The mechanical actions of muscles predict the direction of muscle activation during postural perturbations in the cat hindlimb

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Honeycutt CF, Nichols TR. The mechanical actions of muscles predict the direction of muscle activation during postural perturbations in the cat hindlimb. J Neurophysiol 111: 900 –907, 2014. First published December 4, 2013; doi:10.1152/jn.00706.2013.—Humans and cats respond to balance challenges, delivered via horizontal support surface perturbations, with directionally selective muscle recruitment and constrained ground reaction forces. It has been suggested that this postural strategy arises from an interaction of limb biomechanics and proprioceptive networks in the spinal cord. A critical experimental validation of this hypothesis is to test the prediction that the principal directions of muscular activation oppose the directions responding muscles exert their forces on the environment. Therefore, our objective was to quantify the endpoint forces of a diverse set of cat hindlimb muscles and compare them with the directionally sensitive muscle activation patterns generated in the intact and decerebrate cat. We hypothesized that muscles are activated based on their mechanical advantage. Our primary expectation was that the principal direction of muscle activation during postural perturbations will be directed oppositely (180°) from the muscle endpoint ground reaction force. We found that muscle activation during postural perturbations was indeed directed oppositely to the endpoint reaction forces of that muscle. These observations indicate that muscle recruitment during balance challenges is driven, at least in part, by limb architecture. This suggests that sensory sources that provide feedback about the mechanical environment of the limb are likely important to appropriate and effective responses during balance challenges. Finally, we extended the analysis to three dimensions and different stance widths, laying the groundwork for a more comprehensive study of postural regulation than was possible with measurements confined to the horizontal plane and a single stance configuration.

HUMANS AND CATS RESPOND TO balance challenges, delivered via horizontal support surface perturbations, with directionally selective muscle recruitment and constrained ground reaction forces (Henry et al. 1998; Honeycutt et al. 2009; Honeycutt and Nichols 2010a; Macpherson 1988a,b). It was subsequently suggested that this postural strategy could arise from an interaction of limb biomechanics and proprioceptive networks in the spinal cord (Burkholder and Nichols 2000; Nichols et al. 1999). That is, directional tuning is driven from activation of muscles through autogenic sensory pathways located in the spinal cord. This would imply that the resulting patterns of muscular activation are determined by the architecture (or anatomy) of the musculoskeletal system translating muscle stretch into limb movement (Burkholder and Nichols 2000; Honeycutt et al. 2009, 2012; Nichols et al. 1999). Recently, we tested this hypothesis using the decerebrate cat preparation. Indeed, the directional tunings of force (Honeycutt and Nichols 2010a) and muscular (Honeycutt et al. 2009) responses in the decerebrate cat were found to correspond to responses observed in the intact animal, indicating that the cortex is not necessary for expression of the postural strategy in the intact animal and highlighting that the residual structures, including the spinal cord, are the primary generators of the postural responses. Further evaluation by Bunderson et al. (2010) showed that the strategy of constrained ground reaction forces can arise from a detailed computational model of the feline hindlimb and autogenic length feedback. No particular combination of active muscles was necessary to obtain the observed pattern of force responses, further indicating that musculoskeletal architecture and autogenic length feedback alone can constrain the directional tuning of muscular responses. Finally, we demonstrated that the directional tuning of autogenic muscle spindle receptors in selected muscles is similar to the directional selectivity of the electromyographic (EMG) signals obtained from the same muscles (Honeycutt et al. 2012). Taken together, these reports highlight the importance of musculoskeletal architecture and autogenic length feedback on the postural control strategy.

Still, a critical experimental validation of this hypothesis was to test the prediction that the observed principal directions of muscular activation oppose the directions in which the responding muscles exert their forces on the environment. This was the primary goal of the studies reported here. Specifically, our objective was to quantify the endpoint forces of a diverse set of cat hindlimb muscles and compare them with the directionally sensitive muscle activation patterns generated in the intact and decerebrate cat testing the hypothesis that muscles are activated during postural corrections based on their mechanical advantage. Still, it has been demonstrated that muscles of the wrist can be activated in nonmechanically favorable directions (Fagg et al. 2002; Hoffman and Strick 1999). Another study from the human leg also demonstrated a deviation from the preferred direction of a muscle and its mechanical action (Nozaki et al. 2005). Given the complexity of the postural response, it is possible that muscles are activated outside of their biomechanical advantage to provide stability of the limb, which would argue against our hypothesis. Therefore, we measured the ground reaction forces at the
METHODS

The endpoint of the feline hindlimb in response to intramuscular (IM) stimulation and compared the directions of these forces with the principal directions extracted from the muscle activation tuning curves previously published (Honeycutt et al. 2009). We found that the ground reaction forces due to IM stimulation were indeed directed in opposition to the principal directions of activation of the same muscles except when the activation was dominated by sensory input from a different muscle. These observations indicate that muscle recruitment during postural perturbations is driven, at least in part, by limb architecture. Furthermore, this suggests that sensory sources that provide feedback about the mechanical environment of the limb are likely critical to an appropriate and effective response during postural perturbations.

An additional goal of the work reported here was to extend the analysis to three dimensions and different stance widths (distance between forelimbs and hindlimbs; Torres-Oviedo et al. 2006), laying the groundwork for a more comprehensive study of postural regulation than was possible with measurements confined to the horizontal plane and a single stance configuration. The inclusion of vertical forces also allowed us to provide insight into a finding from previous reports. Some muscles exhibited more variable directional tuning in the horizontal plane than others, including the rectus femoris (RF), sartorius (Sart), and tibialis anterior (TA) in the intact animal (Macpherson 1988b) and medial gastrocnemius (MG), vastus medialis (VM), and vastus lateralis (VL) in the decerebrate cat (Honeycutt et al. 2009). We expected, based on the relevant musculoskeletal anatomy and a modeling study (Bunderson 2008), that forces in the sagittal plane would be substantially greater than those in the horizontal plane at least for some muscles and that the smaller magnitudes in the horizontal plane would be associated with greater variability. Our data support this hypothesis as well. Finally, the amplitude of muscular activation has been shown to increase at more unstable stance widths (Henry et al. 2005; Torres-Oviedo et al. 2006). Therefore, we expected that the ground reaction forces would demonstrate alterations in magnitude but not direction when stance width is altered. Although our results were not consistent in all muscles, our data indicate that changes in muscular activation amplitude may be driven, in part, by the changing architecture of the limb.

METHODS

Experimental setup. Eleven adult cats (3.6–6.4 kg) were used in this study. All experimental procedures and animal care were approved by the Emory Institutional Animal Care and Use Committee. Under isoflurane anesthesia, a tracheotomy was performed to monitor anesthesia levels, and an intravenous line was inserted in the external jugular vein for hydration and drug delivery. IM stimulation electrodes were implanted into the VM, VL, RF, MG, lateral gastrocnemius (LG), TA, biceps femoris (anterior: aBF; middle: mBF; posterior: pBF), semitendinosus (ST), semimembranosus (cranial: cSM), caudofemoralis, gluteus medius (Glut), gracilis (Grac), Sart (anterior: aSart; medial: mSart), and iliopsoas (IL) muscles. Electrode placement was verified at the conclusion of all experiments. Electrodes were constructed from Teflon-coated, braided, stainless steel wire and were suitable for both EMG recordings and IM stimulation.

Animals were decerebrated at the precollicular level with the removal of both cerebral cortices and all brain material rostral to the superior colliculus. The precollicular decerebration produced adequate background tone in the hindlimb musculature to achieve natural leg kinematics without producing stepping or postural behaviors typical of modified and traditional premammillary decerebrations (Grillner and Shik 1973; Honeycutt et al. 2009; Honeycutt and Nichols 2010a). The animal’s head was fixed in a stereotaxic frame, and its tail was secured through a mechanical clamp at the base of the tail. Fixation of the tail allowed for more natural rotation of the hip as opposed to hip fixation with pins. A sling was used to support the animal’s torso and to ensure a proper back alignment. The animal’s body weight was significantly supported through these implements. On average, the animal exerted a downward z-force of 0.98 ± 1.17 N. The toe pads of all four limbs were fixed with glue and tape to four ATI force transducers instrumented to record three-dimensional ground reaction forces and torques. The large, central pad of the foot was not secured to allow natural movement of the foot at longer stance widths. Based on recorded intact animal kinematics, we placed the toe ～1 cm behind the greater trochanter for natural stance conditions.

IM stimulation was taken under three different stance widths (distance between fore and hindlimbs): short (S), natural (N), and long (L). Both the right and left hindlimbs were moved rostrally from the natural stance 5 cm for the short condition and caudally 4 cm for the long condition.

IM stimulation protocol. After proper positioning, five trials of IM stimulation were applied to each muscle individually under each limb condition. A four-pulse train of stimuli at 200 Hz over 20 ms was used to produce a composite twitch contraction in each muscle. This protocol, used previously in the intact (Praat 1995) and decerebrate (Murinas 2003) animal, creates a significant contraction of the muscle for reproducible force outputs but not so strong as to alter limb kinematics significantly to a nonnaturalistic configuration. The stimulation amplitude was chosen to be three times the threshold at which each muscle begins to contract. To confirm this methodology, we evaluated angle measurements at a variety of stimulation amplitudes. We found that the angle generated by increasing stimulation thresholds reached a plateau at ~2 times threshold. We chose 3 times threshold to ensure that we were firmly in the plateau region for reproducible results. IM stimulation electrodes were also suitable for EMG recordings, which were used to ensure that stimulation was isolated to the test muscle.

EMG tuning curve data. Directionally sensitive muscle activation during the postural response has been previously quantified using tuning curves that have been described at length (Honeycutt et al. 2009, 2012; Macpherson 1988b). In brief, the average increase (or decrease) in muscle activation (EMG amplitude) was graphed against the perturbation direction. Tuning curves were further quantified with a principal direction of activation, the direction that the muscle is most strongly activated. The mean and standard deviation of the principal directions observed in the behaving decerebrate cat were obtained from previously published work (Honeycutt et al. 2009) and utilized here for comparison with IM stimulation ground reaction force trajectories obtained in this report.

Data analysis. Force data were analyzed at 35 ms, a time when forces were large enough to be reliably measured but before the influences of force and length pathways could alter the force output of the muscle (Fig. 1). The magnitudes of the three-dimensional force vectors were variable across experiments as electrodes differed in conductance. Therefore, to compare results across experiments, we normalized the magnitude of each three-dimensional force vector to 1 N. Next, we evaluated the data in two planes, x-y (horizontal) and y-z (and its tail in each plane, we report a magnitude and an angle of the response. The magnitude reported is the projected normalized vector of 1 N onto each of the two planes. Therefore, the x-y magnitude is the x-y component of the normalized force vector. Forces (magnitude and angle) were calculated for each plane (x-y and y-z) for all three stance widths (S, N, and L).

Our main hypothesis was that the principal direction of muscle activation should be directed oppositely (180°) from the muscle endpoint ground reaction force. We tested this hypothesis using two
measures. First, we calculated the similarity of the principal direction of muscle activation and muscle endpoint ground reaction forces. Similarity indices have been used in the literature to measure quantitatively the correspondence of two angular measurements (d’Avella and Bizzi 2005). Specifically, the cosine of the angle between the two principal directions is computed. This gives a similarity index that ranges from 1 (perfectly similar) to −1 (perfectly dissimilar). Second, we calculated the angle difference between these two measures for all muscles. We then performed a one-sample t-test that computed whether the angular difference was statistically unique from 180°. We further hypothesized that the ground reaction forces would demonstrate alterations in magnitude but not direction when stance width is altered. We compared mean force angle and magnitude (projection of normalized vector) during three different stance widths across animals. Statistical measures were computed with an ANOVA utilizing a linear mixed-effects model with limb position as an independent effect. Subjects were treated as a random effect to ensure that statistical differences were associated with changes in limb position and not related to variances between animal morphology. Tukey honestly significant difference test was used for all post hoc comparisons. All statistical analyses were performed using the nlme package in R (R Development Core Team, 2006).

RESULTS

Natural stance. IM stimulation produced highly consistent results in all three planes with most muscles demonstrating consistent angles and magnitudes; however, some muscles (VM, RF, TA, and mSart) were more variable in angle across experiments (Table 1). Most muscles showed standard deviations in angle measurements <20°. Magnitude variability across experiments was <0.20 except aBF, mSart, and IL in the x-y plane. The y-z plane shows extremely low variability in magnitudes across experiments with all standard deviations <0.1. In general, both angles and magnitudes were more consistent in the y-z plane across experiments than those values reported in the x-y plane.

Although all muscles have large y-z plane magnitudes, most muscles generated only small actions in the x-y plane with an absence of muscles having strong medial-lateral actions (Figs. 2 and 3). The pBF, Grac, and aSart muscles had the most significant actions in the x-y plane with magnitudes >0.7. The VM, TA, RF, and VL muscles had limited actions in the x-y plane with magnitudes <0.25. Interestingly, those muscles with the most limited action in the x-y plane (VM, TA, RF, and VL) were the muscles that generated medial-lateral forces and showed the largest variability in x-y angle. All muscles had strong activation in the y-z plane with all magnitudes >0.80. The VM, RF, cSM, and LG muscles all had magnitudes of ≥0.99 showing that their primary action was in the y-z plane. Muscles that had small actions in the x-y plane (VM, VL, RF, and TA) had large z-components or forces predominately in the ventral or dorsal direction.

Individual muscle actions. The force actions of individual muscles reflected their anatomy and function in the limb. Although the VM and VL muscles showed strong downward (negative z) action, the x-y projection of the VL muscle was more lateral in action than the VM muscle, corresponding to the anatomic insertion of the VL onto the lateral edge of the patella contrary to the VM muscle insertion medially. Similarly, the LG muscle produced force more medially than the MG muscle corresponding to the gastrocnemius tendon twisted insertion onto the calcaneus. Although both the cSM and ST muscles are hip extensors and knee flexors, the cSM produced a downward force, whereas the ST muscle produced an upward force. The knee flexion action was also stronger in the ST muscle corresponding to its more distal insertion along the

Table 1. Average x-y and y-z magnitudes and angles

<table>
<thead>
<tr>
<th>Muscle</th>
<th>X-Y Magnitude</th>
<th>X-Y Angle, °</th>
<th>Y-Z Magnitude</th>
<th>Y-Z Angle, °</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vastus medialis</td>
<td>0.13 (0.03)</td>
<td>55 (26)</td>
<td>1.00 (0.001)</td>
<td>276 (3)</td>
</tr>
<tr>
<td>Vastus lateralis</td>
<td>0.28 (0.07)</td>
<td>52 (12)</td>
<td>0.99 (0.008)</td>
<td>283 (4)</td>
</tr>
<tr>
<td>Rectus femoris</td>
<td>0.24 (0.16)</td>
<td>52 (53)</td>
<td>1.00 (0.001)</td>
<td>281 (12)</td>
</tr>
<tr>
<td>Medial gastrocnemius</td>
<td>0.58 (0.11)</td>
<td>282 (6)</td>
<td>0.99 (0.008)</td>
<td>235 (8)</td>
</tr>
<tr>
<td>Lateral gastrocnemius</td>
<td>0.60 (0.12)</td>
<td>271 (4)</td>
<td>0.99 (0.034)</td>
<td>234 (10)</td>
</tr>
<tr>
<td>Tibialis anterior</td>
<td>0.26 (0.08)</td>
<td>199 (31)</td>
<td>0.97 (0.019)</td>
<td>96 (8)</td>
</tr>
<tr>
<td>Biceps femoris (anterior)</td>
<td>0.41 (0.21)</td>
<td>321 (13)</td>
<td>0.94 (0.066)</td>
<td>252 (15)</td>
</tr>
<tr>
<td>Biceps femoris (middle)</td>
<td>0.60 (0.17)</td>
<td>311 (5)</td>
<td>0.91 (0.069)</td>
<td>240 (10)</td>
</tr>
<tr>
<td>Biceps femoris (posterior)</td>
<td>0.70 (0.09)</td>
<td>283 (7)</td>
<td>0.98 (0.015)</td>
<td>134 (6)</td>
</tr>
<tr>
<td>Semitendinosus</td>
<td>0.69 (0.07)</td>
<td>261 (9)</td>
<td>0.98 (0.018)</td>
<td>134 (3)</td>
</tr>
<tr>
<td>Semimembranosus (cranial)</td>
<td>0.60 (0.17)</td>
<td>271 (6)</td>
<td>1.00 (0.003)</td>
<td>232 (12)</td>
</tr>
<tr>
<td>Tendinous flexor</td>
<td>0.62 (0.09)</td>
<td>338 (4)</td>
<td>0.80 (0.073)</td>
<td>253 (2)</td>
</tr>
<tr>
<td>Gluteus medius</td>
<td>0.52 (0.10)</td>
<td>343 (7)</td>
<td>0.87 (0.061)</td>
<td>260 (3)</td>
</tr>
<tr>
<td>Gracilis</td>
<td>0.77 (0.07)</td>
<td>245 (6)</td>
<td>0.94 (0.030)</td>
<td>138 (7)</td>
</tr>
<tr>
<td>Sartorius (anterior)</td>
<td>0.95 (0.08)</td>
<td>90 (7)</td>
<td>0.99 (0.006)</td>
<td>14 (12)</td>
</tr>
<tr>
<td>Sartorius (medial)</td>
<td>0.76 (0.15)</td>
<td>130 (19)</td>
<td>0.89 (0.050)</td>
<td>47 (21)</td>
</tr>
<tr>
<td>Iliopsoas</td>
<td>0.32 (0.19)</td>
<td>79 (14)</td>
<td>0.99 (0.010)</td>
<td>288 (11)</td>
</tr>
</tbody>
</table>

Values are means (SD).

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tibia. aBF, mBF, and pBF exhibited distinctive actions. The regions had increasing action in the x-y plane with magnitudes of 0.41, 0.60, and 0.70, respectively. The regions also showed a shift from the lateral action of aBF to a mostly caudally directed force of pBF. Finally, the aBF and mBF regions demonstrated downward z-action, whereas the pBF muscle produced an upward z-action corresponding to its distal insertion along the tibia.

Comparison with EMG activation. The principal directions of EMG muscle activations were generally oppositely directed to the ground reaction forces generated by IM stimulation in the horizontal plane (Fig. 4). The average similarity index across muscles was $-0.91 \pm 0.08$, indicating strong dissimilarity or oppositely directed vectors. Only the Glut muscle had a similarity index greater than $-0.8$. Furthermore, the difference between the principal direction of EMG muscle activation and the ground reaction force across muscles was not significantly different from $180^\circ$ ($t_{11} = 1.05, P = 0.31$).

Effect of stance width alteration. X-y angle was minimally impacted by moving the foot caudally (Fig. 5). Only 5 out of 17 muscles evaluated showed x-y angle changes that were affected by stance condition (VL: $F_{2,8} = 10.7, P = 0.0055$; TA: $F_{2,13} = 18.9, P = 0.0001$; pBF: $F_{2,8} = 8.5, P = 0.01$; Glut: $F_{2,4} = 9.7, P = 0.02$; and Grac: $F_{2,4} = 25.2, P = 0.005$). No differences were found between the short and natural conditions, but there were significant differences between natural and long conditions. With the exception of the case for the TA muscle, x-y angle changes did not exceed $16^\circ$. All muscles show a clockwise (decreasing angle) shift in x-y angle except pBF, which shows a counterclockwise shift.

The x-y magnitude was more significantly impacted by moving the foot, but changes were present in only 41% of muscles evaluated (Fig. 6). The x-y magnitude was significantly impacted by stance condition in seven muscles (VM: $F_{2,4} = 12.1, P = 0.02$; VL: $F_{2,8} = 28.8, P = 0.0002$; MG: $F_{2,8} = 94.6, P < 0.0001$; LG: $F_{2,8} = 56.9, P < 0.0001$; pBF: $F_{2,8} = 82, P < 0.0001$; ST: $F_{2,6} = 36.7, P = 0.0004$; and Grac: $F_{2,4} = 35.5, P = 0.0028$). P values were more significant, and magnitude differences between conditions averaged a 26% shift in magnitude.

With the exception of the VL muscle, y-z magnitudes were not affected by stance condition. In contrast, 60% of y-z angles were strongly impacted by movement of the foot (Fig. 7). Ten of seventeen muscles show a clockwise shift in y-z angle (VM: $F_{2,4} = 12.3, P = 0.02$; VL: $F_{2,8} = 36.0, P = 0.0001$; RF: $F_{2,6} = 10.2, P = 0.01$; TA: $F_{2,13} = 36.1, P < 0.0001$; MG: $F_{2,8} = 57.2, P < 0.0001$; LG: $F_{2,8} = 10.2, P = 0.006$; pBF: $F_{2,8} = 69.2, P < 0.0001$; ST: $F_{2,5} = 91.8, P = 0.001$; Glut: $F_{2,6} = 6.6, P = 0.03$; and Grac: $F_{2,4} = 54.1, P = 0.001$).

**DISCUSSION**

Summary. We found evidence in support of our hypothesis that muscles are activated during balance challenges based on their mechanical advantage. Muscle endpoint ground reaction forces were directed oppositely to the principal direction of muscle activation during postural perturbations. Second, we found that several of the muscles exhibiting directionally variable muscle activation during horizontal perturbations (RF, TA, Sart, MG, VL, and VM) in previous studies (Honeycutt et al. 2009; Macpherson 1988b) exhibited variable ground reaction forces (VM, RF, and TA) and/or had only small actions in the y-z plane (VM, RF, TA, and VL). Furthermore, the reaction forces of 41% of muscles did demonstrate alterations in magnitude when stance width was altered. Taken together, these results indicate that muscle activation during balance challenges is likely strongly influenced by the mechanical actions of each muscle. Therefore, sensory sources that provide feedback about the mechanical environment of the limb are likely critical to an appropriate and effective response during balance challenges.

Implications for muscle recruitment. We sought to determine how the biomechanics of the limb influence appropriate
muscle activation during the active postural response. We hypothesized that muscles are activated during balance challenges based on their mechanical advantage. In support of this hypothesis, we found that muscle activation and endpoint reaction forces were directed oppositely. On average, they generated a $-0.91$ average similarity index, and the angular difference between their vectors was not statistically different from $180^\circ$. Furthermore, both proximal and distal muscles had similar similarity indices indicating that although there is a more complex mechanical transformation from the hip to the foot, proximal muscle activation is still predicted based on their ground reaction forces.

IM stimulation force trajectories correctly predicted architectural (or anatomic) differences between the hamstring (aBF, mBF, pBF, cSM, and ST) and quadricep (VM and VL) muscles in terms of attachments in the skeleton. The intact (Chanaud and Macpherson 1991) and decerebrate (Honeycutt et al. 2009) animal demonstrates regional differences in the biceps femoris muscle leading to directionally unique muscular activation patterns during postural perturbations. Results of the study reported here indicate that the anterior portion of biceps was activated when animals were subjected to lateral perturbations, whereas the middle and posterior portions were activated by more rostrally directed perturbations. We found that these regions also had different endpoint forces that correspond to the directional activation of each region. The anterior portion produced forces that act medially, whereas middle and posterior biceps show increasingly more caudal force trajectories. IM stimulation reaction forces also accurately mirrored mechanical differences between the other hamstring muscles and the quadriceps. Specifically, the cSM muscle was activated more laterally than the ST muscle, and the VM muscle was activated more medially than the VL muscle, corresponding to the IM stimulation forces as well.

The Glut, VL, and LG muscles deviated the most from their predicted direction based on the observed principal direction of their EMG responses. The rationale for this deviation is un-
clear; however, the deviation in LG may be explained by its complex sensory input as well as its anatomy. The LG muscle force was directed straight backward, whereas its principal direction of EMG activation was oriented forward and medi- 
al. On the other hand, the force and principal directions for MG were in direct opposition and oriented backward and outward to forward and inward. Indeed, the similarity index of MG muscular activation onto the LG IM stimulation reaction force was −0.97, which is a better match than the own muscular activation of LG, which resulted in a similarity index of −0.80. The 10° shift was reproducible, as indicated by low standard deviations. We propose that this deviation results from two sources: 1) anatomy of the gastrocnemius tendon; and 2) heteronymous Ia feedback between LG and MG. Previous reports of the torque generated by the muscles of the shank report that the MG muscle has more lateral actions than the LG muscle (Lawrence et al. 1993; Lawrence and Nichols 1999). The more lateral action is likely the result of the crossover of the MG tendon to the lateral side of the calcaneus resulting in a substantial abduction moment from this muscle (Crouch and Smith 1969; Nichols et al. 1993; Parsons 1894). A three-dimensional anatomic model that accounts for the lateral insertion of the MG muscle also predicts a more laterally directed force vector for the medial compared with the lateral head of the gastrocnemius muscle (Burkholder and Nichols 2004). Previous reports on the distribution of group Ia feedback indicated that the LG muscle receives much of its feedback from spindles in the MG muscle but that MG receives little Ia feedback from LG (Eccles et al. 1957). Putting these two factors together, the principal directions for both LG and MG would be expected to correspond more closely to the force direction of MG.

The evidence also supported our second expectation. Specifically, muscles with high variability in their principal direction during postural perturbations (RF, TA, Sart, MG, VL, and VM) exhibit variable ground reaction forces (VM, RF, and TA) and/or had only small actions in the x-y plane (VM, RF, TA, and VL). These muscles all have strong z-forces indicating that their primary role is likely for antigravity support. It is possible that the additional variability associated with these muscles might relate to technical difficulty in achieving homogenous muscle activation across experiments. However, electrode insertion was closely monitored and verified following each experiment with a complete dissection. Furthermore, if the primary cause of the variability was related to experimental differences, we would expect to see it in ≥4 out of 17 muscles.

Our evidence also showed that ground reaction forces in the x-y plane would demonstrate alterations in magnitude but not direction during changes in stance width. Angular differences between short and long stance widths amounted to only an average of 4%, whereas changes in magnitude averaged 29%. In total, 41% of muscles showed a change in x-y magnitude, which is consistent with previous literature demonstrating that 53% of muscles evaluated had alterations in muscular activation magnitude as stance width was altered (Torres-Oviedo et al. 2006). Therefore, it seems likely that the mechanical actions of muscles influence muscular activation changes with stance widths, at least in part.

Sensory implications. If muscles during postural perturbations are indeed activated selectively based on their potential counteracting endpoint point force, the sensorimotor transformation should be able to represent this information. Global sensory systems, such as cutaneous and vestibular feedback, are certainly critical for appropriate postural adjustment (Al- lum et al. 1998; Belozerova et al. 2003; Bolton and Misiaszek 2009; Bouyer and Rossignol 2003; Honeycutt and Nichols 2010b; Horak and Hlavacka 2001; Peterka 2002; Roll et al. 2002; Runge et al. 1998; Stapley et al. 2002); however, they lack selectivity. Conversely, muscle receptors that give muscles specific mechanical feedback would make likely sources to relay information about the internal changes within the muscle that influence its ability to compensate for a perturbation. If a muscle is lengthened through a limb perturbation, that same muscle would generate an opposing force if active. Therefore, muscle spindles, which give feedback on muscle length, would provide important feedback on which muscles can generate counteractive forces. We have previously demonstrated that muscle spindles produce similarly tuned and directed activation patterns to active muscular responses during support surface perturbations (Honeycutt et al. 2012). Furthermore, muscle spindles are known to respond to not only length changes, but also the dynamics of length change (Houk et al. 1981). As the short-latency elements of muscle activation are known to change based on the velocity of the perturbation (Diener et al. 1988), this further implies that muscle spindle feedback may be critical to establishing proper activation patterns. The importance of rapid feedback from muscle spindles is further supported by the correspondence between the principal direction for the LG muscle and force direction of MG, as discussed above.

Comparison with model results. Data from the decerebrate cat do depart somewhat from the predictions of previous models. Several of the muscle forces predicted by a three-dimensional anatomic model have very small nonsagittal actions (Burkholder and Nichols 2004). Our data demonstrate that many of these muscles, including the Grac, ST, and biceps femoris muscles, have strong nonsagittal actions. Although nonsagittal forces are smaller in general than those in the y- and z-dimensions, they are more substantial than the model predicts. This observation suggests an important role for the metatarsophalangeal joint, intrinsic musculature of the foot, and other soft tissue structures (ligaments and fascial struc-
structures) in the production of nonsagittal forces in the hindlimb as they are not present in the current model (Falcon et al. 2011; Stahl and Nichols 2011). Other deviations from model predictions include muscle actions at different stance conditions during locomotion. Previous models have assumed that the actions of the hindlimb musculature rotated with the limb (hip to toe) axis (Kaya et al. 2006) during the step cycle. However, our results indicated that although the force vectors created through stimulation do rotate in the y-z plane as stance condition is altered, they did not show a consistent rotation across muscles. Some muscles were affected dramatically, whereas others showed little or no rotation with stance condition. The quantitative analysis of limb axis rotation effect on the ground reaction forces of individual muscles provided by this report will provide future improvement to model assumptions of muscle ground reaction forces.

Force constraint strategy. Our result that there are few hindlimb muscles that generate substantial mediolateral forces indicates that the biomechanics of individual muscle actions may contribute to the constrained appearance of ground reaction forces during active postural responses as previously proposed (Bunderson et al. 2010; Honeycutt et al. 2009; Honeycutt and Nichols 2010a). The smaller mediolateral forces of the surveyed muscles corresponds to the force constraint strategy observed in the intact (Macpherson 1988a) and decerebrate (Honeycutt and Nichols 2010a) cat. This indicates that the force constraint strategy may arise from the stronger rostral/caudal actions of the limb musculature. This architectural principle has been previously demonstrated in the feasible constraint strategy observed in the cat hindlimb model, which showed that the limb could generate substantially more force in the rostral/caudal direction than medial/laterally (McKay et al. 2007).

These results also indicate the significance that the biomechanics of limb play in constraining the control of movement. More specifically, these results imply that the biomechanics of limb, rather than only neural mechanisms, constrain the kinematic degrees of freedom of the limb. Furthermore, these results extend the analysis to three dimensions and different stance widths allowing a more comprehensive study of postural regulation than was possible with measurements confined to the horizontal plane and a single stance configuration. This expanded data set can be used to improve biomechanical models that have the capability of more rigorously testing hypotheses about the role of biomechanics on movement strategies.

Limitations of hypothesis. Our hypothesis states that muscles are activated based on their potential counteracting force; however, this hypothesis likely does not hold for volitional tasks. It has been demonstrated that muscles of the wrist can be activated in nonmechanically favorable directions (Fagg et al. 2002; Hoffman and Strick 1999). We would argue that the architecture of the wrist is very complex, and mechanical actions are difficult to ascertain. Furthermore, the wrist does not have the same utility for weight support; therefore, its musculature is likely adapted to a different set of mechanical tasks. Still, one study from the human leg also demonstrated a small deviation from the preferred direction of a muscle and its opposing mechanical action (Nozaki et al. 2005). However, all of these reports (including the wrist) were based on data collected during volitional activation of muscles. It is likely that during a voluntary task muscles can be recruited in a variety of directions that may diverge from their strict mechanical action. Cocontraction may be necessary for certain movements. Muscles are often made up of several compartments and certainly many motor unit populations (Sokoloff et al. 1999) such that the mechanical action of a muscle likely can vary over several degrees during different types of motor tasks. Regardless, it is likely that that the sensory mechanisms driving this directional tuning are highly influenced by limb architecture, and limb architecture appears to be organized effectively for the maintenance of posture and stability.

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

AUTHOR CONTRIBUTIONS
C.F.H. and T.R.N. conception and design of research; C.F.H. performed experiments; C.F.H. and T.R.N. analyzed data; C.F.H. and T.R.N. interpreted results of experiments; C.F.H. and T.R.N. prepared figures; C.F.H. and T.R.N. drafted manuscript; C.F.H. and T.R.N. edited and revised manuscript; C.F.H. and T.R.N. approved final version of manuscript.

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