Research Article

Frequency Tuning of the Cervical Vestibular-Evoked Myogenic Potential (cVEMP) Recorded from Multiple Sites along the Sternocleidomastoid Muscle in Normal Human Subjects

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ABSTRACT

Frequency tuning of tone burst-evoked myogenic potentials recorded from the sternocleidomastoid muscle (cervical VEMP or cVEMP) is used clinically to assess vestibular function. Understanding the characteristics of cVEMP is important for improving the specificity of cVEMP testing in diagnosing vestibular deficits. In the present study, we analyzed the frequency tuning properties of the cVEMPs by constructing detailed tuning curves and examining their morphology and dependence on SCM tonic level, sound intensity, and recording site along the SCM. Here we report two main findings. First, by employing nine tone frequencies between 125 and 4,000 Hz, some tuning curves exhibited two distinct peaks, which cannot be modeled by a single mass spring system as previously suggested. Instead, the observed tuning is better modeled as linear summation of two mass spring systems, with resonance frequencies at ~300 and ~1,000 Hz. Peak frequency of cVEMP tuning curves was not affected by SCM tonic level, sound intensity, and location of recording site on the SCM. However, sharpness of cVEMP tuning was increased at lower sound intensities. Second, polarity of cVEMP responses recorded from the lower quarter of the SCM was reversed as compared to that at the two upper sites. While more studies are needed, these

results suggest that cVEMP tuning is mediated through multiple generators with different resonance frequencies. Future studies are needed to explore implications of these results on development of selective VEMP tests and determine the nature of polarity inversion at the lower quarter of SCM.

Keywords: sound activation of vestibular system, canal, otolith, VCR

INTRODUCTION

Sound-evoked vestibular responses have been well documented in humans and animal models (Bickford et al. 1964; Carey et al. 2004; Curthoys et al. 2006; McCue and Guinan 1994; Murofushi et al. 1995; Tullio 1929; Young et al. 1977; Zhou et al. 2004, 2005, 2007; Xu et al. 2009; Zhu et al. 2011, 2012). Because sound stimulation offers simplicity, selectivity, and the capacity to stimulate each labyrinth separately, its potential value in the diagnosis of vestibular disorders has been widely recognized (Colebatch 2001, 2010; Curthoys 2010; Halmagyi et al. 2005; Minor et al. 2001; Rauch 2006; Rosengren et al. 2010; Streubel et al. 2001; Welgampola and Carey 2011; Welgampola and Colebatch 2001, 2005; Zhou and Cox 2004). Among several sound-evoked vestibular responses, the short latency myogenic potential recorded from tonically contracted sternocleidomastoid muscles (SCM) (Colebatch et al. 1994), which is

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known as the cVEMP, has been studied extensively and adopted by vestibular clinics worldwide. Because previous studies suggested that sound primarily activates the vestibular afferents that innervated the saccule, the cVEMP is presently used to assess the saccule function (Murofushi and Curthoys 1997; Kushiro et al. 1999; Colebatch and Rothwell 2004). However, the saccule hypothesis of sound activation of the vestibular system has been challenged by several studies (Carey et al. 2004; Curthoys and Vulovic 2011; Todd et al. 2009; Young et al. 1977; Zhou et al. 2004, 2005, 2007; Xu et al. 2009; Zhu et al. 2011). In particular, Zhu et al. (2011) recently quantitatively examined sound-evoked responses in vestibular afferents that innervated the five vestibular end organs. Their results demonstrated significant sound activation of all five end organs, although with different sensitivities.

The primary goal of the present study was to further test the saccular hypothesis of cVEMP by examining whether sources other than the saccule contribute to generating cVEMP responses. Our approach was to examine the frequency tuning of cVEMP in normal human subjects. The rationale is that frequency tuning of cVEMP reflects the physical resonance of the contributing end organ. McCue and Guinan (1994; in cats) and Zhu et al. (2012; in rats) showed that sound stimulation of the saccular afferents has a unimodal frequency response. Thus, if there in cVEMP tuning curves exhibit multiple peaks, it would indicate that the saccule is not the sole source for cVEMP and other vestibular end organs are involved. While cVEMP frequency tuning has been studied previously, the present study is different in the following three ways. First, instead of using four or six frequencies to generate cVEMP tuning curves, nine frequencies ranging from 125 to 4,000 Hz were employed in the present study. The improved frequency tuning curves revealed that cVEMP is better modeled as linear summation of two mass spring damper systems rather than a single mass spring system (Todd et al. 2000). Second, rather than recording cVEMP tuning from a single location at the upper third of the SCM, we recorded cVEMP responses at three sites along the SCM: the top quarter, the midpoint, and the lower quarter. This experiment was motivated by the possibility that similar to monkey, horse, and rabbit, which all have long SCM, the human SCM may be compartmentalized and have multiple innervation zones. We found that the cVEMP recorded at the lower quarter exhibited reversed polarity as compared to that from the two upper sites. Indeed, we found that the cVEMP at the lower quarter of the SCM exhibited opposite polarity to the cVEMPs at the other two sites. While more studies are needed to rule out alternative

interpretations, these results provide new insight into developing discriminative VEMP tests and interpretative guidelines. Finally, since cVEMP response is also affected by muscle tonic contraction level, sound intensity, and recording site along the SCM (Lim et al. 1995; Li et al. 1999; Sheykholeslami et al. 2001), in the present study, we not only used nine frequencies to construct accurate tuning curves, but also examined whether these factors affect tuning curve morphology.

METHODS

Nine subjects (four female, five male, from 25 to 35 years old) with informed consent participated in this study. They were free from otologic disease and were not taking any medications. The experimental protocol was approved by the Institutional Review Board at the University of Mississippi Medical Center.

During the test, subjects were instructed to sit upright and turn their heads to the right. Tonic SCM contraction was maintained by requiring subjects to push a weighted arm placed against left forehead. Two different levels of tonic contraction were produced by changing weight on the arm. Acoustic tone bursts (8 ms plateau, 1 ms rise/fall) were generated by a CED Power 1401 (Cambridge Electronics Devices, Cambridge, UK). Tone bursts at nine frequencies (125, 250, 350, 500, 750, 1,000, 1,500, 2,000, and 4,000 Hz) were presented at 124 and 118 dB SPL and tone bursts at eight frequencies (250, 350, 500, 750, 1,000, 1,500, 2,000, and 4,000 Hz) were presented at 112 dB SPL. One subject was not tested by tones at 124 dB SPL. Another subject showed no response to sound and his data were not included in our analysis. Tone bursts were randomly delivered to the left ear at a rate of 5 Hz via an insert earphone. An important feature of this stimulation delivery approach is that at each recording site, the pre-stimulus SCM tonic levels were comparable among the nine different tones (within ~5 %). Thus, the SCM tonic level did not contribute to the tuning curves. Tuning curves were measured at two SCM tonic level conditions. For example, at the low tonic level condition, the averaged rectified pre-stimulus EMG across the nine frequencies was 92 ± 3 , 101 ± 3 , and 92 ± 3 mV at the top, middle, and lower quarter of SCM, respectively (subject RY). At the high tonic level condition, the averaged rectified pre-stimulus EMG was 153 ± 3 , 146 ± 3 , and 119 ± 3 mV at the top, middle, and lower quarter of SCM, respectively, for the same subject.

It should be noted that a tuning curve was measured using the same sound intensity in the external ear canal for each tone. There was no correction for transmission of sound through the middle ear for several reasons. First, although near

threshold normal hearing for low frequencies is strongly attenuated by the middle ear, at high intensities like those used in the present study, loudness contours are relatively flat below 2,000 Hz (Fletcher and Munson 1933; ISO 226:2003). Second, the middle ear transfer function expressed as displacement transmission ratio at the stapes indicates that amplitude of stapes displacement remains flat for frequencies <1,000 Hz (Gan et al. 2004). Thus, it is unclear how to make corrections for sound-induced vestibular activations. Third, for most previous VEMP tuning studies, VEMP responses were referred to pressure changes in the external canal. While we acknowledge this as an important issue to be addressed in the future, we took the current approach to be consistent with the literature. However, caution should be taken to interpret the resonance frequency data.

Surface electrodes were placed at three locations along the SCM: the top quarter, the midpoint, and the lower quarter. All active electrodes were referred to the sternum. Electrode impedance was maintained below 5 k Ω . EMG signals were amplified (2,500 gain), band path-filtered (5 to 1,000 Hz), and sampled at 10 kHz (CED Power 1401, Cambridge Electronics Devices). Data were stored on a hard disk for offline analysis.

For each frequency, cVEMP was averaged over 100 repetitions with Spike 2 (Cambridge Electronics Devices) and displayed in SigmaPlot (Systat Software Inc, CA, USA). It should be noticed that positive potentials were shown as upward deflections in this study. Amplitudes of the first (peak I) and second peak (peak II) were measured with respect to the baseline before stimulus onset. SEM is used to describe the dispersion of data.

Two models were used to fit cVEMP response at different frequencies. Equation 1 (French 1971) models frequency tuning of the cVEMP as one mass spring damper system. It was based on the hypothesis that cVEMP originated solely from the saccule. $V(\omega)$ represents the response amplitude of cVEMP (milli-volt), and G is the gain of mass spring damper system. Ω is the frequency of sound stimulus, and ω_0 is the resonant frequency of saccule where sound stimulus evokes the largest response. Q is the quality factor which describes the sharpness of tuning curves.

$$V(\omega) = G \frac{\omega}{\sqrt{(\omega_0^2 - \omega^2)^2 + \frac{\omega^2 \omega_0^2}{Q^2}}} \quad (1)$$

$$V(\omega) = G_1 \frac{\omega}{\sqrt{(\omega_1^2 - \omega^2)^2 + \frac{\omega^2 \omega_1^2}{Q_1^2}}} + G_2 \frac{\omega}{\sqrt{(\omega_2^2 - \omega^2)^2 + \frac{\omega^2 \omega_2^2}{Q_2^2}}} \quad (2)$$

Equation 2 assumes that vestibular end organs with different resonant frequencies might be involved in

the generation of cVEMP. In Eq. 2, ω_1 , ω_2 and Q_1 , Q_2 are the resonant frequency and quality factor for the low- and high-frequency components of cVEMP, respectively. G_1 and G_2 is gain of the two components, respectively. R^2 was computed for each fitting and a significant component is determined if it increases R^2 by >5 %. To determine the relative contributions of the low- and high-frequency components to the cVEMP, we computed a frequency selective index (FSI) at each tone frequency, which was defined as the ratio of the difference between the contributions of the high-frequency component ($A_{hi,f}$) and the low-frequency component ($A_{lo,f}$) and the sum of the two components, i.e., $FSI_f = (A_{lo,f} - A_{hi,f}) / (A_{hi,f} + A_{lo,f})$. $A_{lo,f}$ and $A_{hi,f}$ are computed using the first and second items of Eq. 2, respectively. A FSI_f of 1 indicates that the cVEMP at tone frequency f is contributed only by the low-frequency component. A FSI_f of -1 indicates that the cVEMP at tone frequency f is contributed only by the high-frequency component. A FSI_f of 0 indicates that the two components contribute equally to the cVEMP at the tone with frequency f .

cVEMP tests were also conducted in the same group of subjects with an alternate montage. In these tests, electrodes at the top, middle, and lower quarter of SCM were referenced to an electrode on the right hand. Each subject was tested by one frequency which was found to evoke the largest cVEMP response at 130 dB SPL, respectively.

RESULTS

Figure 1 shows tone-evoked cVEMP responses (subject RY) recorded at the three sites along the SCM. While cVEMP responses at the three sites exhibited similar frequency tuning, polarity of cVEMP responses at the lower quarter site was reversed as compared to that at the two upper sites. To construct tuning curves, amplitudes of cVEMP peak I (Fig. 2A) and peak II (Fig. 2C) were plotted as a function of tone frequency. Since both tuning curves exhibited two distinct peaks, they were not adequately modeled by a single mass spring system (Eq. 1; Fig. 2A, C). However, regression lines based on Eq. 2, which models cVEMP tuning as linear summation of two resonance systems, provided better fits with increased R^2 by 8 and 11 percentage points for peak I and peak II, respectively (Fig. 2B, D). The R^2 values increased by 9.5 and 13.6 % for peak I and peak II tuning curves, respectively. For this subject, the peak I tuning curve was modeled as linear summation of a low-frequency system of 251 Hz and a high-frequency system of 763 Hz ($R^2=0.92$), while the peak II tuning curve can be modeled as linear summation of a low-frequency system of 350 Hz and a high-frequency system of 1,035 Hz ($R^2=0.92$).

FIG. 1. A–C Representative cVEMP responses (subject RY) evoked by three frequencies (125, 1,000, and 2,000 Hz) recorded at the three sites along the SCM: the lower quarter (red), the midpoint (black), and the top quarter (blue). Recordings were made at the high muscle tonic level with 124 dB SPL. Time 0 is tone onset. Upward reflection is positive and downward reflection is negative.

Figures 3 (peak I) and 4 (peak II) show the tuning curves of subject RY at the three sites, three intensities at the high SCM tonic level condition. Polarity of cVEMP responses at the lower quarter of the SCM was reversed as compared to that at the other two sites in all conditions. In each tuning curve, three lines were shown to indicate the contributions of the low-frequency component (blue), the high-frequency component (purple), and the sum of the two (red). Relative contribution of the two components was assessed by computing a FSI, which is plotted as a function of frequency (the panel below each tuning curve). To illustrate individual tuning curve

variability, Figure 5 shows peak I tuning curves of all the subjects at the midpoint of SCM with a sound intensity of 118 dB SPL in the high tonic level condition. Figure 6 summarizes FSI tuning curves at the three sites and at the three intensities. On average, cVEMP responses evoked by tones below 500 Hz were primarily due to activation of the low-frequency system ($FSI > 0.5$). The cVEMP responses evoked by tones above 1,000 Hz were primarily due to activation of the high-frequency component ($FSI < -0.5$).

The parameters used for fitting each subject were summarized in Table 1 for 124 dB SPL. In seven out

FIG. 2. Fitting of representative tuning curves by Eqs. 2 (B and D) and 1 (A and C) (subject RY, the top quarter site, higher tonic level, and with an intensity of 124 dB SPL).

FIG. 3. Fitting tuning curves of peak I (subject RY) as linear summation of two mass spring systems at 124 (A), 118 (B), and 112 dB (C) for each of the three recording sites. For each tuning curve, blue and purple lines are contributions of the low- and

high-frequency components, respectively, and the red line is their sum. Below each tuning curve, frequency selectivity index (FSI) is plotted as a function of frequency.

of eight subjects, their tuning curves exhibit two significant tuning peaks at all the recording sites. For the group, resonant frequency of the low-frequency component was 392 ± 42 , 386 ± 35 , and 333 ± 43 Hz for cVEMPs recorded at the top, midpoint, and the lower quarter of SCM, respectively. The resonant frequency of the high-frequency component was $1,240 \pm 253$, $1,114 \pm 161$, and $1,278 \pm 148$ Hz for cVEMP recorded at the top, midpoint, and lower quarter of SCM, respectively. There were no significant differences among the low and high resonance frequencies among the three sites ($P > 0.1$).

The effects of recording site, SCM tonic level, and tone intensity are summarized in Figures 7 and 8. First, ANOVA analysis shows that SCM tonic level has no effect on f_1 , f_2 , Q_1 , and Q_2 ($P > 0.05$, data not shown). Thus, data obtained from the two tonic levels were combined in later analysis. As shown in Figure 7 (peak I), f_1 and f_2 were neither affected by recording site (f_1 : $P = 0.185$; f_2 : $P = 0.985$) nor by stimulus intensity (f_1 : $P = 0.066$; f_2 : $P = 0.813$). For the tuning curves of cVEMP peak I recorded at the top quarter, the midpoint, and the lower quarter of SCM, f_1 was 418 ± 18 , 416 ± 18 , and 373 ± 20 Hz, respectively, and f_2 was

$1,205 \pm 73$, $1,188 \pm 71$, and $1,192 \pm 80$ Hz, respectively. Similarly, for the tuning curves of cVEMP peak I recorded at 124, 118, and 112 dB, f_1 was 371 ± 19 , 403 ± 19 , and 433 ± 19 Hz, respectively, and f_2 was $1,188 \pm 72$, $1,232 \pm 73$, and $1,165 \pm 79$ Hz, respectively. Neither Q_1 nor Q_2 was affected by recording site (Q_1 : $P = 0.258$; Q_2 : $P = 0.704$). Stimulus intensity has a significant effect on Q_1 ($P < 0.001$), but not on Q_2 ($P = 0.087$). Q_1 at 124, 118, and 112 dB was 1.4 ± 0.1 , 1.7 ± 0.1 , and 2.3 ± 0.1 , respectively. There is no interaction between stimulus intensity and recording site for f_1 , f_2 , Q_1 , and Q_2 ($P > 0.474$).

Similar results were obtained for peak II (Fig. 8). Recording site has no effect on peak frequency (f_1 : $P = 0.967$; f_2 : $P = 0.948$) and quality factor (Q_1 : $P = 0.106$; Q_2 : $P = 0.844$). Stimulus intensity has no significant effect on f_1 or f_2 (f_1 : $P = 0.252$; f_2 : $P = 0.102$), but a decrease in intensity significantly increases quality factor (Q_1 : $P = 0.001$; Q_2 : $P = 0.003$). At 124, 118, and 112 dB, Q_1 was 1.3 ± 0.1 , 1.4 ± 0.1 , and 1.8 ± 0.1 , respectively, and Q_2 was 1.6 ± 0.1 , 1.8 ± 0.1 , and 2.1 ± 0.1 , respectively. There is no interaction between stimulus intensity and recording site for f_1 , f_2 , Q_1 , and Q_2 ($P > 0.254$).

FIG. 4. Fitting tuning curves of peak II (subject RY) as linear summation of two mass spring systems at 124 (A), 118 (B), and 112 dB (C) for the three recording sites. For each tuning curve, blue and purple lines are contributions of the low- and high-

frequency components, respectively, and the red line is their sum. Below each tuning curve, frequency selectivity index (FSI) is plotted as a function of frequency.

The effects of recording site and intensity on latency and duration are summarized in Figure 9. Recording site had a significant effect on response latency and duration ($P < 0.001$), but tone intensity had no effect on response latency ($P = 0.09$) and duration ($P = 0.677$). There is no interaction between stimulus intensity and recording site for latency ($P = 0.07$) and duration ($P = 0.06$). Latency of peak I from the lower quarter recording site was shorter than peak I recorded from the midpoint (14.7 ± 0.2 vs. 17.3 ± 0.1 ms, $P < 0.001$) and the top quarter (14.7 ± 0.2 vs. 16.3 ± 0.2 ms, $P < 0.001$). Latency of peak I from the midpoint recording site was shorter than that of the top quarter site ($P < 0.001$). Peak II latency from the lower quarter site was shorter than peak II from the midpoint site (23.8 ± 0.2 vs. 28.9 ± 0.2 ms, $P < 0.001$) and the top quarter site (23.8 ± 0.2 vs. 28.4 ± 0.2 ms, $P < 0.001$), but latency of peak II from the top quarter site was not significantly different from peak II recorded at the midpoint ($P = 0.114$). Duration of peak I–peak II recorded at the lower quarter was reduced as compared to the response duration at the midpoint (9.1 ± 0.2 vs. 11.6 ± 0.2 ms, $P < 0.001$) and the top quarter (9.1 ± 0.2 vs. 12.1 ± 0.2 ms, $P < 0.001$). The response

duration at the midpoint recording site was also shorter than that of the top quarter site ($P = 0.014$).

Adoption of wrist reference has no effect on the polarity of cVEMP at the top, middle, and lower quarter of SCM. Compared to the positive–negative waveform of cVEMP recorded from the upper two sites of SCM, the polarity of cVEMP found at the lower quarter site remained to be reversed in all the subjects tested.

DISCUSSION

In this study, we extended cVEMP frequency tuning analysis by constructing more detailed tuning curves, examining their morphology and characterizing their dependence on sound sensitivity, recording site, and SCM tonic level in normal human subjects. There are two main findings in this study. First, we found evidence that cVEMP tuning is better modeled as linear summation of two mass spring systems. Second, cVEMP responses recorded at the lower quarter exhibited reversed polarity as compared to that recorded at the two upper sites.

FIG. 5. Tuning curves of peak I obtained from the midpoint of SCM at 118 dB at the high tonic level condition for each subject. Blue, purple, and red lines are regression lines fitted by Eq. 2 for the low-frequency component, high-frequency component, and their linear sum, respectively.

FIG. 6. Averaged frequency selectivity curves obtained from the three recording sites for three intensities. Error bars refer to SEM.

TABLE 1

Summary of tuning curve fitting parameters at 124 dB at the high tonic level condition

Subject	Top quarter				Midpoint				Lower quarter			
	f_1	Q_1	f_2	Q_2	f_1	Q_1	f_2	Q_2	f_1	Q_1	f_2	Q_2
AH	311	2.0	818	0.7	342	1.5	944	0.8	310	1.0	1,619	2.0
JG	481	0.7	N/A	N/A	475	0.8	N/A	N/A	495	0.9	2,034	2.5
KT	470	0.3	1,392	2.0	296	0.4	1,514	1.7	261	0.6	1,218	2.1
KA	271	1.5	653	1.5	253	1.5	699	3.0	234	1.5	976	2.0
MT	573	2.0	1,484	0.4	508	3.0	898	0.7	465	0.6	1,270	2.0
RO	465	1.4	2,694	0.8	477	1.4	1,968	1.7	327	3.0	N/A	N/A
RY	251	3.0	763	1.0	431	1.0	928	3.0	426	1.0	944	3.0
WI	316	1.1	878	2.0	308	0.8	845	1.6	150	1.0	884	0.9
Mean	392	1.5	1,240	1.2	386	1.3	1,114	1.8	333	1.2	1,278	2.1
SEM	42	0.3	253	0.2	35	0.3	161	0.3	43	0.3	148	0.2

For each subject, f_1 and f_2 stand for the resonant frequency of the low- and high-frequency components, respectively; Q_1 and Q_2 stand for the quality factor of the low- and high-frequency components, respectively. Top quarter, midpoint, and lower quarter refer to the three sites we record cVEMP over the SCM

N/A not applicable

Since the properties of cVEMP tuning are related to the physical properties of the vestibular end organs that are activated by sound, cVEMP tuning analysis has been employed to characterize the putative cVEMP peripheral generators. Motivated by the hypothesis that sound primarily activates the saccule (Curthoys et al. 2006; Murofushi et al. 1995; Murofushi and Curthoys 1997), Todd et al. (2000) modeled cVEMP

tuning as a single mass spring system and reported a resonant frequency of ~300 Hz. However, later studies showed that cVEMP tuning curves were characterized by a dominant peak between 400 and 800 Hz (Akin et al. 2003; Janky and Shepard 2009; Murofushi et al. 1999; Rauch et al. 2004; Timmer et al. 2006; Todd et al. 2009; Welgampola and Colebatch 2001). In the present study, cVEMP tuning curves were constructed with more tone

FIG. 7. A–D Effects of stimulus intensity and recording site on frequency tuning of peak I. Error bars refer to SEM. Statistical significance ($P < 0.05$) is indicated by asterisk. For details, see text.

FIG. 8. A–D Effects of stimulus intensity and recording site on frequency tuning of peak II. Error bars refer to SEM. Statistical significance ($P < 0.05$) is indicated by asterisk. For details, see text.

frequencies between 125 and 4,000 Hz. Since four out of nine detailed tuning curves showed two distinct peaks, they were better modeled as linear summation of two mass spring systems. Our results show that cVEMPs are generated by activation of two components: one resonating at ~ 300 Hz and the other at $\sim 1,000$ Hz. The low-frequency component accounts for more than 75 % ($FSI > 0.5$) of the overall response for tones below 500 Hz, whereas the high-frequency component makes up more than 75 % ($FSI < -0.5$) of the overall response

for tones above 1,000 Hz. Similar double tuning peaks were in a recent study (Zhang et al. 2012), which found a tuning peak around 100 Hz in addition to a dominant peak around 600 Hz. The 100-Hz peak was considered to be of utricle origin and the 600-Hz peak of saccular origin. Compared to the study of Zhang et al., our tuning peaks are located at higher frequencies. This difference might be due to the different frequency ranges chosen by the two studies (50–1,200 vs. 125–4,000 Hz). Nevertheless, these new results are consistent

FIG. 9. Effects of recording site on latency of peak I (A) and peak II (B) and the duration (C) between the two peaks. Error bars refer to SEM. Statistical significance ($P < 0.05$) is indicated by asterisk.

with accumulating evidence from animal model studies indicating that the saccule is not the sole generator of sound-evoked vestibular responses (Carey et al. 2004; Curthoys and Vulovic 2011; Wit et al. 1984; Young et al. 1977; Zhou et al. 2004, 2005, 2007; Xu et al. 2009). In particular, a recent study by Zhu et al. (2011) showed that 73 % irregular utricle afferents as well as 43 % irregular canal afferents were sensitive to clicks. This multiple generator hypothesis of sound-evoked vestibular responses is also consistent with our previous studies on frequency tuning of bone- and air-conducted VEMP recorded from extraocular muscles (oVEMP), which showed that the tuning peak of air-conducted oVEMP (~1,000 Hz) was much higher than the tuning peak of bone-conducted oVEMP (~400 Hz) (Donnellan et al. 2010; Lewis et al. 2010). We hypothesize that utricle and canals also contribute to cVEMP responses. However, it is premature to associate the low-frequency component and the high-frequency component in our study to a specific vestibular end organ. Additional studies in humans to further explore this hypothesis may provide important insight for improving clinical VEMP tests.

In addition to characterize tuning properties of the cVEMP, we found that the cVEMP responses recorded at the lower quarter site exhibited a negative–positive waveform, rather than the typical positive–negative waveform observed in the two upper sites. There are two interpretations for the polarity reversal in cVEMPs recorded at the lower quarter of the SCM. One interpretation is that the polarity reversal is resulted from volume conduction of motor unit action potentials. Indeed, it has been shown that volume conduction can lead to polarity inversion near the muscle–tendon junction while the polarity of waveforms simultaneously recorded from the middle and top portions of muscle remains unchanged (Roeleveld et al. 1997; Dumitru and King 1991). The other interpretation is that the SCM is compartmentalized, and there exist multiple innervation zones (McLoon 1998). Previous studies showed that the endplates in horse SCM and rabbit SCM were organized into multiple bands 1–3 cm apart throughout the length of muscle (Zenker et al. 1990; McLoon 1998). Given the length of human SCM muscle, it is possible that human SCM is also compartmentalized. However, it has not been shown in published reports. Furthermore, as initial positivity of cVEMP responses reflects sound-induced inhibitory modulation of ipsilateral SCM motoneurons, initial negativity of cVEMP responses reflects sound-induced excitatory modulation of ipsilateral SCM motoneurons (Colebatch and Rothwell 2010). Although Uchino et al. found in cats that some IPSPs evoked by electrical stimulation of saccule nerve were preceded by an EPSP (Kushiro et al. 1999), it remains to be determined whether human SCM receives similar excitatory input from the ipsilateral vestibular pathways.

Therefore, it is an open question on how the polarity reversal is generated, and future studies are needed to solve the nature of polarity reversal observed in the present study. It is also worth noting that the possibility of having differential nerve innervation zones along the SCM is not directly related to the hypothesis of multiple generators of cVEMP because a single end organ (e.g., the saccule) may contribute to all parts of the SCM but with different connectivity patterns.

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