Frequency response of vestibular reflexes in neck, back, and lower limb muscles

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The present study aims to characterize the frequency response of vestibular reflexes in neck, back, and lower limb muscles. J Neurophysiol 110: 1869–1881, 2013. First published July 31, 2013; doi:10.1152/jn.00196.2013.—Vestibular pathways form short-latency disynaptic connections with neck motoneurons, whereas they form longer-latency disynaptic and polysynaptic connections with lower limb motoneurons. We quantified frequency responses of vestibular reflexes in neck, back, and lower limb muscles to explain between-muscle differences. Two hypotheses were evaluated: 1) that muscle-specific motor-unit properties influence the bandwidth of vestibular reflexes; and 2) that frequency responses of vestibular reflexes differ between neck, back, and lower limb muscles because of neural filtering. Subjects were exposed to electrical vestibular stimuli over bandwidths of 0–25 and 0–75 Hz while recording activity in sternocleidomastoid, splenius capitis, erector spinae, soleus, and medial gastrocnemius muscles. Coherence between stimulus and muscle activity revealed markedly larger vestibular reflex bandwidths in neck muscles (0–70 Hz) than back (0–15 Hz) or lower limb muscles (0–20 Hz). In addition, vestibular reflexes in back and lower limb muscles undergo low-pass filtering compared with neck-muscle responses, which span a broader dynamic range. These results suggest that the wider bandwidth of head-neck biomechanics requires a vestibular influence on neck-muscle activation across a larger dynamic range than lower limb muscles. A computational model of vestibular afferents and a motoneuron pool indicates that motor-unit properties are not primary contributors to the bandwidth filtering of vestibular reflexes in different muscles. Instead, our experimental findings suggest that pathway-dependent neural filtering, not captured in our model, contributes to these muscle-specific responses. Furthermore, gain-phase discontinuities in the neck-muscle vestibular reflexes provide evidence of destructive interaction between different reflex components, likely via indirect vestibular-motor pathways.

vestibular reflexes; neck muscles; bandwidth; electrical vestibular stimulation; neural filtering

THE FREQUENCY CONTENT of the oscillatory activity of a muscle may provide insight regarding the neural source driving that muscle. For example, corticomuscular oscillations have generally been observed in the 15- to 30-Hz bandwidth (Baker et al. 1997; Murthy and Fetz 1992; Salentius et al. 1997; Salmelin and Hari 1994), whereas muscular oscillations arising from the reticular structures appear to occur in the 10- to 20-Hz range (Blouin et al. 2007; Grosse and Brown 2003). Recently, vestibular reflexes elicited via electrical stimuli were found to influence lower limb muscles during standing balance over a bandwidth of 0–25 Hz (Dakin et al. 2007, 2011). This bandwidth corresponds with the known dynamic range of the vestibular system (Armand and Minor 2001; Huterer and Cullen 2002), suggesting that the vestibular system influences motor activity mainly over a 0- to 25-Hz bandwidth. The short-latency, high-frequency neck-muscle responses to vestibular stimuli (Colebatch et al. 1994; Lee et al. 2008; Murofushi et al. 2002; Watson and Colebatch 1998; Wu et al. 1999), however, suggest that the frequency response of vestibular reflexes operates at higher frequencies for the neck muscles. These differences between body regions likely result in muscle-dependent variation in the expression of vestibular reflexes. The present study aims to characterize the frequency response of vestibular reflexes across neck, back, and lower limb muscles and to determine the mechanisms underlying potential between-muscle differences.

Differences in the frequency response between the neck, back, and lower limb vestibular reflexes may be due to differences in 1) the underlying firing rates of these muscles, or 2) the neural pathways mediating these reflexes. Motor unit firing rate is known to affect the amplitude (Kilner et al. 2000; Ushiyama et al. 2010) and frequency bandwidth of brain-muscle correlations (Ushiyama et al. 2012). The firing rates of neck muscles motor units are generally higher (10–15 Hz; Falla and Farina 2008; Falla et al. 2010; Schomacher et al. 2012) than those of lower limb muscles (6–8 Hz; Dalton et al. 2009; Mochizuki et al. 2006, 2007) and therefore may be responsive to vestibular stimuli over a wider bandwidth. Based on these firing rate data, we hypothesized that the bandwidth of vestibular reflexes will be larger for the neck muscles than for the lower limb muscles. We first evaluated this hypothesis experimentally by estimating the coherence between the input electrical stimulus and muscle activity to determine the bandwidth of vestibular reflexes in each muscle. We then used a simple computational model composed of a population of vestibular afferents and a motoneuron pool to determine whether changes in firing rate alone could explain these differences.
Vestibulospinal pathways and time delays between their activation and subsequent excitation/inhibition of motoneurons differ across muscles. Vestibular signals form short-latency disynaptic excitatory and inhibitory connections with neck motoneurons (Shinoda et al. 1997) via different vestibular nuclei (medial vs. lateral) and neural pathways (medial vs. lateral vestibulospinal) compared with lower limb muscles (Shinoda et al. 2006; Wilson and Peterson 1981; Wilson and Schor 1999). The dynamic behavior of these pathways varies; compared with medial vestibular neurons, lateral vestibular neurons have a lower sensitivity over a wide range of frequencies and demonstrate a “cutoff” behavior by synchronizing their firing with the depolarizing phase of high-frequency sinusoidal stimuli (Uno et al. 2003). As such, these differences may filter the descending vestibular signals to varying degrees. In addition, the potential for multiple vestibular pathways projecting on neck muscles (see reviews by Wilson and Schor 1999 and Goldberg and Cullen 2011) may result in their destructive interaction due to a fixed time delay between them (Matthews 1994). This manifests itself as discontinuities in the phase relationship between an input stimulus and the muscles response (Matthews 1994). To determine whether the frequency response of vestibular reflexes varies between muscles, we characterized the gain and phase response of the vestibular reflexes across muscles. We hypothesized that the gain and phase of vestibular reflexes will differ between muscles because of different neural pathways and the resulting neural filtering between the vestibular system and the motoneurons of the neck, back, and lower limbs. This hypothesis was not explored with our simple computational model due to the limited information regarding the properties of various vestibular pathways.

METHODS

Subjects

Eleven healthy subjects (10 males, 1 female; age 20–48 yr; mass 73 ± 10 kg; height 1.73 ± 0.10 m; mean ± SD) with no self-reported history of neurological disorders or injuries took part in 2 separate experiments (8 in experiment 1; 4 in experiment 2; 1 subject performed both experiments). The experimental protocol was explained before each experiment, and all subjects gave written, informed consent. The experiment conformed to the Declaration of Helsinki and was approved by the University of British Columbia’s Clinical Research Ethics Board.

Vestibular Stimuli

Vestibular stimulation was delivered to subjects using carbon rubber electrodes (~9 cm²) in a binaural bipolar arrangement. The electrodes were coated with Spectra 360 electrode gel (Parker Laboratories, Fairfield, NJ) and secured over the subject’s mastoid processes with an elastic headband. The stimuli were delivered as analog signals via a data acquisition board (PXI-6289; National Instruments, Austin, TX) to an isolated constant-current stimulator (STMISOL; Biopac, Goleta, CA). The signals were generated offline using MATLAB (The MathWorks, Natick, MA), and identical signals were presented to all subjects.

Subjects were exposed to 90-s filtered white noise stochastic vestibular stimuli (SVS) of two bandwidths (0–25 and 0–75 Hz). The signals were normalized to provide equal amplitude per frequency, resulting in different root-mean-square (RMS) amplitudes for each bandwidth: 0.83 mA (0–25 Hz) and 1.38 mA (0–75 Hz). The 0- to 25-Hz stimulus was considered our baseline condition since it had been used previously to characterize the frequency response of the vestibular reflexes in the lower limb muscles (Dakin et al. 2007, 2010). The 0- to 75-Hz stimulus was chosen to examine the short-latency (approximately 13–17 ms) and higher-frequency vestibular reflexes previously observed in neck muscles following 500-Hz short-tone acoustic stimuli (Wu et al. 1999) or transient electrical vestibular stimulation (see Rosengren et al. 2010 for review).

Protocol

The 1st experiment (8 participants) was performed to evaluate the bandwidth and frequency response of vestibular reflexes across the different muscles (i.e., our main hypotheses). Subjects stood on a force plate (Bertec 4060-80; Bertec, Columbus, OH) with their feet parallel and medial malleoli 2–3 cm apart. For each trial, subjects were instructed to stand relaxed, lean forward slightly, close their eyes, hold their arms by their sides, and rotate their torso and head axially to the left (i.e., leftward yaw). Torso yaw was maintained at 30° using a torso-mounted laser (level with T2), and head yaw was maintained at 90° relative to the feet (60° relative to the torso) using a head-mounted laser. The head was also rotated in extension such that the Reid plane was tilted up by 18° horizontally. This head position maximizes the postural response to binaural bipolar electrical vestibular stimulation in the anterior-posterior direction (Cathers et al. 2005; Fitzpatrick and Day 2004) along the line of action of the right gastrocnemius and soleus muscles. Orienting the head 60° to the left (relative to the torso) ensured activation of the right sternocleidomastoid (r-SCM) and the left splenius capitis (l-SPL) muscles. Each 90-s stimulus (0–25 and 0–75 Hz) was applied twice in a random order. Two additional trials were performed without vestibular stimulation, one before and one after all stimulation trials, to estimate the frequency characteristics of the muscles in the tested posture and to evaluate the effects of the digital filters (see Signal Analysis).

The first experiment applied an ideal axial torsion of the neck (60°) and torso (30°) to ensure activity in the measured neck and back muscles. However, responses across muscles may be affected by the variation in descending commands and/or propriospinal reflex contributions specific to maintaining this posture. Therefore, the 2nd experiment (4 participants) was performed to evaluate and eliminate any confounding effects of the amount of muscle length on the different responses across muscles. We investigated this effect in the same 2 neck muscles (r-SCM and l-SPL) using a custom-built axial-torsion device. Subjects wore a helmet that was restrained in yaw but could move in all other degrees of freedom. This was accomplished using two universal joints and a telescoping shaft, allowing subjects to sway freely while applying torque through the neck at any orientation (Dakin 2012). Subjects performed three tasks: 1) head free with the head (60° relative to the torso) and torso (30° relative to the feet) rotated to the left (HF-60), thereby replicating experiment 1; 2) head restrained with the neck (60° relative to the torso) and torso (30° relative to the feet) rotated to the left (HR-60) and applying a torque generating equivalent muscle activity of r-SCM to that of task 1; and 3) head restrained with the head and torso facing forward (0°; HR-0) and applying a torque generating equivalent muscle activity of r-SCM to that of task 1. These tasks were chosen to evaluate, first, the effects of restraining the head in yaw using the torsion device (task 2 vs. 1) and, second, the variation in neck-muscle length between the two orientations (i.e., 60 vs. 0°, task 3 vs. 2). Only the 90-s, 0- to 75-Hz stimulus was applied in this experiment and repeated three times to improve the frequency response estimates of vestibular reflexes (coherence, gain, and phase; see Signal Analysis). The torques required to match muscle activity across conditions were established in preliminary experiments performed without stimulation where the RMS of the electromyogram (EMG) was matched to the HF-60 condition (task 1).
Data Collection

In the first experiment, EMG was collected from neck, back, and lower limb muscles. Intramuscular EMG was recorded in the r-SCM and l-SPL using pairs of 0.05-mm wire (Stablohm 800A; California Fine Wire) inserted under ultrasound guidance (MicroMaxx; Sonosite; Blouin et al. 2007). One of the two wires of each electrode had 2–3 mm of exposed wire. This electrode design allowed the recording of multunit EMG potentials. All wires were placed near the center of the horizontal cross-section of a muscle. In the r-SCM, the wire always remained superficial to the readily identifiable cleidomastoid subvolume (Kamibayashi and Richmond 1998). Both wires were inserted at about the C4 level. Surface EMG was recorded for the right erector spinae (r-ESP) muscle 2–3 cm laterally from midline at the level of L3. Surface EMG was recorded for the right solas (r-SOL) and medial gastrocnemius (r-mGAS) muscles; r-mGAS was recorded for comparison with previous studies. All EMG signals were amplified (×200–2,000; NeuroLog; Digitimer, Hertfordshire, United Kingdom) and band-pass filtered (intramuscular 0.05–1,000 Hz; surface 10–1,000 Hz). EMG, vestibular stimuli, and force plate data were then digitized and recorded at 2,000 Hz via a digital data acquisition board (PXI-4495; National Instruments) using a custom LabVIEW software program (National Instruments).

In the second experiment, EMG was collected from the r-SCM, l-SPL, and r-mGAS muscles only. Intramuscular EMG was recorded in the r-SCM and l-SPL while both surface and intramuscular EMG was recorded in the r-mGAS. We recorded both electrode types in r-mGAS to eliminate the possibility that differences observed between neck and lower limb muscles in experiment 1 were an artifact of using surface and intramuscular electrodes. After all, the spectral properties of surface EMG differ from those of intramuscular EMG (reviewed in De Luca 1997 and Farina et al. 2004). From these experiments, we observed equivalent frequency responses of vestibular reflexes (coherence, gain, and phase; see Signal Analysis) for both electrode types of the r-mGAS during the 0–75-Hz stimulus for all conditions (see head-free results in Fig. 1). This indicated that it was possible to provide a direct comparison of responses from the two electrode types across different muscles in experiment 1.

Signal Analysis

Raw neck EMG data were first high-pass filtered using a phaseless eighth-order Butterworth digital filter (−3 dB at 100 Hz). The high cutoff was necessary to eliminate the stimulation artifact given the close proximity of the neck intramuscular electrodes to the vestibular stimulation site. To ensure that digital filtering did not substantially alter the spectral characteristics of the rectified signals, the effect of increasing cutoff frequencies (40, 60, 80, and 100 Hz) was evaluated using data from the 0–25-Hz stimulation condition. The frequency response of vestibular reflexes (coherence, gain, and phase; see below) for all muscles was unaffected with exception of a slight drop in gain at all frequency points with increasing cutoff frequency, thus ensuring that responses were unaffected by a high-pass cutoff of 100 Hz.

Repeated trials within each subject were concatenated to create either 180- (experiment 1) or 270-s (experiment 2) data records. Data from all subjects (EMG and force) were then concatenated again to create a single pooled data set for each condition. Before concatenation, the filtered EMG of each trial was normalized by its vector norm to ensure equal contributions to the averaged response from all subjects. Both the individual and pooled EMG data were full-wave rectified, and the autospectra for the SVS and the activity of each muscle as well as the cross-spectra between SVS and activity of each muscle were calculated. Data were sectioned into segments of 1 s before calculating the auto- and cross-spectra, and then the spectra were averaged in the frequency domain. This yielded a frequency resolution of 1 Hz for all spectra. A repeated-measures ANOVA was performed on the frequencies of the peak values extracted from the EMG autospectra of experiment 1 to evaluate the effects of muscle type and stimulation (5 muscles × 3 stimuli: 0–25 Hz, 0–75 Hz, and no stimulation). Pairwise comparisons with a Bonferroni correction were made to evaluate differences between combinations of two muscles. For the l-SPL, an increased frequency resolution was necessary to evaluate the frequency response of the vestibular reflexes at low frequencies. For these purposes, data were reprocessed with a segment length of 4 s, yielding a frequency resolution of 0.25 Hz.

Coherence was calculated to explore the bandwidth of the frequency response of the vestibular reflexes across muscles. Given that the input signals were filtered white noise, coherence is a measure of the linear relationship between the electrical vestibular (input) and muscle activity (output) signals across the frequencies considered. At each frequency point, coherence ranges from 0 for systems with no linear relation to 1 for linear systems without noise (Pintelon and Schoukens 2001). The 95% confidence limit for coherence spectra was derived from the number of disjoint segments (individual subjects: 180 and 270, pooled subjects: 1,440 and 1,080; for experiments 1 and 2, respectively) to indicate frequencies where coherence was significantly different from 0 (Halliday et al. 1995). Significant changes in SVS-EMG coherence between different muscles were identified using a difference of coherence (DoC) test (Amjad et al. 1997). The DoC test was applied on the Fisher transform (tanh⁻¹) of the coherence (square root of the coherence) values, evaluated for the 0–75-Hz stimulus condition and compared with a χ²-distribution with k-1 degrees of freedom (k is the number of muscles included in the comparison). Since we were interested in the coherence difference across the entire stimulus bandwidth (0–75 Hz), pairwise comparisons were made to highlight differences between neck-neck, neck-back, and neck-lower limb muscle combinations. Two additional DoC tests were performed separately for r-SCM and l-SPL coherence.
obtained from experiment 2 to evaluate the effects of head restraint (i.e., HF-60 vs. HR-60) and head orientation (i.e., HR-60 vs. HR-0). A significance level of $\alpha = 0.05$ was used for all analyses.

Gain- and phase-frequency estimates were calculated from the pooled data to describe the frequency response of the vestibular reflexes at frequencies with significant coherence. Gain and phase indicate the magnitude and timing of the output EMG relative to the input SVS and were assessed with two primary objectives: 1) to identify muscle-dependent filtering behavior as shown by gains and phases that decrease with increasing frequency; and 2) to identify the destructive interaction of multiple reflex pathways as shown by phase-frequency discontinuities. Both would support the hypothesis that pathway-dependent neural filtering plays a role in the variation of vestibular reflexes across muscles. Gains were normalized within each muscle to the mean gain at the lowest frequency point (1 Hz) across the three stimulus conditions allowing comparison across muscles. As a result, the gain is plotted without units. Normalized gains that remained close to one up to higher frequencies indicate vestibular reflexes that can respond over a wider bandwidth. Finally, coherence-, gain-, and phase-frequency estimates of SVS-to-horizontal forces were also calculated, and they verified that the postural responses were similar to previous studies (Dakin et al. 2010; Mian and Day 2009). As a result, the force data were not presented in this manuscript.

Vestibular Afferent-Motoneuron Pool Model

To assess whether differences in motoneuron properties explained the different frequency responses we observed in neck and lower limb muscles, we built a computational model of the vestibular reflexes (Fig. 2A) and then varied the motoneuron firing rates and action potential durations to encompass the behavior of the neck and lower limb motoneurons. Coherence, gain, and phase estimates were then used to evaluate our hypothesis, where it was expected that the lower motoneuron firing rates and longer action potential durations would limit the coherent bandwidth of SVS-EMG coupling. It is noted here that we did not attempt to fit the model to the experimental data but simply evaluated whether the simulated results matched any of the muscle-dependent variations.

The model consisted of a pool of 300 motoneurons that received input from 2 sources: tonic supraspinal input and vestibular afferent input. Development details of the model (i.e., tonic supraspinal input, vestibular afferent input, and motoneuron pool) can be found in the Appendix, and information regarding the model simulation and parameter variation are provided here.

Model simulation. The tonic input ($R^T$) was varied to reflect the physiological range of motor unit firing rates in neck (10–15 spikes per second; Farina and Falla 2008; Falla et al. 2010; Schomacher et al. 2012), back (7–8 spikes per second; Barkhaus et al. 1997), and lower limb (6–8 spikes per second; Dalton et al. 2009; Mochizuki et al. 2006, 2007) muscles at the contraction levels expected in these conditions (i.e., 15–30% maximal voluntary contraction). To examine the effects of motoneuron firing rate on the frequency response of the vestibular reflexes, we simulated firing frequencies ranging between 6 and 18 spikes per second. The required $R^T$ input was established in preliminary simulations with natural vestibular afferent output (i.e., without electrical stimulation).

To simulate EMG signals, the motoneuron spike signals were replaced by motor unit action potential (MUAP) waveforms. We used the same wave shape as implemented by Stegeman et al. (2010) where the half-pulse wave shape was defined by:

$$h(t) = 5 \sin \left( \frac{\pi t}{d/2} \right) e^{[-(t/d^2) - 1]} \quad \text{for } 0 < t \leq d/2$$

which was mirrored in time and amplitude for $d/2 < t < d$ and aligned with the motoneuron spike signals at $t = d/2$. The duration ($d$) of the MUAP was varied between 8 and 24 ms, reflecting the variation in

![Fig. 2. A: schematic of the vestibular-afferent motoneuron (MN)-pool model that simulates the electrical vestibular stimulus experiments. The motoneuron pool is innervated by vestibular afferents (irregular and regular) as well as a tonic input. The output is the summation of motor unit action potentials (MUAPs) that correspond to the motoneuron spiking. B: motor unit action potential profiles used for 1 of the stimulation parameter variations. EMG, electromyography.](http://jn.physiology.org/)

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spectral properties (Stegeman et al. 2010) and durations (Boonstra and Breakspear 2012) expected in small (i.e., neck) and large (i.e., lower limb) muscles, respectively (Fig. 2B). The damping factor (r) smooths the onset of the wave shape and was set at 0.18 ms as used in previous studies (Boonstra and Breakspear 2012; Stegeman et al. 2010). The amplitude of each MUAP was randomly distributed between 0 and 1 to account for variation in the number and position of the muscle fibers within each motor unit with respect to measurement electrodes (Boonstra and Breakspear 2012). In addition, the polarity of the MUAPs was equally divided between being initially positive or negative. The model output was the summation of all MUAPs delayed by 15 ms to approximate neck-muscle vestibular reflex latencies reported in the literature (Rosengren et al. 2010). Additional delays were simulated to represent back and lower limb muscle latencies; however, these data were not presented, as their effect was limited to changes in the phase slope only.

The vestibular afferent-motoneuron pool model was implemented in MATLAB (The MathWorks) and simulated using the MATLAB Distributed Computing Toolbox and Engine for parallel processing on a 12-core computer. The effect of 3 variables on the coherence, gain, and phase estimates was evaluated: stimulus bandwidth (0–25 and 0–75 Hz), motor unit firing rate (6, 8, 12, 16, and 18 spikes per second), and MUAP duration (8, 12, 16, 20, and 24 ms). Each stimulus bandwidth and motor unit firing rate combination was simulated 10 times each for a total of 100 simulations. The MUAP convoluted was then performed on each simulation for the 5 MUAP durations, generating a total of 500 simulation results. The vestibular afferent model was simulated for 90 s using the experimentally applied vestibular signals with a discrete time step of 0.1 ms. The resultant spike train was downsampled by 10, whereby the spike trains were summed over bins of 1 ms. The motoneuron pool model was then run with a discrete time step of 1 ms. To avoid potential start-up effects, the 1st 1 s was removed from the data.

Coherence, gain, and phase estimates were calculated in the same manner as described for the experimental data, although only 89 segments of 1 s were used. In the frequency domain, this produced a resolution of 1 Hz. A repeated-measures ANOVA was performed on the frequency of the peak gain and phase discontinuities, which occurred at the same frequency point, as well as the magnitude of the peak gain during the 0- to 75-Hz condition to assess the effects of motor unit firing rate and MUAP duration (5 rates x 5 durations). These particular features of gain and phase were chosen because they were similar to responses observed in the experimental data.

RESULTS

Experimental

Filtered, unrectified EMG signals from all subjects reflected typical multunit recordings and showed no obvious pattern of covariance between the stimuli and muscle EMG data (see sample data from experiment 1 in Fig. 3A). The matching shapes of the autospectra of the rectified EMG for the stimulation and nonstimulation data indicated successful filtering of the stimulation artifact in neck muscles (Fig. 3B; note the autospectra of the nonstimulation trials were offset from the stimulation trials only for better visualization). The repeated-measures ANOVA showed a significant effect of muscle type (F_{1,28} = 46.6, P < 0.001) and no significant effect of stimulus (F_{2,14} = 2.7, P = 0.103) on the frequency of autospectra peaks. Within these individual data, the autospectra of neck muscles showed peak values at higher frequencies (r-SCM: 16.4 ± 2.0 Hz, l-SPL: 13.3 ± 1.5 Hz) relative to the back (r-ESP: 7.5 ± 2.4 Hz) and lower limb muscles (r-mGAS: 11.0 ± 1.9 Hz, r-SOL: 8.4 ± 0.9 Hz) when averaged across conditions (0–25 Hz, 0–75 Hz, and no stimulation). A pairwise comparison revealed no significant difference between the no-stimulation and either SVS condition for all muscles.

Frequency bandwidth of vestibular reflexes. Significant SVS-EMG coherence was observed for all muscles in experiment 1 (Fig. 4A). Overall, the bandwidth of coherent frequencies was larger for neck muscles compared with lower limb and
Fig. 4. Coherence, gain, and phase frequency estimates for all muscles elicited by the 2 stimuli: 0–25 and 0–75 Hz. Pooled responses include all subject data (n = 8). A: r-SCM. B: l-SPL. C: r-ESP. D: r-mGAS. E: r-SOL. Coherence, gain, and phase frequency estimates for l-SPL (B) using a segment length of 4 s are included as insets in each plot to detail the low-frequency responses. The horizontal, segmented lines in the coherence plots represent the level above which the coherence is significant. Solid lines in the phase plots were fitted onto the different regions of linearly decreasing phase for visualization using a linear regression algorithm. SVS, stochastic vestibular stimuli.
back muscles. In both lower limb muscles, well-defined peaks in coherence spectra were observed at 4–8 and 11–16 Hz with no significant coherence >30 Hz. In the r-ESP muscle, significant coherence was limited to a range of about 1–15 Hz. A distinct peak was seen at 2 Hz in r-ESP for all conditions, and sporadic significant coherence was found around 7–8 Hz. Coherence was significant up to ~70 Hz in both r-SCM and l-SPL. Both muscles exhibited coherence peaks between 5 and 8 Hz. In the r-SCM, coherence decreased below significance between 18 and 25 Hz and thereafter remained significant up to 75 Hz with a broad peak at about 40–50 Hz. In the l-SPL, an additional low-frequency peak was observed at ~2 Hz, which was more clearly revealed when analyzing the data with a frequency resolution of 0.25 Hz (Fig. 4B, inset in l-SPL data). These observations were confirmed by the DoC tests, which revealed significant differences in the coherence estimates between the five muscles at almost all stimulated frequencies (Fig. 5A). Pairwise DoC revealed coherence in l-SPL to be larger than r-SCM at low frequencies (<20 Hz) and r-SCM to be larger than l-SPL at high frequencies (30–70 Hz). Coherence in both neck muscles was larger than in r-ESP at all frequencies where significant differences were detected. The magnitude of the DoC test was largest when comparing neck and lower limb muscles, with larger coherences in lower limb muscles at low frequencies (<20 Hz) and presence of coherence in neck muscles at high frequencies (30–70 Hz).

To determine whether these differences in coherence across muscles were due to differences in muscle length, we examined the potential effects of muscle length on the bandwidth of the neck vestibular reflexes. The SVS-EMG coherence of the neck muscles in experiment 2 was similar to the results of experiment 1, with the exception of a decrease in l-SPL coherence at low frequencies (<20 Hz). This was attributed to a single subject where coherence was below significance at almost all frequencies below 20 Hz; all other subjects demonstrated the characteristic low-frequency peak. More importantly, the bandwidth of coherence was unaffected by either the restriction of head yaw (HR-60) or head orientation (HR-0). A slight reduction in coherence was observed in restrained conditions relative to the head-free condition; however, the DoC test revealed significant differences at only sporadic frequency points (no more than 6 nonadjacent points) across the entire bandwidth for either comparison (i.e., head restraint and head orientation) in both muscles.

**Gain and phase responses of the vestibular reflexes.** Gain and phase estimates of the vestibular stimuli-to-EMG relationship for all muscles in experiment 1 were plotted at frequency points with significant coherence (Fig. 4). Back and lower limb muscle gain and phase indicate a low-pass behavior, where phase decayed monotonically over one or two regions and gain was flat up to a cutoff frequency of approximately 16, 14, and 3 Hz for r-SOL, r-mGAS, and r-ESP, respectively. In r-SCM, a discontinuity in phase was observed over the region where coherence was not significant. This discontinuity was characterized by a positive shift in phase lag by ~90° at 20 Hz. Within each region (the low, 1–15 Hz, and high, 22–62 Hz, frequencies), phase decreased linearly and monotonically, although with different slopes (Fig. 4). These were accompanied with gains that were flat and close to 1 in the low-frequency region and that increased by a factor of ~2 in the high-frequency region. In l-SPL, three separate linear regions of monotonically decreasing phase were defined by inflection points at 19 and 36 Hz (Fig. 4). An additional phase discontinuity (positive shift of ~90°) was observed at ~4 Hz, which was more clearly revealed when analyzing the data with a frequency resolution of 0.25 Hz (Fig. 4B, insets in l-SPL data). l-SPL gains peaked at ~10 Hz to a maximum of 2–3 over a range of 6–18 Hz but otherwise remained close to 1 for all other frequency points. The gain and phase of both neck muscles were comparable across all subjects with the exception of the r-SCM phase discontinuities, which were observed in 10 of 12 subjects.

We then examined whether the gain and phase characteristics of the r-SCM and l-SPL muscles observed in experiment 1 were associated with the length of these muscles. Gain and phase estimates of these two neck muscles in the head-free condition (HF-60) of experiment 2 were similar to the results of experiment 1 (Fig. 6), with the exception of a less-defined low-frequency (~10-Hz) peak in the l-SPL. This was attributed to the same subject with coherence that fell below the significance threshold at low frequencies, and therefore no gain peak was found. The most important result of this experiment was that gain and phase responses were unaffected by either the restraint in yaw (HR-60) or head orientation (HR-0).
Together with the coherence results, this indicates that differences in neck-muscle length were not responsible for the variation in bandwidth or frequency response of reflexes observed across the different muscles.

**Simulation**

The model yielded coherence and gain/phase relationships (Fig. 7) that captured some but not all of the behavior observed in the experimental data. The simulated stochastic stimuli (Fig. 7A) elicited coherence profiles that closely matched the stimulus-dependent experimental findings in the r-SCM. At the largest stimulus bandwidth (0–75 Hz), two distinct peaks were observed in the coherence at ~14 and 40 Hz, separated by an intermediate dip ~20 Hz. A low-frequency peak in the gain and a phase discontinuity (in the form of an inflection point), which occurred at the same frequency between 10 and 20 Hz, were similar but not identical to the experimental neck data (Fig. 7A). Above 20 Hz, the monotonically decreasing phase captured the simulated delay of 15 ms.

Increasing the motor unit firing rates (Fig. 7B) decreased the coherence across the entire bandwidth. The frequency of the 1st coherence peak increased with firing rate and at the highest rate (18 spikes per second) the peak aligned with the firing frequency of the motor units. Since the firing rate was simulated as an increase in the descending input, the relative contribution from the vestibular afferent activity at all other frequencies decreased. The frequency of the gain peak and phase discontinuity ($P < 0.001, F_{4,36} = 64.6$) and the magnitude of the gain peak ($P < 0.001, F_{4,36} = 34.4$) increased (Fig. 7B) with motor unit firing rate. Increasing the MUAP duration resulted in further reductions in coherence across the entire bandwidth, although in particular at the highest frequencies (Fig. 7C). The magnitude of the gain peak ($P < 0.001, F_{4,36} = 507.0$) decreased with increasing MUAP duration (Fig. 7C) but did not affect the frequency of the gain peak and phase discontinuity ($P = 0.115, F_{4,36} = 2.0$). These results indicate that although both input parameters affected the gain and phase responses, neither was able to mimic the variation in frequency responses observed experimentally across the measured muscles.

**DISCUSSION**

The aim of this study was to characterize the frequency response of vestibular reflexes in different muscles. We specifically evaluated two hypotheses: 1) that variation in the bandwidth of vestibular reflexes between muscles are due to the muscle-specific motor unit properties such as firing rate and action potential durations; and 2) that the gain and phase of vestibular reflexes will differ between muscles because of pathway-dependent neural filtering. The frequency response of the neck-muscle vestibular reflexes exhibited coherence across higher bandwidths compared with back and lower limb muscles. Our mathematical model, however, did not support our first hypothesis: variations in motor unit properties do not appear to be the primary determinants of coherence bandwidth. The discontinuities we observed in the stimulus-EMG phase relationships of both neck muscles confirmed our second hypothesis and suggest that neural filtering may be responsible for the muscle-specific responses to vestibular reflexes.

**Muscle-Dependent Bandwidth of Vestibular Reflexes: Neural Mechanisms?**

Based on our numerical model, motor unit firing rates and action potential duration do not solely explain the observed differences in bandwidth between neck and lower limb vestibular reflexes. Although both motor unit properties had an effect on the coherence, variations in firing rate and motor unit waveform were unable to replicate the apparent low-pass
behavior, limiting the response bandwidth to <25 Hz, observed in back and lower limb muscles. Nonetheless, the model captured the high-bandwidth behavior of neck muscles along with some aspects of the gain and phase characteristics. These results lead us to suggest that additional filtering in the neural pathways plays a role in the back and lower limb muscles. Although neural filtering was not included in our model, our experimental data support this hypothesis. The frequency response of vestibular reflexes in the back and lower limb muscles behave as a low-pass filter with a flat gain up to 14–16 Hz in r-mGAS and r-SOL and 3 Hz in r-ESP. In contrast, neck-muscle frequency responses were characterized by gains near or above 1 up to 70 Hz with discrete changes in phase.

One possible neurophysiological mechanism responsible for the differential filtering of the vestibular reflexes is the transmissibility of head movement information provided by the different vestibular nuclei to either neck or lower limb motoneurons. During in vitro current stimulation in the guinea pig, the response dynamics of lateral vestibular nucleus neurons (LVNn) are fairly flat across a wide bandwidth (approximately 0–50 Hz) compared with medial vestibular nucleus neurons (MVNn), which drop off in firing rate sensitivity after the peak frequency of resonance (~10 Hz; Uno et al. 2003). However, LVNn possess lower firing rate sensitivities to input currents at all frequencies and often demonstrate a cutoff behavior where neurons synchronize their firing with the depolarizing phase of the current input and no response is measured. It is conceivable that similar phenomena play a role in our experiments whereby the LVNn connecting to lower limb motoneurons are limited or unable to transfer the high-frequency vestibular signals induced by the electrical stimulation.

An alternative mechanism is that the spinal circuitry prohibits high-frequency vestibular information from reaching the back and lower limb motoneurons. In neck muscles, disynaptic connections form both short-latency excitatory and inhibitory pathways between the labyrinth and motoneurons (Wilson and Schor 1999; Wilson and Yoshida 1969). Single vestibulospinal neurons branch to multiple neck motoneurons whereby muscles activate in synergy to respond to individual canal stimulation (Perlmutter et al. 1998; Shinoda et al. 1992, 2006). On the other hand, vestibulospinal and reticulospinal tracts connect to lower limb motoneurons via direct connections, which are exclusively excitatory and indirect connections via interneurons (Davies and Edgley 1994; Grillner et al. 1970; Lund and Pompeiano 1968; Pompeiano 1972; Shinoda et al. 1986; Wilson and Yoshida 1969). In humans, vestibular input mod-
ulates the transmission of spinal reflex pathways, facilitating reciprocal Ia inhibition, group I nonreciprocal inhibition, and Ia presynaptic inhibition (Iles and Pisini 1992; Kennedy and Inglis 2002; Rossi et al. 1988), suggesting convergence of vestibular and peripheral signals on interneurons. Spinal interneurons transferring vestibular information may therefore limit signal transmission to below ~25 Hz as observed in our experiments.

From a functional standpoint, the flat gain observed in neck muscles indicates a system that responds effectively at all frequencies. However, since the majority of these frequencies lie above the frequency response of the mechanical system the muscle is controlling, it might at first seem difficult to assign functional value to these responses (Matthews 1994). From a biomechanical control aspect, it would seem logical that neck muscles can respond to sensory input across a wider bandwidth; after all, the dynamic range of the sensor must be greater than the actuator, which must be greater than the underlying mechanical system to ensure effective control (Franklin et al. 2009). In lower limb muscles, vestibular reflexes function over a bandwidth of 25 Hz in upright stance, whereas the system mechanics (i.e., SVS-to-mechanical sway) extend only as high as ~5 Hz (Dakin et al. 2010; Fitzpatrick et al. 1996). The bandwidth of head motion (i.e., position) during both voluntary whole body movements and imposed force perturbations to the head contains frequency components up to 20 Hz (Grossman et al. 1988; Pozzo et al. 1990; Viviani and Berthoz 1975). Therefore, to maintain head-neck stabilization, vestibular influence over neck-muscle activation must function across a much higher bandwidth than it does for lower limb muscles.

Neck-Muscle Responses: Evidence of Multiple Reflex Pathways

Our results suggest that multiple reflex pathways connect the vestibular system to the neck muscles and that these pathways may differ between different neck muscles. In L-SPL, gain peaked at ~10 Hz and phase shifted by 90° at ~4 Hz, whereas in r-SCM gain remained more or less flat and phase shifted by 90° at ~20 Hz. This phase shift in the neck muscles differed considerably from the phase response observed in the lower limb muscles and did not change when reorienting the head forward. Furthermore, the variation across neck and lower limb muscles was shown not to be due to electrode type because vestibular reflex responses in a lower limb muscle (r-mGAS) were equivalent when using the two recording techniques.

Similar behavior has been observed in the stretch reflex of the human wrist during mechanical perturbations and attributed to the interaction of two reflex mechanisms separated by a fixed delay (Matthews 1993). At frequencies where the two components are out of phase, destructive interference occurs without invoking inhibition. For our results, this would imply a delay of ~100 ms in L-SPL and ~25 ms in r-SCM and leads to a question of what two (or more) reflex components could induce this response in neck-muscle control. In the lower limb of humans, the short- and medium-latency vestibulomyogenic components have been hypothesized to originate from, respectively, the otoliths via reticulospinal pathways and the semicircular canals via vestibulospinal pathways (Britton et al. 1993; Cathers et al. 2005). Although canal/otolith interaction cannot be excluded in neck muscles, this is unlikely because both sensory end organs generate excitatory and inhibitory postsynaptic potentials in neck motoneurons with response latencies as low as 1 ms during electrical stimulation of hair cells (Shinoda et al. 1997; Uchio et al. 1997; Wilson and Maeda 1974).

Another possibility is that multiple pathways transmitting similar information are involved, yielding destructive interaction caused by a time delay between them. Neuroanatomical and neurophysiological evidence from animal studies regarding the neck-muscle vestibular reflexes support this proposal. Although neck-muscle motoneurons connect to the labyrinth via strong disynaptic pathways, more indirect circuits make dominant contributions to vestibular neck reflexes (reviewed in Goldberg and Cullen 2011; Wilson and Schor 1999). For one, neck muscles receive many different patterns of excitatory and inhibitory input from the separate canals and maculae, and these inputs arrive via multiple vestibulospinal tracts (Shinoda et al. 1997; Sugiochi et al. 2004). In addition, transection of the medial vestibulospinal tract in decerebrate cats results in almost no change in the dynamic properties of either the canal-driven vertical vestibulocolic reflex (Thomson et al. 1995) or the otolith spinal reflexes (Miller et al. 1982) for many muscles. Such responses point toward additional pathways contributing to the reflex responses, and several experimentally supported hypotheses have been put forward, including interneuron connections from additional vestibulospinal, i.e., bulbospinal, pathways (Bankoul et al. 1995; Isu et al. 1996), input from the interstitial nucleus of Cajal (Fukushima et al. 1994), and input via reticulospinal pathways (Peterson et al. 1980). Although the vestibular reflexes in neck muscles observed in this study point toward the contribution of multiple pathways, additional animal experiments would be needed to test this hypothesis during head-neck balancing actions.

Study Limitations

A limitation of this study is the inability of our model to replicate exactly the features of the vestibular reflex responses of any of the experimentally measured muscles. This may be due to several physiological features not implemented in this study. First, the lack of full bandwidth dynamics of vestibular afferent firing during electrical stimulation were not included because no literature currently exists to describe this behavior up to 75 Hz. However, because these properties would most likely affect both neck and lower limb muscle responses, this exclusion would not change our results. Second, the effects of task dependence were not included in the model where ongoing vestibular input contributing to motoneuron drive is required to evoke vestibular reflexes, at least in the lower limb muscles (Fitzpatrick et al. 1994; Luu et al. 2012). As a result, even if the input provided by the natural firing of vestibular afferents did not contribute to the motoneuron pool, a vestibular reflex would still be evoked by the electrical stimulation. Finally, the motoneuron pool model lacks the detail of more complex models (Williams and Baker 2009) such as variable motoneuron recruitment thresholds and persistent inward currents. Nonetheless, despite the above limitations, because the model was unable to replicate the primary experimentally observed muscle-dependent variation in responses [i.e., coherence bandwidth, phase (dis)continuity], it was considered sufficient to

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evaluate and disprove our first hypothesis. Future efforts to simulate the physiological mechanisms thought to be responsible for the neural filtering and/or multiple reflex pathways may help to identify the exact mechanisms responsible for the muscle-dependent variation of vestibular reflexes.

Regarding the interpretation of our experimental results, we cannot rule out the possibility that part of the observed responses to electrical vestibular signals are of a nonvestibular origin (Mian et al. 2010; Spiegel 1942). Although nonvestibular afferents may contribute to responses from the current study, the short-duration and therefore high-frequency characteristics of vestibular-evoked myogenic potentials using short-duration (2-ms) electrical and nonelectrical stimuli (see Rosen gren et al. 2010 for review) suggest that our responses are of a vestibular origin.

Conclusions

The frequency response of vestibular reflexes in neck muscles showed both a larger bandwidth and discontinuities in phase that were not present in vestibular reflexes in back or lower limb muscles. Moreover, differences between the two neck muscles tested here indicate that vestibular reflexes are different in anatomically related muscles. Our numerical model showed that variations in motor unit firing rates between muscles do not substantially influence the coherence bandwidth. Instead, the extended dynamic range of neck-muscle vestibular reflexes indicates the presence of additional neural filtering in lower limb muscles. Discontinuities in the gain and phase responses of vestibular reflexes in neck muscles provide evidence of reflex component interaction and additional support for alternative vestibular-motor pathways to the well-established direct disynaptic connections.

APPENDIX

Tonic Supraspinal Input

The motoneuron pool received neural input from 100 tonically firing descending fibers to produce baseline postural muscle activity. Tonic supraspinal input was converted into spike trains for each fiber via a stochastic Poisson process, such that each neuron received input with the same spike rate statistics but with a different random realization (Schuurmans et al. 2009).

Vestibular Afferent Input

A vestibular afferent model was used to estimate the modulated output of a population of 100 vestibular afferents during electrical stimulation (Goldberg et al. 1984; Smith and Goldberg 1986). The afferent model captured the variability observed in the interspike interval (ISI) among regular, intermediate, and irregular firing afferents. Discharge regularity was defined by the normalized coefficient of variation ($c_v$), which was calculated by dividing the standard deviation of the ISI by the average ISI and normalized to a standard ISI (15 ms) to account for the variability of $c_v$ within each afferent (see Goldberg et al. 1984 for more details). Five afferent types were defined and distributed evenly among the 100 afferents and having $c_v$ distributed in roughly octave steps ranging from 0.026 to 0.51 (Smith and Goldberg 1986). This captured the range of afferents recorded in squirrel monkey otoliths (Fernandez and Goldberg 1976) and semicircular canals (Goldberg and Fernandez 1971).

No literature was found that quantified afferent firing rates due to externally applied electrical vestibular stimulation. Stimulation of monkey vestibular afferents using anodal and cathodal currents of 70 $\mu$A in the perilymphatic space explored the entire dynamic range (0–300 spikes per second; Goldberg et al. 1984). Equivalent afferent output can be achieved with kinetic stimuli on the order of $\pm 150^\circ/s^2$ (Goldberg and Fernandez 1971). Fitzpatrick and Day (2004) combined this information with human perception experiments (Fitzpatrick et al. 2002) to estimate the afferent spike rate during constant current electrical vestibular stimulation and suggested a 1-mA current was equivalent to 4 spikes per second.

The vestibular afferent model used a voltage input signal ($V_{ip}$) to represent electrical stimulation, whereas our experiments applied current stimuli (in milliampers). Depending on the regularity of the simulated afferents, an input $V_{ip}$ signal as low as 10 mV (irregular) and as high as 120 mV (regular) can generate afferent spike rates at the upper limit of the dynamic range (300 spikes per second; see Smith and Goldberg 1986; Fig. 4B). Averaged across the entire population of 100 afferents in our model, a voltage input of 0.55 mV will generate a modulation in afferent firing rate of 4 spikes per second, the estimated equivalent of 1 mA. Therefore, all input stimuli were scaled by 0.55 to capture the effects of externally applied vestibular stimulation.

Several studies have shown that the dynamic behavior of canal afferents due to electrical stimulation is composed of a static phase advance at all frequencies and a modest increase in gain with increasing frequencies (Ezure et al. 1983; Goldberg et al. 1982; Kim et al. 2011). Since these dynamics have only been described up to 20 Hz, and considering that our stimuli extended up to 75 Hz, they were not included in the model.

Motoneuron Pool

The motoneuron pool model was based on a discrete time integrate and fire model (MacGregor and Oliver 1974) and included sodium and potassium conductances. Arrival of descending and vestibular afferent input increases the synaptic conductances by an amount (0.03 and 0.01, respectively) representing the amplitude of the postsynaptic potential (Bashor 1998). Spike generation was modeled as a discrete event (i.e., having no shape) and occurred when the membrane potential exceeded the threshold. The threshold of each neuron was variable with first-order dynamics and was dependent on the membrane potential, thereby capturing cell accommodation. The model was parameterized according to previous efforts (Bashor 1998; Schuurmans et al. 2009).

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DISCLOSURES

G. P. Siegmund owns shares in a consulting company, and both he and the company may derive benefit from being associated with this work.

AUTHOR CONTRIBUTIONS

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