of muscle. We performed intraxial recordings from medical gastrocnemius (MG) and biceps femoris (BF) muscle spindles in anesthetized cats. MG and BF muscle spindles were chosen because 1) their nerves were readily accessible, and 2) their functional and regional diversity allow us to evaluate any differences due to these parameters. We evaluated the response properties of these afferents when the whole limb was perturbed in different directions and from different initial stance widths and velocities. We compare our muscle spindle responses with electromyographic (EMG) recordings obtained from intact animals (Macpherson 1988) and anesthetized decerebrate cats (Honeycutt et al. 2009a) under the same conditions. Finally, we varied length input parameters to determine afferent sensitivity to different perturbation amplitudes. We relate these findings to those obtained from receptors in isolated muscle preparations (Houk et al. 1981b). The results have appeared in a thesis (Honeycutt 2009), and preliminary results have appeared in abstract form (Honeycutt et al. 2007, 2009b; Martin et al. 2005).

METHODS

Surgery. Data were collected from four purpose-bred female cats (weights 2.5–4.5 kg) and were used in accordance with the Emory and Wright State University Laboratory Animal Care and Use Committee. Animals will be referred to by the date of the experiment (3/20, 3/22, 2/12, and 2/14). Anesthesia was induced with ketamine-xylazine (10 and 1 mg/kg) and maintained with 1–3% isoflurane once intubated. An intravenous line for fluid and drug delivery and a blood pressure transducer were inserted into the external jugular and carotid artery vessels, respectively. To ensure deep anesthesia, heart rate, blood pressure, oxygen and carbon dioxide saturation, temperature, and respiratory rate were monitored continuously along with the withdrawal reflex. The right hindlimb was dissected at the hip and knee to expose the MG and BF nerves. The nerve sections related to all three portions (anterior, middle, and posterior) of the BF muscle were isolated from nerve sections that contained other hamstring muscles. Each dissected portion was electrically stimulated to determine its innervations and confirm isolation of the BF muscle. Sections of the nerve that appeared mixed or could not be conclusively identified were not included. Finally, a laminectomy was performed to expose the L5–L7 spinal processes. To achieve a natural hip rotation, the spine and hips were secured with clamps at the L4 and L7 spinal processes. The muscle nerve was stimulated at a low frequency (2 pps), and the afferent behavior was observed. Pausing or acceleration of afferent firing during twitch contractions indicated muscle spindle or tendon organs, respectively (Fig. 1). Tendon organs were only evaluated in the MG muscle. Twitch occurrence and force was measured from ground reaction forces collected from a force transducer located beneath the foot. Afferents with these characteristics were studied further for their responses to limb translations as described below. A few additional afferents identified to be spindles from the lower limb were further evaluated. The muscle of origin was identified by palpation. Recordings during gentle brushing and/or blowing on the skin ensured the afferent was not cutaneous. Finally, the afferent must have response characteristics of a spindle (described above).

Positioning and perturbations. The animal’s head was fixed in a stereotaxic frame while the spine and hips were secured with clamps. Anesthesia was induced with ketamine-xylazine (10 and 1 mg/kg) and maintained with 1–3% isoflurane once intubated. An intravenous line for fluid and drug delivery and a blood pressure transducer were inserted into the external jugular and carotid artery vessels, respectively. To ensure deep anesthesia, heart rate, blood pressure, oxygen and carbon dioxide saturation, temperature, and respiratory rate were monitored continuously along with the withdrawal reflex. The right hindlimb was dissected at the hip and knee to expose the MG and BF nerves. The nerve sections related to all three portions (anterior, middle, and posterior) of the BF muscle were isolated from nerve sections that contained other hamstring muscles. Each dissected portion was electrically stimulated to determine its innervations and confirm isolation of the BF muscle. Sections of the nerve that appeared mixed or could not be conclusively identified were not included. Finally, a laminectomy was performed to expose the L5–L7 spinal processes. To achieve a natural hip rotation, the spine and hips were secured with clamps at the L4 and L7 spinal processes. The muscle nerve was stimulated at a low frequency (2 pps), and the afferent behavior was observed. Pausing or acceleration of afferent firing during twitch contractions indicated muscle spindle or tendon organs, respectively (Fig. 1). Tendon organs were only evaluated in the MG muscle. Twitch occurrence and force was measured from ground reaction forces collected from a force transducer located beneath the foot. Afferents with these characteristics were studied further for their responses to limb translations as described below. A few additional afferents identified to be spindles from the lower limb were further evaluated. The muscle of origin was identified by palpation. Recordings during gentle brushing and/or blowing on the skin ensured the afferent was not cutaneous. Finally, the afferent must have response characteristics of a spindle (described above).

Afferent identification and recording. Glass micropipettes (1.2-mm outside diameter, 20- to 30-MΩ direct current resistance, 2 M potassium acetate) were advanced into dorsal rootlets (L6–L7) supported in continuity on metal hooks while either the MG or BF muscle nerve was electrically stimulated through bipolar cuff electrodes [stimulus pulse duration 0.4 ms, frequency 2 pulses per second (pps), strength 2.5× threshold for muscle contraction]. When intraxial penetration yielded orthodromic actions potentials that were readily discriminable (typically ≥20-mV amplitude) and stable over time (>5 min), we tested the afferent identity. Orthodromic action potentials (typically ≥20-mV amplitude) produced by either MG or BF nerve stimulation designated the afferent muscle of origin. Brief conduction delays indicated the afferent to be proprioceptive. Delays ranged 1.4–2.2 ms for the MG muscle and 0.77–1.03 ms for the BF muscle. Based on measures of nerve length, these conduction delays correspond to conduction velocities of 67–91 m/s in the MG muscle and 75–98 m/s in the BF muscle. To define afferents as muscle spindles and tendon organs, the muscle nerve was stimulated at a low frequency (2 pps), and the afferent behavior was observed. Pausing or acceleration of afferent firing during twitch contractions indicated muscle spindle or tendon organs, respectively (Fig. 1). Tendon organs were only evaluated in the MG muscle. Twitch occurrence and force was measured from ground reaction forces collected from a force transducer located beneath the foot. Afferents with these characteristics were studied further for their responses to limb translations as described below. A few additional afferents identified to be spindles from the lower limb were further evaluated. The muscle of origin was identified by palpation. Recordings during gentle brushing and/or blowing on the skin ensured the afferent was not cutaneous. Finally, the afferent must have response characteristics of a spindle (described above).

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at its base and attached to an immobile bar above the pelvis. The toe pads of the hindlimb were secured with glue and tape to the surface of the perturbation platform. The large central pad of the foot was not secured to allow natural rotation of the metatarsophalangeal joint during perturbations. Kinematic measurements of standing posture of intact cats were observed and quantified. Joint angle measurements and toe position with respect to the greater trochanter were measured. Based on an average of these measures, the toe was placed 1 cm behind the greater trochanter for the initial (natural) stance condition. The natural turnout of the animal’s foot was chosen as the position with the least amount of medial-lateral resistance.

Once positioned, the hindlimb was perturbed in 16 directions using 2 linear motors (x: rostral/caudal, y: medial/lateral). Motor position, recorded simultaneously through the motor encoder, was monitored closely to ensure a linear perturbation. Perturbations consisted of a 400-ms ramp followed by a 2,000-ms hold and a 400-ms ramp return. The foot was perturbed 4 cm during 400 ms to be comparable with data previously collected in the intact (Macpherson 1988) and decrebrate (Honeycutt et al. 2009a) animal. MG afferents were subjected to further evaluation. Following a full set of 16 directions of data collection, the limb was moved either 3 cm rostrally (short-stance width) or caudally (long-stance width) from the initial stance width (natural), and a new set of 16 direction perturbations were applied to MG afferents at the new starting stance width. Perturbations of varying velocity (0.1, 0.2, 0.4, and 0.8 mm/ms) but constant 4-cm amplitude were applied along with perturbations of varying amplitude (0.5, 0.75, 1, 2, 4, and 6 cm) but constant duration of 400 ms. These final 2 perturbation types were only completed in the rostral (90°) direction using 1 motor to ensure a perfectly linear perturbation at faster velocities.

Data collection and tuning curve analysis. Records of afferent membrane potential, motor positions (x- and y-motors), and initiation of perturbation were collected and stored using Cambridge Electronic Design (CED) Power1401 and Spike2 software. Spike2 was also used to discriminate action potentials and export data to MATLAB for further analysis. Instantaneous firing rate (IFR), inverse of the time of the current spike minus the time of the previous spike, was calculated for all perturbations. Tuning curve analysis was applied to the 16 direction perturbation data from the MG and BF afferents. The number of spikes that occurred during the perturbation (400 ms) was calculated followed by a subtraction of the number of spikes during the same time period of background before the perturbation. The increase or decrease in activity was then graphed against perturbation direction to create a tuning curve (see Fig. 3). Tuning curves were also created using the mean IFR, calculated during the same time period of 400 ms and maximum IFR achieved during the entire perturbation. Latencies were calculated as the time when the first spike achieved greater than twice the background firing rate.

All three types of tuning curves (number of spikes, mean IFR, and maximum IFR) were calculated at different stance conditions and then further quantified with breadths and principal directions. Breadth, the area under the normalized afferent tuning curve, is a quantification of range of directions a muscle is active. A large breadth represents an afferent that is active over many perturbation directions. The principal direction represents the maximum direction of activation. To calculate the principal direction, afferent responses from each direction were converted to a vector and x- and y-components and then averaged to find the primary vector or principal direction of the response.

Parameter analysis. Two different parameter conditions were collected: varying amplitude constant duration and varying velocity constant amplitude. The first (varying amplitude constant duration) was quantified at six different time intervals. We evaluated afferents during the ramp (dynamic) and hold (steady-state) phases of the perturbation. These time periods correspond to the initial dynamic (0–150 ms), middle dynamic (150–300 ms), last dynamic/early steady-state (300–450 ms), early steady-state (450–600 ms), middle steady-state (800–950), and late steady-state (1,500–1,650 ms) phases. The mean IFR during each of these time intervals was then graphed against the mean position of the perturbation platform during the same time period (Fig. 6). The second parameter condition (varying velocity constant amplitude) was quantified during short latencies 0–80 ms for comparison with results obtained in the intact animal. However, as amplitude is not the same at short latencies due to the varying speed of the perturbation, we additionally calculated the firing rate at the end of the perturbation when all afferents are at the same amplitude. These values were then graphed against the velocity of the ramp perturbation to determine any correlations. It was noted that some afferents did show vibration sensitivity. However, statistical comparison of several parameters (latencies, firing rates, background firing, principal directions, breadth, etc.) did not generate significance. Therefore, all afferents were evaluated identically.

Statistics. We quantified differences in key parameters of afferent firing (firing rate, tuning curve principal direction, and breadth) across different stance widths and velocities. A linear mixed-effect model with stance width or velocity as the independent factors was used with parameters of afferent firing treated as dependent factors. In all analyses, each afferent was treated as a random factor. If statistical significance was found, a Tukey honestly significant difference was used for post hoc comparisons. Statistical analyses were performed using R (R Development Core Team, 2006). All statistical tests were made at a significance level of P < 0.05.

RESULTS

Directionally sensitive firing. Both MG and BF muscle spindles were sensitive to endpoint displacements of the hindlimb responding uniquely to each perturbation direction (Fig. 2). IFR was calculated during ramp, hold, and return perturbations in 16 directions. During the initial ramp, MG and

![Fig. 2. Individual medical gastrocnemius (MG) and bicep femoris (BF) muscle spindle firing patterns in response to 16 directions of whole limb perturbation. IFR in response to all 16 directions of perturbation are depicted from an MG (top) and BF (bottom) muscle spindle afferent.](image-url)
BF afferents either increased firing from background (22.5–157.5°) or decreased and sometimes extinguished firing (180–0°). Increases in firing rate gradually ascended until a maximum was achieved at 90° for MG and 67.5° for BF. Firing rate was completely extinguished for perturbations in the opposite directions 247.5–270° for MG and 202.5–337.5° for BF. During the hold phase of the perturbation, firing rate stabilized above background firing in the afferents. The return phase of the perturbation showed subtle direction-dependent properties (MG afferent 45–112.5°) but generally was less sensitive to perturbation direction than the initial ramp phase.

Quantification of the ramp phase (1st 500 ms of perturbation) created broad tuning curves that were similar in direction and breadth across afferents and experiments (Fig. 3, Table 1). The firing pattern in response to the ramp phase of the perturbation was similar across MG afferents in the same experiment and across experiments (Fig. 3B, Table 1). MG tuning curves were directed rostrally and slightly medially in both limbs (right limb 80°, left limb 90°; Table 1). BF afferents were also found to be broadly and similarly tuned within and across experiments (Fig. 3D, Table 1).

BF tuning curves were generally directed rostrally (90°) and were slightly larger in breadth than the MG tuning curves. Afferents fell into two classes according to the extent of adaptation of responses during the hold phase of the ramps. Fifty percent of the afferents generated considerable adaptation during the steady-state phase typically returning firing to or just slightly elevated from background firing. The remaining afferents demonstrated directionally sensitive steady-state (hold-phase) firing (incomplete adaptation). This occurred in both MG [Fig. 2, top: cell 10 (3/20); Fig. 3A, cell 7 (2/14)] and BF [Fig. 2, bottom: cell 3 (2/14); Fig. 3C: cell 13 (2/12)] afferents. The populations, which are designated type A and type B, respectively, were discernible based on conduction time. Type B afferents had on average 0.3-ms longer conduction times than type A afferents. Afferents of both types responded comparably with respect to directional tuning. To illustrate their similarity, both types of afferents are depicted in all figures.

**Sensitivity to stance width.** Alteration of starting stance width affected firing rates but not the direction or breadth of MG afferent tuning curves (Fig. 4). Both type A (Fig. 4A, right) and type B (Fig. 4A, left) afferents showed similar

### Table 1. Quantification of muscle spindle afferents

<table>
<thead>
<tr>
<th></th>
<th>Medial Gastrocnemius</th>
<th>Biceps Femoris</th>
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<tbody>
<tr>
<td>Number of afferents</td>
<td>9</td>
<td>3</td>
</tr>
<tr>
<td>Right or left limb</td>
<td>Left</td>
<td>Right</td>
</tr>
<tr>
<td>Principal direction</td>
<td>80 (2.9)</td>
<td>104 (5.8)</td>
</tr>
<tr>
<td>Breadth</td>
<td>5.1 (0.3)</td>
<td>4.8 (0.9)</td>
</tr>
<tr>
<td>Average min. latency, ms</td>
<td>35 (4)</td>
<td>69 (70)</td>
</tr>
<tr>
<td>Average MaxIFR, pps</td>
<td>169 (60)</td>
<td>146 (23)</td>
</tr>
<tr>
<td>Average MaxIFR time, ms</td>
<td>386 (40)</td>
<td>359 (51)</td>
</tr>
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Values are means (SD). min., Minimum; MaxIFR, maximum instantaneous firing rate; pps, pulses per second.
sensitivities to initial stance width. Background firing rate and maximum IFR during the ramp phase (dashed lines) of the perturbation were diminished with longer stance widths, corresponding to shorter MG muscle lengths (Fig. 4A). This result was consistent across all MG afferents with statistically smaller responses to shorter MG muscle lengths (Fig. 4B, right) as starting stance width increased. Despite the amplitude differences with stance condition, the principal direction (P = 0.65) and breadth (P = 0.11) were not significantly different across stance conditions.

Varying velocity with constant amplitude. Both type A (Fig. 5A, bottom) and type B (Fig. 5A, top) spindle afferents increased IFR in response to increasing velocity (Fig. 5A). Quantification of short latency (0–80 ms) firing in all MG muscle spindles was statistically different across all groups except between 0.2 and 0.4 mm/ms conditions (Fig. 5B, left). Afferents also produced larger dynamic responses as velocity was increased. Mean IFR was calculated at perturbation termination when all afferents were at the same amplitude of 4 cm but had been subjected to different prior velocities. Despite being at the same amplitude, all MG afferents showed statistically significant, velocity-related increases in IFR at perturbation termination (Fig. 5B, right).

Varying amplitude with constant duration. MG muscle spindles were more sensitive to stretch during the initial phases of perturbation when the platform has been displaced <1 cm (Fig. 6). Both type A (Fig. 6A, bottom) and type B (Fig. 6A, top) afferents showed heightened sensitivity to smaller perturbations. This heightened sensitivity was prevalent for all muscle spindles during the early time period (0–150 ms). Approximately half of muscle spindles also showed enhanced sensitivity during later time periods (Fig. 6B: cells 9 and 8). These cells show a transition in sensitivity between 0.75 and 1.0 cm of platform displacement. The other half of the sensitivity of cells changed mostly with time and was less related to the platform displacement (Fig. 6B: cells 12 and 10). Type A (cells 12 and 9) and type B (cells 10 and 8) cells were also differentiated by later time periods. Type A cells quickly adapted during the hold phase of the perturbation. This led to a low sensitivity (horizontal line) to platform displacement during the hold phase of the perturbation (Fig. 6: last 3 conditions). Type B cells did not rapidly adapt and therefore remained sensitive to platform displacements during those time periods (Fig. 6B: positive slopes).

Additional afferent responses. In addition to MG and BF muscle spindle afferents, three additional muscle spindle afferents were observed as well as one MG Golgi tendon organ. Through palpation, muscle spindle afferents were identified in the anterior compartment of the shank (tibialis anterior), the lateral foot, and posterior compartment of the shank (plantaris; Fig. 7, A–C). The tibialis anterior muscle spindle responded with tuning in the posterior and slightly medial direction, whereas the lateral foot muscle spindle responded most strongly in the medial direction. Finally, the plantaris muscle spindle responded with similar directions as the MG muscle spindle, but the orientation is more laterally. Of the 10 MG tendon organs that were recorded from, only 1 fired in response to perturbation.

DISCUSSION

We hypothesized that the direction-, stance-width-, and velocity-sensitive muscle response during support surface perturbations are driven largely by rapid autogenic proprioceptive pathways. The primary objective of this study was to obtain direct evidence for our hypothesis by establishing that muscle spindle receptors in the intact limb could provide appropriate information to drive the muscle response to whole body pos-
tural perturbations. We found that both MG and BF muscle spindle receptors provide directionally sensitive feedback that when quantified generates broad tuning curves. The diversity of the location and function in the lower limb of these afferents indicates that these results will likely be representative of many muscles of the hindlimb. This is supported by our additional recordings of muscle spindles in other muscles of the hindlimb resulting with distinctive tuning curves oriented correspondingly to their unique function in the limb. For example, the tibialis anterior and lateral foot muscle spindles respond in the posterior and medial directions, respectively. MG and BF muscle spindles never responded in these orientations.

MG muscle spindle afferents were further evaluated and found to be sensitive to stance width and velocity within the intact limb. The sensitivities of muscle spindle afferents to direction, stance width, and velocity are remarkably similar to response characteristics of whole muscle responses during postural disturbances in the intact human and cat. (Diener et al. 1988; Henry et al. 2001; Macpherson 1988; Torres-Oviedo et al. 2006). Our second objective was to compare in situ muscle afferent responses with those performed in isolated muscle to determine if the heightened sensitivity of muscle spindles to small perturbations (Hasan and Houk 1975). When isolated muscles are stretched following a period of rest at constant length, muscle spindle receptors increase firing linearly with stretch amplitude up to \(0.5\) mm. Following this, afferents become less sensitive to stretch at larger amplitudes. Following the stretch, the responses adapt substantially during the hold period but generally remain sensitive to amplitude during all time periods of the perturbation. Primary and secondary receptors show this pattern of response, but the extent of adaptation is more pronounced for primary endings. Primary endings are also less sensitive to amplitude at later time periods.

The geometric characteristics and mechanical filtering actions of the hindlimb musculature might have prevented the expression of the heightened sensitivity of muscle spindles to small perturbations, and therefore it was important to confirm this result in situ. Our objective was to observe and compare our muscle receptor recordings with those from isolated muscle to help classify the receptors and to gain insight into the functional significance of these nonlinear properties. There were at least two reasons to believe that these properties would be transformed in our experimental situation. First, there is a geometric transformation between muscular length and endpoint position (Grillner and Shik 1973; Joyce et al. 1969; Rack and Westbury 1969) that should result in scaling of the “small-signal” range of spindle receptors when expressing the perturbation with respect to endpoint position rather than muscular length. Second, there is a greater amount of in-series connective tissue in the intact limb that could mechanically filter the

**Sensitivity to small stretches: comparison with isolated muscle.** Muscle spindle receptors have a heightened sensitivity to small isolated muscle stretches (Hasan and Houk 1975). When isolated muscles are stretched following a period of rest at constant length, muscle spindle receptors increase firing linearly with stretch amplitude up to \(-0.5\) mm. Following this, afferents become less sensitive to stretch at larger amplitudes. Following the stretch, the responses adapt substantially during the hold period but generally remain sensitive to amplitude during all time periods of the perturbation. Primary and secondary receptors show this pattern of response, but the extent of adaptation is more pronounced for primary endings. Primary endings are also less sensitive to amplitude at later time periods.
responses and potentially obscure the transition in sensitivity (Nichols 1984; Stahl and Nichols 2011).

We found that MG muscle receptors did show a transition to higher sensitivity at small displacements (<1 cm) of the paw. In analogy with the experiments of Hasan and Houk (1975) in isolated muscle, we recorded from muscle spindles in situ while perturbations of varying amplitude but same duration were applied to the paw. After quantification, we found that all MG receptors had increased sensitivity to small distances at the earliest time frame and that 50% of those afferents studied maintained this sensitivity at later time points (Fig. 6). The transition occurred at 10 mm of endpoint displacement compared with 0.5 mm in isolated muscle. This distance is reasonable assuming the displacement of the endpoint was distributed among the joints of the hindlimb and that in-series connective tissue would effectively increase the transition length. We conclude that displacements of up to ~1 cm are within the range of high sensitivity to muscle spindle receptors. This is functionally significant because it demonstrates that muscle spindles are highly sensitive to small disturbances of the limb.

Population identification. Two different types of afferents were observed that appear analogous to primary and secondary afferent endings. The receptors evaluated here all showed adaptation during the hold period and higher sensitivity to smaller stretches, but some (type A) showed greater adaptation and a more pronounced transition in sensitivity between low and high amplitudes of stretch. Type B receptors showed greater sensitivity to the amplitude of the displacement during the steady-state response. Type B afferents also had longer conduction times, on average 0.3 ms, indicating that these two types compare favorably with response properties of primary and secondary muscle spindles observed by Hasan and Houk (1975) and Houk et al. (1981a). These authors showed that
primary endings exhibited greater adaptation during the hold phase of stretch, greater dynamic sensitivity, and more pronounced transition in sensitivity as amplitude of stretch increased. The responses of secondary endings, in contrast, were more closely proportional to the length change. The results presented here indicate that these distinctive response properties of muscle spindle receptors are expressed at the level of the intact limb and are therefore suited to providing efficient and important information about postural sway and perturbation during quiet standing. Type A receptors are suited to the detection of the onset of sway, whereas type B receptors are suited to detecting the extent of postural disturbances.

**Correspondence of muscle spindle responses to whole muscle responses during postural disturbances.** The tuning curves obtained from the recordings reported here correspond well to those obtained from EMG responses in intact (Macpherson 1988) and unanesthetized decerebrate cats (Honeycutt et al. 2009a; Honeycutt and Nichols 2010) during horizontal postural perturbations. The average principal direction for EMG tuning curves of the right MG muscle in the decerebrate preparations was 105° (Honeycutt et al. 2009a). The principal directions for the MG spindle responses reported here varied from 104 to 111°. The average principal directions for the anterior and posterior BF muscle are 112 and 91°, respectively, in the decerebrate cat (Honeycutt et al. 2009a). The average value for BF muscle spindle responses in the present study was 89.5°, which corresponds well with the responses from the posterior BF muscles in the decerebrate preparations. In addition, the principal direction of MG and BF spindles are complementary to the direction of force exerted by these muscles on the ground (Honeycutt 2009; Lawrence et al. 1993; Nichols et al. 1993). Specifically, intramuscular stimulation of the MG and posterior BF muscles demonstrate that they exert horizontal plane forces oriented at 282 ± 6 and 283 ± 7°, respectively (Honeycutt 2009). These directions are oriented 180° opposite to muscle afferent spindle firing. This is important as it suggests that muscle spindle responses may relate information about the direction that the muscle can most significantly direct force. Furthermore, as these perturbations were in the horizontal plane, these results highlight the possibility that muscle spindle afferents could provide important feedback about the nonsagittal actions of muscle (Lawrence et al. 1993).

Responses from receptors and muscles were also broadly tuned, like those seen in the intact animal, with tuning curves occupying ~25% of the perturbation space. Breadth was somewhat smaller for the muscle spindle recordings (MG: 4.8–5.1, posterior BF: 5.2–5.6) vs. EMG tuning curve breadths (MG: 6.5, posterior BF: 8.2) (Honeycutt et al. 2009a). There are at least two possible reasons for these differences. First, more variability would be expected from the decerebrate preparations (as well as the intact animal undergoing dynamic postural disturbances), given the spontaneous and triggered movements that sometimes occur. Second, motoneurons receive inputs from synergistic muscles as well, and these muscles may have had similar, but not identical, principal directions (Lawrence et al. 1993; Lawrence and Nichols 1999). When these sources of feedback combine, larger breadths are created that may be functionally advantageous for added stability.

Muscle spindle receptors also generated the same amplitude adjustments to stance width and perturbation velocity seen in the intact human and cat during postural adjustments. When a shorter stance width (less stable) is enforced in the intact cat, MG generates larger muscular responses (Torres-Oviedo et al. 2006). An analogous observation was made in human subjects (Henry et al. 2001). Firing rate amplitude in isolated muscle spindles is dependent on initial starting length (Houk et al. 1981b); therefore, we expected to see this same relationship in the intact limb. As anticipated, our results show that the background firing rate and the maximum IFR achieved during a perturbation is related to stance width, increasing with shorter stance widths (Fig. 4). We reported no differences in principal direction or breadth. Increased muscle activity was also observed as perturbation velocity is increased in the human (Diener et al. 1988). The muscle spindle afferent evaluated here showed the same trend of enhanced firing rate resulting from increasing perturbation velocity. This increase occurred at the same latency as the increase observed in humans. These results indicate that muscle spindles could encode the necessary information to generate the observed amplitude adjustments to stance width and perturbation velocity.

One major difference between the EMG responses and spindle recordings was that complementary inhibitory responses were seldom observed in the recordings from receptors. This difference may be due to the presumed lack of γ-motor activity in the anesthetized preparation and consequent lower background firing of the receptors making quantification of inhibitory tuning curves rarely possible. However, when observed, the inhibitory portion of the afferent response was generally oriented 180° opposite to the excitatory portion (Fig. 3). This is in contrast with the inhibitory portion of EMG tuning curves observed in the decerebrate animal, which were often noncollinear with excitatory responses (Honeycutt et al. 2009a). This indicates that the inhibitory response likely receives convergent input from other sensory sources, possibly resulting from feedback from antagonistic muscles (Honeycutt 2009).

It is possible that the loss of γ-drive in these anesthetized animals could affect the sensitivity of muscle spindle responses to an extent to be dissimilar from the intact, behaving animal. However, the distribution of length feedback among the muscles of the hindlimb, specifically the triceps surae group, has been shown to be nearly identical under anesthetized and unanesthetized (decerebrate) conditions (Nichols 1999). Furthermore, although γ-activation likely would affect global sensitivity, we are not aware of any evidence to suggest it would do so in a directionally specific manner. Only one Golgi tendon organ afferent fired in response to perturbation. Although it is likely that these afferents provide important feedback during postural corrections, the anesthetized preparation does not provide the appropriate environment to evaluate properly the contributions of these afferents.

**Implications for postural control.** Postural stability is a complex neural behavior that relies on input from a variety of sensory systems. Local variables must be orchestrated to control the motion of the body relative to the center of mass (Lockhart and Ting 2007). The results presented here and in previous publications (Honeycutt et al. 2009a; Honeycutt and Nichols 2010) suggest that the whole body response during a postural perturbation results largely from autogenic feedback from muscle spindle receptors. We have shown that muscle spindle receptors show the same directional, stance-width, and velocity sensitivities as present in the intact animal and human.
indicating that spindle receptors potentially provide a major source of input to the motoneurons during postural corrections.

Muscle spindle receptors are ideally suited for this function given their high sensitivity to small postural disturbances, making them excellent detectors of a developing disturbance. Furthermore, this increased sensitivity to small stretches in both primary and secondary endings is most apparent when perturbations are preceded by a period of relative constant muscular length (Haftel et al. 2004; Huyghues-Despointes et al. 2003), as occurs during quiet standing. Other sources of sensory feedback are available such as Golgi tendon organs, cutaneous, vestibular, visual, joint, and proprioception from other limbs. Our preliminary results from Golgi tendon organs indicate that they are sensitive to perturbations and stance width. All of these sources are available to modulate the magnitudes of the local responses (Honeycutt and Nichols 2010; Ting and Macpherson 2004) and to provide an integrated response of the body. Our results do not minimize the importance of these sensory sources, but rather they mount evidence that muscle spindle afferents possess critical information about direction, stance width, and velocity that may play an important role in driving appropriate muscle responses during balance disturbances.

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DISCLOSURES

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AUTHOR CONTRIBUTIONS


REFERENCES


