Tuning of the ocular vestibular evoked myogenic potential to bone-conducted sound stimulation

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Zhang AS, Govender S, Colebatch JG. Tuning of the ocular vestibular evoked myogenic potential to bone-conducted sound stimulation. J Appl Physiol 112: 1279–1290, 2012. First published February 2, 2012; doi:10.1152/japplphysiol.01024.2011.—Ocular vestibular evoked myogenic potentials (oVEMPs) are a recently described clinical measure of the vestibulo-ocular reflex. Studies demonstrating differences in frequency tuning between air-conducted and bone-conducted (BC) oVEMPs suggest a separate vestibular (otolith) origin for each stimulus modality. In this study, 10 healthy subjects were stimulated with BC stimuli using a hand-held minishaker. Frequencies were tested in the range of 50–1,000 Hz using both a constant-force and constant-acceleration method. Subjects were stimulated at the mastoid process and the forehead. For constant-force stimulation at both sites, maximum acceleration occurred around 100 Hz, in differing axes. Both forms of stimulation had low-frequency peaks of oVEMP amplitudes (constant force: mastoid, 80–150 Hz; forehead, 50–125 Hz; constant acceleration: mastoid, 100–200 Hz; forehead, 80–150 Hz), for both sites of application, despite differences in the magnitude and direction of evoked head acceleration. For mastoid stimulation, ocular responses changed from out of phase to in phase for 400 Hz and above. Our results demonstrate that BC stimuli show tuning around 100 Hz, independent of stimulus site, that is not due to skull properties. The findings are consistent with an effect on a receptor with a resonance around 100 Hz, most likely the utricle. oVEMP amplitudes (constant force: mastoid, 80–150 Hz; forehead, 50–125 Hz; constant acceleration: mastoid, 100–200 Hz; forehead, 80–150 Hz), for both sites of application, despite differences in the magnitude and direction of evoked head acceleration. For mastoid stimulation, ocular responses changed from out of phase to in phase for 400 Hz and above. Our results demonstrate that BC stimuli show tuning around 100 Hz, independent of stimulus site, that is not due to skull properties. The findings are consistent with an effect on a receptor with a resonance around 100 Hz, most likely the utricle.

Both air-conducted (AC) and bone-conducted (BC) sounds can activate vestibular receptors in normal human subjects. Evidence of this excitation can be recorded as myogenic potentials, either through the vestibulo-collic reflex [cervical vestibular evoked myogenic potentials or cVEMPs (6)] or, more recently, through the vestibulo-ocular reflex [ocular vestibular evoked myogenic potentials or oVEMPs (14, 25, 32)]. The vestibulo-ocular reflex response to BC stimulation, in particular, has a very low threshold (33). Both AC and BC-evoked oVEMPs have been used clinically in the detection of vestibular disease (5, 11, 14, 27).

In animals, 500-Hz BC stimulation has been shown to activate both otolith (utricle and saccule) organs (7). In humans, BC sound elicits larger oVEMP and cVEMP responses at lower frequencies, in the range of 100–250 Hz (4, 34). This is in contrast to the tuning seen for AC stimulation, which has been shown to have a broad peak around 500 Hz for both the oVEMP (4, 34) and cVEMP (1, 17, 22, 23, 31, 37) responses.

The mechanism by which AC and BC stimuli activate the otolith receptors is not definitively known. Von Békésy (36) proposed that activation of saccular receptors by AC sound occurred as a consequence of eddy currents set up in the endolymph by movement of the stapes. Tuning might then be expected to be strongly influenced by the properties of middle ear transmission, but it is still evident even when middle ear effects are allowed for (34, 39). For BC stimuli, the effects of skull resonances must be allowed for, if the properties of the vestibular receptors themselves are to be determined (30). Todd et al. (34) observed similar tuning patterns for both the oVEMP and the cVEMP when using a given AC or BC stimulus modality. The authors reasoned that the effect must be common to both reflexes, and this led them to propose that the two patterns of resonance reflected the mechanical properties of the two otolith organs. Approximation of the observed patterns of resonance was possible using models of otolith structure, with the higher resonance arising from the saccule, and the lower from the utricle. This principle, if confirmed, would be an important new insight into otolith function, as well as a potential means of selective activation.

This study was designed to provide a detailed assessment of vestibular tuning in response to BC stimuli, as assessed using the oVEMP reflex. Constant-force stimuli were used, corresponding to the usual means of eliciting oVEMPs in clinical applications. Tuning was also assessed using constant-acceleration stimuli to remove skull transmission effects. We compared BC tuning at two sites of stimulation: the mastoid and at the forehead (Fz), to ensure that any findings were not peculiar to a single location.

MATERIALS AND METHODS

Subjects. The sample population consisted of 10 healthy subjects (6 men, 4 women; range: 19–34 yr) with no history of vestibular, hearing, or neurological impairment. All subjects were recruited from the staff and students at the Prince of Wales Hospital and gave informed consent, according to the Declaration of Helsinki before the start of the experiment. The study was approved by the local ethics committee (Human Research Ethics Committee, Northern Network, South Eastern Sydney and Illawarra Area Health Service, NSW, Australia). Subjects included in this study were also tested using AC stimulation, as previously reported (39).

BC stimuli. BC stimuli were generated using custom software and a CED laboratory interface (1401plus, Cambridge Electronic Design, Cambridge, UK). Subjects were presented with sinusoidal tone bursts for 100–200 repetitions at a rate of ~5 Hz. BC sound was delivered using a hand-held minishaker (model 4810, Bruel and Kjaer, Denmark) with an attached perspex rod. The frequencies tested were 50, 80, 100, 125, 150, 200, 400, 500, 600, 800, and 1,000 Hz. A 10-ms stimulus duration (rise, hold, fall: 2, 6, and 2 ms, respectively) was used for all frequencies ≥100 Hz. Longer duration stimuli were used for 80 Hz (2, 6, and 4.5 ms) and 50 Hz (2, 6, and 12 ms) to ensure the delivery of a complete cycle of sound. All 10 subjects were stimulated over either the left (n = 6) or right (n = 4) mastoid process with the minishaker held normal to the skin surface. In five subjects, the...
minishaker was also placed on the frontal bone at Fz (approximately the midpoint of the head at the hairline). The minishaker was held by the experimenter for the duration of the recording with ~1–2 kg of force (31). In all instances, the initial polarity of the stimulus was in the “positive” direction (i.e., the first movement of the perspex rod was away from the motor of the minishaker). For the first set of experiments, a constant peak force level of 134 dB re 1 μN was maintained across all frequencies. The required drive levels were determined using an artificial mastoid (Bruel and Kjaer model 4930, including a type 8000 impedance head). In the second set of experiments, BC stimulus intensities were adjusted across all frequencies to achieve an initial peak head acceleration of ~0.1 g. The maximum input to the motor was limited to 60 V peak to peak to minimize waveform distortion and subject discomfort.

**Accelerometry.** Triaxial accelerometers (model 2228C-Y, Endevco) were placed over the temporal bones immediately superior to the ear in the line of the external auditory meatus and secured using tight elastic bandages. For both mastoid and forehead stimulation, there was no significant difference between the magnitudes of acceleration in the naso-occiput (x), vertical (z), and interaural (y) axes for the two sides of the head, so these were averaged. (The direction of interaural acceleration was the same for both sides for mastoid stimulation, but opposite for Fz stimulation). For the second set of experiments, adjustments for mastoid stimulation were based on the largest peak lateral head acceleration for the first 5 ms following stimulus onset. BC stimulation, especially at Fz, produced accelerations of the skull in all three axes, so stimulus intensity was adjusted according to the peak triaxial root mean squared (RMS) acceleration. Initial pilot studies revealed a longer latency of the initial head acceleration following Fz stimulation, and so the largest peak RMS acceleration was based on the first 7.5 ms following stimulus onset. Fourier analysis of the acceleration was performed over the initial 10 ms following the stimulus onset (15 ms for 50 Hz), using the dominant direction of acceleration. For mastoid stimulation, we analyzed the interaural axis for all frequencies, and, for Fz stimulation, we used the naso-occipital axis to 400 Hz and the interaural above this (Fig. 1). Given the sampling rate and the duration, this led to bin widths of ~80 Hz.

**oVEMPs.** Surface electrodes (Cleartrace 1700–030, Conmed) were placed inferior to both eyes. The active electrode was located over the inferior orbital margin, and a reference electrode was placed immediately below it. An earth electrode was placed on the sternum. Subjects were positioned upright, and gaze direction was 30° above horizontal for the duration of the recording. The recorded EMG was amplified using D150 amplifiers (Digitimer, Welwyn Garden City, UK), band-pass filtered (5–1,000 Hz), and sampled using a second CED1401 laboratory interface at 10 kHz from 10 ms before to 60 ms after stimulus onset. Data collection was performed using SIGNAL (version 2.15, Cambridge Electronic Design, Cambridge, UK) software. Response size was assessed using three amplitude parameters: the initial negative peak (n1), the initial negative-positive peaks (n1-p1), and the largest consecutive peak-to-peak (P-P) amplitude over the entire oVEMP response. Measurements were taken from both eyes following stimulation at either the mastoid or forehead.

**Data analysis.** Statistical analysis was performed using SPSS (version 18.0.0, SPSS, Chicago, IL). Analyses of oVEMP amplitudes were performed using both raw and normalized (N) values. Raw amplitudes were used to compare responses between stimulus methods (constant force vs. constant acceleration) and stimulus sites (mastoid vs. forehead). Absent responses were assigned a 0–μV value. Testing the small responses obtained at higher frequencies showed that there was no significant difference from zero whether using this method \( t_{(f_{max})} = 1.0–2.0, P > 0.073 \) or amplitudes taken at the mean latency \( t_{(f_{max})} = 1.2–2.3, P > 0.062 \). For each subject, oVEMP amplitudes were normalized by expressing responses at each frequency \( f \) as a ratio of the largest measured amplitude \( (f_{max}) \) before averaging \( [i.e., N(f) = \text{amplitude}(f)/\text{amplitude}(f_{max})] \). Two-way within-subjects ANOVA (eye and frequency as factors) were used for analysis of tuning effects using mastoid stimulation. Tuning effects following forehead stimulation were assessed using one-way within-subjects ANOVA (frequency as a factor). The initial n1 and p1 peaks were considered for analysis of latency values, and, due to absent responses at higher frequencies, a between-subject ANOVA was used. Post hoc paired and unpaired t-tests were used, where appropriate, to compare amplitudes and latencies. One-sample t-tests were used to compare acceleration values \( (\pm 600 \text{ Hz}) \) to zero. A Bonferroni-corrected critical \( P \) value was used to control family-wise error. For the dominant axes of head acceleration, linear regressions were performed for each subject using the applied and measured skull frequencies for both mastoid and forehead stimulation. Correlations were performed between the magnitudes of head accelerations in each

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![Image](http://jap.physiology.org)
axis and n1-p1 oVEMP tuning following constant-force stimuli delivered at both sites of stimulation. Values are reported in the text as means ± SD and are displayed in Figs. 1–8 as means ± SE.

RESULTS

Accelerometry: constant force. For mastoid stimulation, the magnitude of head acceleration was greatest overall in the y-axis [main effect of axis: $F_{(2,18)} = 64.8, P < 0.001$]. Below 600 Hz, acceleration was predominantly in the y-axis, while stimulation at frequencies >600 Hz produced similar magnitudes of acceleration in all three axes (Fig. 1A). Head acceleration was largest at 125 Hz for the interaural y-axis (0.15 ± 0.03 g), at 150 Hz for the naso-occipital x-axis (0.07 ± 0.03 g), and at 200 Hz for the vertical z-axis (0.04 ± 0.01 g). The magnitude of head acceleration at 800 Hz for the x-axis was not significantly different from zero [$t_{(8)} = 2.2, P = 0.059$]. All other head accelerations ≥600 Hz for both sites of stimulation were greater than zero [$t_{(4–9)} = 2.3–27.8, P < 0.048$]. The magnitude of head acceleration decreased significantly with increasing frequency [main effect of frequency: $F_{(10,90)} = 23.8, P < 0.001$]. For the dominant y-axis, applied frequencies were highly correlated with skull acceleration measured using the Fourier transform ($r^2$ range: 0.901–0.995). For mastoid stimulation, acceleration in the y-axis was equal in magnitude and occurred simultaneously, indicating translational acceleration of the head (Fig. 2A).

For forehead stimulation, head acceleration was greatest overall for the x-axis [main effect of axis: $F_{(2,8)} = 65.4, P < 0.001$]. Skull acceleration occurred predominantly in this axis for frequencies <400 Hz, while, >400 Hz, acceleration was predominantly in the y-axis (Fig. 1B). Acceleration was largest at 80 Hz for the x-axis (0.10 ± 0.01 g), at 1,000 Hz for the y-axis (0.05 ± 0.01 g), and at 100 Hz for the z-axis (0.04 ± 0.01 g). The magnitude of head acceleration decreased signif-
Mastoid stimulation: constant force. Maximum oVEMP amplitudes were observed at low frequencies from 80 to 150 Hz for all measurements (Fig. 4). Overall, the largest mean amplitude occurred at 80 Hz in the contralateral eye (n1-p1: 13.1 ± 7.9 μV; n1: 5.5 ± 3.1 μV; largest P-P: 24.7 ± 12.8 μV). For the ipsilateral eye, mean amplitudes were largest at 100 Hz (largest P-P: 16.4 ± 4.4 μV) and 150 Hz (n1-p1: 14.0 ± 5.2 μV; n1: 5.8 ± 3.3 μV). For both eyes, amplitudes at these frequencies for all three measurements were significantly larger than amplitudes at 50 Hz [t(9) = 2.4–6.5, P < 0.038].

For n1-p1, n1, and largest P-P amplitudes, increasing frequency decreased oVEMP amplitudes [main effect of frequency: F(10,90) = 45.7–101.3, P < 0.001]. While both eyes showed similar patterns of frequency tuning for n1-p1 and n1 measurements, minor differences were observed [interaction between eye and frequency: F(10,90) = 3.1–7.8, P < 0.005]. For n1-p1 peaks, amplitudes at 200 Hz were significantly larger for the ipsilateral eye than the contralateral eye [t(9) = 5.7, P < 0.001; Fig. 4A]. In contrast, amplitudes at 400 Hz were generally larger for the contralateral eye [t(9) = 3.3, P = 0.009, trend due to Bonferroni correction]. There was no significant difference in amplitude between the eyes from 80 to 150 Hz [t(9) = 1.5–2.1, P > 0.05 for all cases]. There was a significant positive correlation between oVEMP n1-p1 tuning for the contralateral eye and the magnitude of acceleration for all three axes (x-axis: r = 0.91, P < 0.001; y-axis: r = 0.91, P < 0.001; z-axis: r = 0.70, P = 0.024). Similar findings applied to the oVEMP n1-p1 tuning for the ipsilateral eye (x-axis: r = 0.86, P < 0.001; y-axis: r = 0.90, P < 0.001; z-axis: r = 0.72, P = 0.012).

Both the n1 and p1 responses usually began earlier for the contralateral eye [main effect of eye: F(1,1173) = 117.7 and F(1,p1:176) = 213.6, P < 0.001], and this effect was more marked at lower frequencies [interaction between eye and frequency: F(10,1173) = 9.0 and F(10,p1:176) = 15.5, P < 0.001: Fig. 5A]. At 200 Hz and below, both peaks occurred significantly earlier for the contralateral eye than the ipsilateral eye [t(9) = 5.4–25.9, P < 0.001]. There was no significant difference in latencies between the eyes at 400 Hz [t(9) = 0.8 and 1.6, P > 0.05] and for frequencies ≥500 Hz [t(6–15) = 0.08–1.9, P > 0.05 for all cases]. The largest mean difference in n1 and p1 latencies between the eyes was at 50 Hz (n1 contralateral: 10.4 ± 0.5 ms; n1 ipsilateral: 15.0 ± 2.6 ms; p1 contralateral: 16.0 ± 0.7 ms; p1 ipsilateral: 21.8 ± 1.5 ms).

Mastoid stimulation: constant acceleration. Tuning effects were still observed in all subjects following constant-acceleration stimuli delivered at the mastoid (Fig. 6). For the contralateral eye, the largest mean amplitudes occurred at 100 Hz (largest P-P: 18.7 ± 10.4 μV) or 150 Hz (n1-p1: 8.3 ± 4.2 μV; n1: 3.9 ± 1.9 μV). Mean amplitudes for the ipsilateral eye were largest at 100 Hz (largest P-P: 13.2 ± 3.5 μV), 150 Hz (n1-p1: 12.3 ± 5.4 μV), or 200 Hz (n1: 5.1 ± 2.2 μV). For the ipsilateral eye, n1-p1 and n1 amplitudes at their respective peak frequencies were significantly larger than at 50 Hz [t(9) = 3.3–3.8, P < 0.004].

Increasing frequency decreased the amplitudes for n1-p1, n1, and largest P-P measurements [main effect of frequency: F(10,90) = 16.7–57.2, P < 0.001]. Despite similar tuning curves for both eyes, the n1-p1 and n1 measurements showed some differences [interaction between eye and frequency: F(10,90) =
5.1–10.1, \( P < 0.001 \). For n1-p1 measurements, amplitudes were larger for the ipsilateral eye at 150 Hz \( t(9) = 2.4, P = 0.04 \), trend due to Bonferroni correction] and 200 Hz \( t(9) = 3.8, P = 0.004 \). In contrast, n1-p1 amplitudes were larger for the contralateral eye at 400 and 1,000 Hz \( t(9) = 3.1 \) and 3.4, \( P = 0.007 \) and 0.01, trends due to Bonferroni correction].

Similar to the constant-force method, n1 and p1 latencies generally began earlier for the contralateral eye [main effect of eye: \( F_{1,10} = 62.2 \) and \( F_{1,10} = 147.7, P < 0.001 \) for both cases], and this effect was more pronounced in the low-frequency range [interaction between eye and frequency, \( F_{10,10} = 6.3 \) and \( F_{10,10} = 10.3, P < 0.001 \)]. Latencies for the n1 and p1 peaks were significantly earlier for the contralateral eye at and below 200 Hz \( t(9) = 3.5–13.6, P < 0.008 \) for all cases], whereas there was no significant difference in latencies between the eyes at 400 Hz and above \( t(9) = 0.2–1.9, P > 0.05 \) for all cases: Fig. 5A].

**Mastoid stimulation: constant force vs. constant acceleration.** For mastoid stimulation, n1-p1 raw amplitudes from the contralateral and ipsilateral eyes were larger at 80 Hz [contralateral: \( t(9) = 3.6, P = 0.006 \); ipsilateral: \( t(9) = 3.0, P = 0.014 \), trends due to Bonferroni correction]. 100 Hz [contralateral: \( t(9) = 3.8, P = 0.001 \).
Fig. 5. Mean latencies for the initial n1 and p1 peaks following mastoid (A) and forehead (B) stimulation using constant-force and constant-acceleration methods. Both n1 and p1 peaks from the two eyes are out of phase at low frequencies and have a shorter latency contralaterally. At 400 Hz and above, there is no latency difference between the two sides for either peak. For forehead stimulation, both n1 and p1 peaks occurred earlier at the low-frequency range around 200 Hz. Mean latencies increased significantly from 200 to 400 Hz, with a second minimum around 200 Hz. While mean latencies varied substantially for different frequencies, for a given frequency, SEs of the means were low, indicating that the range of latencies was narrow. Values are means ± SE.

For all three measurements, increasing frequency decreased oVEMP amplitudes [main effect of frequency: $F_{(10,40)} = 65.3–101.9$, $P < 0.001$]. Post hoc analysis showed that, at 100 Hz, n1-p1 amplitudes were significantly larger than at 50 Hz and all frequencies >150 Hz [$t_{(4)} = 6.6–50.3$, $P < 0.005$ in all cases]. There was no significant difference in average amplitudes between 80 and 125 Hz [$t_{(4)} = 1.0–2.0$, $P > 0.05$ for all cases]. Normalized n1-p1 oVEMP amplitudes were positively correlated with the magnitude of acceleration in the x-axis ($r = 0.99$, $P < 0.001$) and z-axis ($r = 0.97$, $P < 0.001$), but not the y-axis ($r = −0.33$, $P = 0.324$).

In contrast to mastoid stimulation, n1 and p1 latencies were not significantly different between the eyes [main effect of eye: $F_{(1,41)=5.8} = 0.1$ and $F_{(1,41)=72} = 0.2$, $P > 0.05$ for both cases]. The mean n1 and p1 latencies, averaged over both eyes, occurred earliest at 200 Hz (n1: 9.9 ± 0.3 ms; p1: 14.1 ± 2.7 ms), but showed a second minimum at 600 Hz (Fig. 5B). For the n1 latency, responses at 200 Hz appeared earlier compared with either 400 Hz (11.9 ± 0.2 ms) or 500 Hz [11.1 ± 0.5 ms; $t_{6–7} = 4.4$ and 11.2, $P < 0.01$ for both cases]. The mean p1 latency occurred earlier at 200 Hz compared with 400 Hz [18.3 ± 1.9 ms; $t_{4} = 3.5$, $P = 0.006$].

Forehead stimulation: constant acceleration. BC oVEMP tuning at the forehead using constant RMS head acceleration
(Fig. 8) produced similar peak tuning to that of constant-force stimuli. The largest mean amplitudes were at 80 Hz (largest P-P: 18.9 ± 5.3 μV), 100 Hz (n1-p1: 15.3 ± 5.4 μV), or 150 Hz (n1: 6.4 ± 2.1 μV). Overall, increasing frequency decreased the n1-p1, n1, and largest P-P amplitudes [main effect of frequency: \( F_{(10,40)} = 22.6 – 62.5, P < 0.001 \)]. The n1-p1 amplitudes at 100 Hz were significantly larger than amplitudes at all frequencies [\( t_{(4)} = 6.8 – 31.6, P < 0.005 \) in all cases]. There was no significant difference between amplitudes from 50 to 150 Hz [\( t_{(4)} = 0.5 – 2.5, P > 0.05 \) in all cases].

There was no significant difference in the latency of the n1 and p1 responses between the eyes [main effect of eye: \( F_{(1,n=72)} = 0.01 \) and \( F_{(1,p=78)} = 0.02, P > 0.05 \) for both cases]. The mean n1 response, averaged between the eyes, occurred earliest at 500 Hz (9.7 ± 0.4 ms), significantly earlier than at 400 Hz [11.8 ± 0.3 ms; \( t_{(4)} = 20.1, P < 0.001 \): Fig. 5B]. There was, however, no significant difference in the latency of n1 between 500 Hz and either 200 Hz (9.9 ± 0.3 ms) or 600 Hz [10.0 ± 0.8 ms; \( t_{(4)} = 0.9 \) and 1.5, \( P > 0.05 \) in both cases]. For p1 latencies, the earliest peak occurred at 200 Hz (12.8 ± 0.6 ms). Latencies at 200 Hz
appeared significantly earlier than at 400 Hz (18.3 ± 1.9 ms), 500 Hz (15.8 ± 0.9 ms), and 600 Hz (15.1 ± 1.4 ms; \( t_{(4)} = 4.3–8.2, P < 0.002 \) in all cases).

**Forehead stimulation: constant force vs. constant acceleration.** Constant-acceleration stimuli produced larger n1-p1 raw amplitudes following forehead stimulation at 400 Hz \( t_{(4)} = 6.0, P = 0.004 \), 500 Hz \( t_{(4)} = 5.7, P = 0.005 \) (trend due to Bonferroni correction), and 600 Hz \( t_{(4)} = 4.0, P = 0.016 \) (trend due to Bonferroni correction). There was no significant difference in n1-p1 amplitudes between stimulus methods from 50 to 200 Hz and ≥800 Hz \( t_{(4)} = 0.2–2.0; P > 0.05 \) for all cases). There was no significant difference in the frequencies that elicited the largest raw n1-p1 amplitudes when comparing constant-force and constant-acceleration stimulus methods \( t_{(4)} = 2.4, P = 0.08 \).

**Constant force: mastoid vs. forehead stimulation.** Overall, raw n1-p1 amplitudes were similar for both eyes following mastoid stimulation and forehead stimulation across the majority of frequencies tested. Comparison of n1-p1 raw amplitudes showed they were slightly larger at 600 Hz for the contralateral eye using mastoid stimulation compared with forehead stimulation \( t_{(4)} = 3.0, P = 0.042 \) (trend due to Bonferroni correction). In the five subjects who had both sites of stimulation using the constant-force
method, there was no significant difference in the frequencies that elicited the largest raw n1-p1 amplitude using forehead stimulation and either eye with mastoid stimulation [contralateral: $t(4) = 1.6, P = 0.178$; ipsilateral: $t(4) = 1.9, P = 0.129$].

**Constant acceleration: mastoid vs. forehead stimulation.** At 400 and 500 Hz, raw n1-p1 amplitudes were generally larger following stimulation at the forehead compared with responses from the ipsilateral eye following mastoid stimulation [$t(4) = 3.3$ and $4.3, P = 0.012$ and 0.03, trends due to Bonferroni correction]. For forehead stimulation, raw n1-p1 amplitudes were also larger at 125 Hz [$t(4) = 6.8, P = 0.002$] and 150 Hz [$t(4) = 4.3, P = 0.012$, trend due to Bonferroni correction] compared with the contralateral eye following mastoid stimulation. At 800 Hz, n1-p1 raw amplitudes tended to be larger for the contralateral eye following mastoid stimulation than at the forehead [$t(4) = 3.0, P = 0.039$, trend due to Bonferroni correction]. In the five subjects who had both sites of stimulation using the constant-acceleration method, frequencies that
elicited the largest raw n1-p1 amplitudes showed no significant difference between forehead stimulation and either eye using mastoid stimulation [contralateral: t_(adj) = 0.7, P = 0.546; ipsilateral: t_(adj) = 0.3, P = 0.749].

DISCUSSION

BC oVEMP tuning was examined using both constant-force stimuli and stimuli adjusted to induce constant head acceleration. Constant-force output is a simpler measure of stimulus intensity, but head acceleration is the stimulus detected by the otolith organs. The present study found a peak around 100 Hz for BC oVEMP amplitudes using both types of stimulation, confirming that the observed tuning effects were not solely due to attenuation of head acceleration with increasing frequency. While more observations are required to define the response below 50 Hz, our observations confirm the findings of previous studies, which have reported a low-frequency peak in BC oVEMP tuning (4, 34). Donnellan et al. (9) reported largest BC oVEMP amplitudes at 400-Hz stimulation, although this finding is likely be a consequence of the use of a Radioear B-71 for stimulus generation as its output falls rapidly below 300 Hz (22). BC stimulation at both the mastoid process and Fz have both been used in clinical settings (11, 15). This study has shown that both the mastoid and Fz are effective sites for eliciting oVEMPs, and, for constant-force stimuli, BC oVEMPs demonstrate broadly similar tuning maxima for stimulation at both sites, with largest responses from 50 to 150 Hz, declining steeply above 200 Hz. Much oVEMP testing has used 500 Hz for both AC and BC stimulation, but the tuning profile for BC oVEMPs indicates that low frequencies are more effective stimuli. At both the mastoid process and at Fz, some subjects had small or absent n1-p1 responses for 500-Hz BC stimulation, whereas responses at 100 Hz were present in all subjects and were larger by a factor of 3 or more.

Head acceleration. Overall levels of head acceleration decreased with increasing frequency, despite keeping peak force levels constant. At 500 Hz and below, mastoid stimulation evoked predominantly interaural acceleration, consistent with previous reports (2, 26). For forehead stimulation at 200 Hz and below, the predominant acceleration was in the naso-occipital axis. At 600 Hz and above, the acceleration became less directional, but predominantly in the interaural direction. These findings are similar to those of Cai et al. (2), who reported that the predominant direction of acceleration for both sites declined with frequency but was still evident at 500 Hz. Unlike Cai et al. (2), we found that skull frequency closely followed the applied frequency, a difference probably due to the somewhat longer period over which we assessed frequency. The “bowing” of the skull in the interaural axis at higher frequencies of Fz stimulation has been previously reported by Iwasaki et al. (15) using 500-Hz tone bursts and contrasts with the direction of interaural acceleration produced by mastoid stimulation for this and lower frequencies. Given these contrasting directions of induced acceleration, particularly for low frequencies, it is notable that the overall pattern of BC oVEMP tuning for both sites of stimulation was so similar.

In the present study, only the acceleration occurring in the first 5–7 ms was considered relevant for generation of the short-latency n1-p1 response once conduction time through the vestibulo-ocular reflex pathway was allowed for. No studies of oVEMP signal conduction times through neural pathways have been performed to date. Estimates of conduction from inner ear to brain stem based on latencies of wave V of the brain stem auditory evoked potential give an approximate conduction time of 5.7 ms (3). Conduction along motoneurons in the oculomotor nerve, ~44 mm long (13), combined with transmission across the neuromuscular junction, gives a conduction time from brain stem to extraocular muscles of ~1 ms. The n1 and p1 peaks have maxima occurring at ~10 and 15 ms, respectively, leaving an effective initial stimulus period of 5–7 ms for tuning effects to take place. Intervals of 5 ms for mastoid stimulation and 7.5 ms for Fz stimulation were thus concentrated upon when analyzing acceleration levels, with the longer period used for Fz stimulation due to the longer latency of the first peak of acceleration for this site.

Origins of the tuning of vestibular-evoked potentials. The cVEMP is also known to demonstrate frequency tuning to both AC and BC stimuli, and previous studies reported broadly similar frequency tuning to that observed for the oVEMP. BC cVEMPs show peak sensitivity at lower frequencies than for AC stimuli (34). BC stimulation has been found in guinea pigs to be very effective in activating irregularly firing otolith afferents, although only one frequency (500 Hz) was reported (7). Initial studies in humans reported that, for BC cVEMPs, the largest amplitudes occurred in the range 200–400 Hz (29, 38), but these were influenced by the mechanical properties of the bone vibrator used (24). Our study has shown that constant-force stimuli evoke the largest accelerations around 100 Hz. A recent study by Jombik et al. (18) has confirmed the effectiveness of such frequencies. Vibration at around 100 Hz can unmask vestibular deficits (12, 19) and may operate, at least in part, through the resonance peak we have shown.

Todd et al. (33) reported tuning of the oVEMP response to near 100 Hz, even when acceleration was kept constant. A subsequent study by Todd et al. (34) reported the largest responses were at 100 Hz for both oVEMP and cVEMP reflexes produced by BC stimulation. The oVEMP and cVEMP responses arise from separate neural pathways, forming part of the vestibulo-ocular and vestibulo-collic reflexes, respectively, and Todd et al. (34), therefore, proposed that tuning of vestibular-evoked potentials arose primarily from resonance of the two otolith organs. Resonance of the otolith end organs as a consequence of their structures has long been recognized (8, 10). A study modeling otolith function calculated sacular otoconial resonance at 650 Hz (16). Using a biomechanical model, Todd et al. (34) estimated the resonances of the utricle and sacculle at 162 and 986 Hz, respectively, with the lower resonant frequency for the utricle being largely explained by its looser attachment to the underlying bone and thus reduced stiffness (35). The estimated resonances of Todd et al. for the otolith organs were similar to the peak resonances demonstrated for their BC and AC oVEMP tuning, respectively. We have confirmed that low-frequency tuning is present for oVEMPs produced by both mastoid and forehead stimulation, even when initial acceleration is controlled. Given that the n1 response with the infraorbital montage mainly arises from the contralateral vestibular apparatus (15) and the pattern of latency differences, the end organ responsible appears to be activated synchronously by frontal stimulation, but shows differential responses for lateral acceleration. The earlier n1 contralaterally found here and by Cai et al. (2) implies that the
ipsilateral vestibular end organ is excited by the initial acceleration directed toward the opposite side. The utricle responds to accelerations in the horizontal plane, but its medial part is larger than the lateral (28), making it more responsive to contralaterally directed accelerations. The saccule lies in the sagittal plane and shows no such anatomical asymmetry in the horizontal plane (20). A plausible interpretation of these and previous results, therefore, is that the BC oVEMP elicited by stimulation frequencies <200 Hz is mainly dependent on utricular activation. Stimulation at 400 Hz and above showed no such latency asymmetry, consistent with an effect dependent on the saccule, but directionality was lost for these higher frequencies.

Zheng et al. (39) have recently reinvestigated tuning of the AC oVEMP using a wider range of frequencies than previous studies. Like previous studies, they reported a dominant tuning peak around 500–600 Hz. However, unlike other studies, they also found a smaller peak at 100 Hz, findings that they suggest supported proposed differing resonances of the saccule and the utricle. The selective activation of the saccule by AC sound would then be a consequence, of both its proximity to the stapes (36), as well as its resonance (34). Responses to BC would not be expected to depend on the relationship to the stapes, given that the whole of the vestibular apparatus is likely to be accelerated identically. Like Jombik et al. (18), we believe that the main mechanism of vestibular activation by BC stimulation is a consequence of inertia of the otolith organs, which also explains its selectivity for afferents arising from these end organs. Surprisingly perhaps, no clear increase in oVEMP response amplitudes was obvious around 500 Hz, corresponding to saccular excitation, when constant-acceleration stimuli were used. However, for both the n1 and n1-p1 responses for mastoid and, more so, for forehead stimulation, there is the appearance of a broad secondary response around 400 Hz for constant-acceleration stimuli. The smaller response around this frequency, even when acceleration is kept constant, may reflect the relatively weaker sacculo-ocular projections compared with utricular projections (20). The second minimum for n1 and p1 latencies around 600 Hz could also be explained by saccular excitation.

Our study has shown that, whether applied to the mastoid bone or at the forehead, the most effective frequency for eliciting oVEMPs is close to 100 Hz. This frequency minimizes the intensity of vibration required and, for this reason alone, should be considered for clinical applications. For constant-force stimulation, the largest responses produced from both sites were of similar sizes, with out-of-phase responses with mastoid stimulation and in-phase responses with Fz stimulation. At higher frequencies, the responses became smaller and occurred synchronously following mastoid stimulation, consistent with a different origin from those evoked by lower frequencies. If our tentative conclusions are confirmed, there is the prospect of separate assessment of the two otolith organs, based on their frequency sensitivity.

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AUTHOR CONTRIBUTIONS


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