Click-evoked responses in vestibular afferents in rats

Hong Zhu,1 Xuehui Tang,1 Wei Wei,1 William Mustain,1 Youguo Xu,1 and Wu Zhou1,2,3

Departments of 1Otolaryngology and Communicative Sciences, 2Neurology, and 3Anatomy, University of Mississippi Medical Center, Jackson, Mississippi

Submitted 3 January 2011; accepted in final form 19 May 2011

Zhu H, Tang X, Wei W, Mustain W, Xu Y, Zhou W. Click-evoked responses in vestibular afferents in rats. J Neurophysiol 106: 754–763, 2011. First published May 25, 2011; doi:10.1152/jn.00003.2011.—Sound activates not only the cochlea but also the vestibular end organs. Research on this phenomenon led to the discovery of the sound-evoked vestibular myogenic potentials recorded from the sternocleidomastoid muscles (cervical VEMP, or cVEMP). Since the cVEMP offers simplicity and the ability to stimulate each labyrinth separately, its values as a test of human vestibular function are widely recognized. Currently, the cVEMP is interpreted as a test of saccule function based on the assumption that clicks primarily activate the saccule. However, sound activation of vestibular end organs other than the saccule has been reported. To provide the neural basis for interpreting clinical VEMP testing, we employed the broadband clicks used in clinical VEMP testing to examine the sound-evoked responses in a large sample of vestibular afferents in Sprague-Dawley rats. Recordings were made from 924 vestibular afferents from 106 rats: 255 from the anterior canal (AC), 202 from the horizontal canal (HC), 177 from the posterior canal (PC), 207 from the superior vestibular nerve otolith (SO), and 83 from the inferior nerve otolith (IO). Sound sensitivity of each afferent was quantified by computing the cumulative probability of evoking a spike (PC), 207 from the superior vestibular nerve otolith (SO), and 83 from the inferior nerve otolith (IO). Sound sensitivity of each afferent was quantified by computing the cumulative probability of evoking a spike (PC). We found that clicks activated irregular afferents (normalized coefficient of variation of interspike intervals >0.2) from both the otoliths (81%) and the canals (43%). The order of end organ sound sensitivity was SO = IO > AC > HC > PC. Since the sternocleidomastoid motoneurons receive inputs from both the otoliths and the canals, these results provide evidence of a possible contribution from both of them to the click-evoked cVEMP.

VEMP testing is presently interpreted as a test of otolith function. In particular, the air-conducted click-evoked cVEMP is believed to be a test of saccule function (for recent review, Colebatch 2010; Curthoys 2010; Rosengren et al. 2010). This theory is based on the animal studies of the Curthoys group (Murofushi et al. 1995; Murofushi and Curthoys 1997; for review, see Curthoys 2010; Curthoys and Vulovic 2011), who recorded vestibular afferent responses to clinical VEMP stimuli. They reported that air- or bone-conducted clicks and short tone bursts only activate the saccule (Curthoys et al. 2006; Murofushi et al. 1995; Murofushi and Curthoys 1997). However, there is accumulating evidence for sound activation of the other vestibular end organs (Carey et al. 2004; Curthoys and Vulovic 2011; Wit et al. 1984; Xu et al. 2009; Young et al. 1977; Zhou et al. 2004, 2005, 2007). Since the cVEMP is routinely performed in vestibular clinics worldwide, it is important to determine the extent to which sound activates different vestibular end organs. The present study was intended to address the issue by quantitatively examining vestibular afferent responses to air-conducted brief clicks used in clinical cVEMP testing. A large number of vestibular afferents were recorded from identified vestibular end organs. Cumulative probability of evoking a spike (CPE) (Broussard et al. 1995) was computed for each afferent to quantitatively assess relative

THE VESTIBULAR SYSTEM responds naturally to head acceleration. Because the vestibular end organs are connected to the auditory end organs by continuous fluid pathways within the membranous labyrinth, the vestibular system also responds to sound stimulation. In 1929, Tullio first showed that sound directly stimulated the cristae of the fenestrated semicircular canals in pigeons. Young et al. (1977) further demonstrated that long-duration tones activated all five vestibular end organs in squirrel monkeys with intact labyrinths. Over the past decades, vestibular afferent responses to different sound stimuli have been studied in other animal models with intact labyrinths [McCue and Guinan (1994, 1995, 1997) in cats; Murofushi et al. (1995), Murofushi and Curthoys (1997), Curthoys et al. (2006), and Curthoys and Vulovic (2011) in guinea pigs; and Carey et al. (2004) in chinchillas]. These neurophysiological studies provide a neural basis for sound activation of the vestibular system.

Address for reprint requests and other correspondence: H. Zhu, Dept. of Otolaryngology and Communicative Sciences, Univ. of Mississippi Medical Center, 2500 North State St., Jackson MS 39216 (e-mail: hozhu@umc.edu).
sound sensitivity of different vestibular end organs. Our results show that clicks activated a large number of irregular afferents from both the otoliths (81%) and the canals (43%). Since the SCM motoneurons receive inputs from both the canals and the otoliths (for reviews, see Uchino et al. 2005; Wilson and Schor 1999), these results call into question which vestibular organs are responsible for the eVEMP responses.

METHODS

Surgical procedures and single-unit recording of vestibular afferents. Adult male Sprague-Dawley rats weighing 250–350 g (Harlan Sprague-Dawley, Indianapolis, IN) were used in this study. All procedures were approved by the Institutional Animal Care and Use Committee at the University of Mississippi Medical Center. All surgical procedures were performed aseptically. The first surgery was to implant a head holder on the skull for stabilization of a rat’s head during recording experiments. Each rat was anesthetized with pentobarbital sodium (50 mg/kg ip) and the head was held in a stereotaxic instrument (David Kopf Instruments, Tujunga, CA) by a pair of ear bars with a 45–deg tip to prevent rupture of the tympanic membrane. A midline dorsal cranial skin incision was made, soft tissues were cleared, and the head was leveled between the skull suture landmarks, bregma and lambda. The head holder was a small stainless steel cylinder that was rigidly attached to the skull and secured in place with three stainless steel machine screws trepanned through the skull and adhered with dental acrylic. Each rat was housed individually after the surgery. In our experience, the use of ear bar during head holder surgery can cause edema of ear canals, which could influence the delivery of air-conducted sound through the earphone. Therefore, vestibular nerve recording was performed 7 days after the surgery to allow a full recovery.

After recovery from the head holder surgery, the rat was anesthetized with pentobarbital sodium (50 mg/kg ip) and the head was stabilized on a stereotaxic frame with the implanted head holder by a Kopf stereotaxic carriage. The animal’s core body temperature was monitored and maintained at 36–37°C with a heating pad (Frederick Haer, Bowdoinham, ME). The left side occipital bone was opened and the cerebellum exposed. With the assistance of a surgical microscope, the cerebellar hemisphere including the flocculus and the paraflocculus was removed carefully by aspiration to allow access to the eighth nerve by a microelectrode (Lasker et al. 2008; Murofushi et al. 1995; Murofushi and Curthoys 1997; Yang and Hullar 2007).

A microelectrode (Sutter Instruments, Novato, CA) filled with 3 M sodium chloride (10–20 MΩ) was mounted on a microdrive and positioned over the superior or inferior vestibular nerve under visual control with the assistance of the surgical microscope. Signals were amplified and filtered by a MNAP system (Plexon, Dallas, TX).

Vestibular afferent characterization. Each animal’s body was restrained in a nylon jacket and stabilized in the stereotaxic instrument. The jacket was secured on the stereotaxic plate to prevent the animal’s body from moving. The stereotaxic instrument was mounted on a custom-made rotation device that allowed us to deliver head rotations in pitch, roll, and yaw planes. In the neutral position, the head was tilted 30 deg nose down and 15 deg left ear down (Blankens and Torigoe 1989; Daunicht and Pellionisz 1987).

To obtain an unbiased sample of vestibular afferents, we isolated every encountered nerve fiber that was spontaneously active. To determine the vestibular end organ that an afferent innervated, we first used the pitch and roll combinations to bring each canal into the plane of earth-horizontal rotation and tested whether the afferent’s firing rate was modulated during sinusoidal earth-horizontal rotations (Estes et al. 1975). Second, we recorded the afferent’s responses during sinusoidal pitch rotations (dynamic vertical head tilt) (Goldberg and Fernández 1975; Young et al. 1977). The rotational stimuli were about ~0.5–1 Hz with amplitude of ~10 deg and peak velocity of ~60 deg/s. Single-unit data along with horizontal and vertical head-positio
were then delivered to the ear ipsilateral to the afferent being recorded. The clicks (0.1 ms, 80 dB SL re ABR threshold) of alternating polarity (rarefaction or condensation) were delivered at a rate of 5 Hz. Rarefaction clicks (pull) move the oval window outward. Condensation clicks (push) move the oval window inward. Typically, 150 trials were obtained for each condition (100 ms prestimulus to 100 ms poststimulus).

Data acquisition and data analysis. Extracellular voltage signals were amplified and filtered by the MNAP system. The amplified extracellular voltage trace was sampled by a CED Power 1401 system (Cambridge Electronics Devices, Cambridge, UK) at 20 kHz with 16-bit resolution and a temporal resolution of 0.01 ms. Signals of horizontal and vertical head positions and click stimulation were sampled at 1 kHz. These signals were stored on a hard disk for off-line analyses. Data analysis was performed on personal computer workstations using Spike 2 (Cambridge Electronics Devices), MatLab (The MathWorks, Natick, MA), and SigmaPlot/SigmaStat (Systat Software, San Jose, CA).

Vestibular afferents were classified as regular, intermediate, or irregular afferents based on the CV*τ. An afferent was classed as a regular afferent if its CV*τ was <0.1, an intermediate afferent if its CV*τ was between 0.1 and 0.2, and an irregular afferent if its CV*τ was >0.2 (Goldberg et al. 1984; Young et al. 1977). To quantify an afferent’s response to head rotation, we extracted the fundamental response from the averaged data using FFT analysis. Gain and phase relative to head velocity were calculated at the fundamental stimulus frequency (~0.5–1 Hz). In the present study, the exact canal sensitivity plane was not determined. Instead, the canal sensitivities were corrected trigonometrically according to the formula described by Hullar and Minor (1999).

Vestibular afferent sound sensitivity was quantified by the CPE analysis, which was first developed by the Lisberger group to analyze electrical pulse-evoked responses in the abducens neurons (Broussard et al. 1995). By adopting this approach, we were able to obtain a detailed structure of sound-evoked response and accurately compute its latency and amplitude. To compute the CPE, we first measured the latency between the click and the foot of the first action potential after click onset for 150 clicks. The latencies were then arranged in ascending order and paired with an ascending series of probabilities ranging in equal increments from 1/150 to 1.0. To estimate the time course of the probability of firing after click onset, we plotted the probability of firing as a function of the time after click onset (Fig. 1C, “click”). To take into account the probability that the afferent would have fired in the absence of clicks, we performed the same analysis beginning 30 ms before click onset (Fig. 1C, “no click”). Linear regression was used to fit a line to the no-click data, and the Y-value of the line was subtracted from each click value to yield the probability of evoking a spike as a function of time (Fig. 1D). The latency of the click-evoked response was defined as the onset of the abrupt increase in firing probability (Fig. 1D). The amplitude of the afferent response was measured by the height of the rapid change in firing probability. The duration of response was measured by the duration of the rapid change in firing probability.

An afferent was classified as sound sensitive if its CPE was larger than 0.1, i.e., clicks increase firing probability by 0.1. Our results showed that vestibular afferent sound sensitivity formed a continuum.
ranging from no response to strong response. Based on CPEs, vestibular afferents were classified into four categories of sound-sensitive groups: none (CPE < 0.1), low (CPE 0.1–0.4), intermediate (CPE 0.4–0.7), and strong (CPE > 0.7). Thus the CPE analysis not only allows us to quantitatively assess an afferent’s sound sensitivity but also allows us to assess relative sound sensitivity among vestibular end organs, which is important for understanding the neural basis for VEMP testing.

RESULTS

Single-unit recordings were made from 924 vestibular afferents from 106 rats. Among them, 255 afferents innervated the AC, 202 afferents innervated the HC, 177 afferents innervated the PC, 207 afferents innervated the otoliths (the utricle and 1/3 of the saccule) in the superior vestibular nerve (SO), and 83 afferents innervated the otolith in the inferior vestibular nerve (IO, saccule). In this study, we did not perform the tests to further assign the SO afferents into the utricle and the saccule.

CPE analysis of vestibular afferent sound sensitivity. Figure 1 shows responses of a HC afferent to rarefaction clicks. This afferent increased its firing rate to ipsilateral earth-horizontal rotation (Fig. 1A). The peristimulus histogram exhibits a short-latency click-evoked excitatory response (Fig. 1B). The CPE as a function of time was computed to measure the latency and amplitude of the click-evoked response (Fig. 1, C and D). As indicated by the arrows in Fig. 1D, the afferent responded to rarefaction clicks with a latency of 0.57 ms (solid arrow) and an amplitude of 0.65 (CPE difference between the shaded and solid arrows), i.e., clicks increased the firing probability by 0.65.

Sound sensitivity (CPE) of different vestibular end organs. We studied vestibular afferent sound sensitivity to both rarefaction and condensation clicks. A paired t-test revealed that there was no difference in CPE of the two types of clicks (P = 0.54). Thus only the CPE results for rarefaction clicks are presented in detail in Figs. 1–4. The CPE results of condensation clicks are summarized in the text.

First, we examined the effects of discharge regularity (CV*) and afferent location on vestibular afferent sound sensitivity. Figure 2 shows that CPEs formed a continuum ranging from no response to strong response for each end organ. A two-way ANOVA revealed significant effects of regularity (CV*; P < 0.001) and afferent location (P < 0.001) on CPE, as well as a significant interaction between the two factors (P < 0.001). Regular afferents essentially had no response to sound stimulation (averaged CPE: 0.00 ± 0.01). Post hoc analysis (Student-Newman-Keuls) showed that irregular afferents had larger CPEs than regular afferents (0.35 ± 0.01 vs. 0.00 ± 0.01, P < 0.001) and intermediate afferents (0.35 ± 0.01 vs. 0.03 ± 0.03, P < 0.001). For the irregular afferents, CPEs were significantly different among end organs. The irregular SO and IO afferents had similar CPEs (0.55 ± 0.02 vs. 0.58 ± 0.06, P > 0.05), but they had larger CPEs than the irregular canal afferents (AC: 0.33 ± 0.02, P < 0.001; HC: 0.22 ± 0.02, P < 0.001; PC: 0.06 ± 0.03, P < 0.001). Among the irregular canal afferents, the AC afferents were more sensitive to clicks than the HC (P < 0.001) and PC afferents (P < 0.001). Thus the CPE analysis established an order of sound sensitivity among vestibular end organs, i.e., SO > IO > AC > HC > PC. Although the irregular afferents were more likely to be
Table 1. Click sensitivity of vestibular afferents from different end organs

<table>
<thead>
<tr>
<th>Afferent</th>
<th>Regular</th>
<th>Intermediate</th>
<th>Irregular</th>
</tr>
</thead>
<tbody>
<tr>
<td>SO</td>
<td>1/101 (1%)</td>
<td>2/4 (50%)</td>
<td>85/102 (83.3%)</td>
</tr>
<tr>
<td>IO</td>
<td>0/37 (0%)</td>
<td>0/4 (0%)</td>
<td>31/42 (73.8%)</td>
</tr>
<tr>
<td>AC</td>
<td>0/140 (0%)</td>
<td>0/9 (0%)</td>
<td>62/106 (58.5%)</td>
</tr>
<tr>
<td>HC</td>
<td>0/116 (0%)</td>
<td>0/12 (0%)</td>
<td>35/74 (47.3%)</td>
</tr>
<tr>
<td>PC</td>
<td>0/110 (0%)</td>
<td>0/11 (0%)</td>
<td>4/56 (7.1%)</td>
</tr>
</tbody>
</table>

Data are presented as fractions [the number of afferents that were sound sensitive, i.e., cumulative probability of evoking a spike (CPE) > 0.1]; numbers in parentheses refer to the percentage of afferents that were sound sensitive. Regular, normalized coefficient of variation of interspike intervals (CV*) < 0.1; intermediate, 0.1 ≤ CV* ≤ 0.2; irregular, CV* > 0.2. AC, anterior canal afferents; HC, horizontal canal afferents; PC, posterior canal afferents; SO, otolith afferents in superior vestibular nerve; IO, otolith afferents in inferior vestibular nerve (saccule). See text for statistical analysis.

sound sensitive, we found no significant correlation between CPE and CV*.

Second, we assessed relative sound sensitivity among vestibular end organs by using two more measurements, the percentage of sound-sensitive units (CPE > 0.1) and the percentage of strong sound-sensitive units (CPE > 0.7). Both measurements revealed the same order of sound sensitivity among vestibular end organs. The SO (83.3%) and IO (73.8%) showed a higher proportion of sound-sensitive units than the AC (58.5%), HC (47.3%), or PC (7.1%) (P < 0.003, χ²; Table 1).

Proportions of strong sound sensitive units were significantly different among end organs (P < 0.001, χ²; Fig. 3). The IO (54.8%) and SO (40.2%) had a higher proportion of strong response units than the AC (16.0%, P < 0.004), HC (10.8%, P < 0.004), and PC (5.4%, P < 0.004). There was no significant difference between the IO and SO (P > 0.05) and among the canals (P > 0.05). The response rate of PC (5.4%) was significantly different from that of regular unit (0%, P < 0.005, χ²).

Third, we computed averages of the peristimulus histograms to compare the population responses of different end organs (Fig. 4). Consistent with the above analysis based on CPE, the regular and intermediate afferents showed little or no responses to clicks, whereas the irregular afferents exhibited large responses. During an interval of 1 ms, clicks evoked larger responses in the irregular IO (636.0 spike/s) and SO afferents (588.6 spike/s), but clicks also evoked significant responses in the irregular AC (408.9 spike/s) and HC afferents (286.9 spike/s).

The same results were obtained for condensation clicks. Two-way ANOVA showed that there was no significant effect of polarity on the CPEs of sound-sensitive units (P = 0.92) and no significant interaction between polarities and afferent locations (i.e., end organs, P = 0.94). Similar to rarefaction clicks, the CPEs for condensation clicks were significantly different among vestibular end organs (P < 0.001, post hoc Student-Newman-Keuls). The averaged CPEs of irregular afferents to condensation clicks were 0.56 ± 0.03, 0.55 ± 0.06, 0.34 ± 0.03, 0.23 ± 0.03, and 0.06 ± 0.03 for the SO, IO AC, HC and PC, respectively. The order of vestibular end organs’ sound sensitivity to condensation clicks was SO = IO > AC > HC > PC, which is the same as that of rarefaction clicks.

Duration of click-evoked responses. Duration of click-evoked responses was measured for both rarefaction and condensation clicks. Averaged durations of each end organ are shown in Table 2. Two-way ANOVA analysis revealed significant effects of afferent location (P < 0.001) on duration but no significant effect of polarity (P = 0.1) and no significant interaction between the two factors (P = 0.96). Post hoc Student-Newman-Keuls analysis showed that the durations of the evoked responses of IO afferents were significantly shorter than those of SO (P < 0.001), AC (P = 0.002), and HC afferents (P < 0.001). There was no significant difference between SO and AC afferents, but the durations of the responses of HC afferents were significantly longer than those of AC (P = 0.018) and SO afferents (P = 0.016) (IO < SO < AC < HC). The durations of the responses of the four PC units were not significantly different from those of IO but were significantly shorter than the other afferents.

Latency of click-evoked responses. Latency of click-evoked responses was measured for both rarefaction and condensation clicks. Although click polarity exhibited no effects on the amplitude of click-evoked response, it did exhibit a significant effect on the latency. Furthermore, the polarity effect varied from afferent to afferent. Afferents recorded from both superior and inferior vestibular nerves fell into two categories; some afferents had shorter latency to rarefaction clicks (pull afferents), whereas the others had shorter latency to condensation clicks (push afferents). Both “pull” and “push” afferents were found in the canals and the otoliths. Figure 5, A and B, shows the latency distributions for rarefaction and condensation clicks, respectively, for different vestibular end organs. For the total of 220 sound-sensitive afferents, the percentage of push afferents was significantly higher than that of pull afferents (66.4 vs. 33.6%, P < 0.001, z-test). The distribution of the two types of afferents was not significantly different among the end organs (P = 0.477, χ²). Although the finding of both push and pull otolith units in the same labyrinth is unexpected because the canal afferents in the same labyrinth have the same polarity.

Fig. 3. Distributions of irregular vestibular afferents (CV* > 0.2) that exhibited none (CPE < 0.1), low (CPE 0.1–0.4), intermediate (CPE 0.4–0.7), and strong (CPE > 0.7) responses to rarefaction clicks. SO, otolith units in the superior vestibular nerve; IO, otolith units in the inferior vestibular nerve.
Averaged latencies of the evoked responses of afferents innervating different end organs are shown in Table 2. A two-way ANOVA revealed significant effects of polarity ($P < 0.05$) and afferent location ($P < 0.001$) on latency but no significant interaction between the two factors ($P = 0.847$). Latencies of the responses of condensation clicks were shorter than those of rarefaction clicks ($0.62 \pm 0.02 \text{ ms} \text{ vs.} 0.67 \pm 0.01 \text{ ms}, P < 0.05$). Latencies were different among afferents innervating different end organs (Table 2). The latencies of the responses of SO afferents were significantly longer than those of IO afferents ($P < 0.001$, post hoc Student-Newman-Keuls). The latencies of the evoked responses were not significantly different between AC and HC afferents ($P > 0.05$). Latencies were shorter for the responses of otolith afferents than those for AC and HC afferents ($P < 0.001$, IO < SO < AC = HC). The latencies of the responses of the four sound-sensitive PC units were not different from those of SO and IO afferents but were significantly lower than those of AC and HC afferents. Figure 5C shows the distribution of the pull and push latency differences for afferents innervating different end organs. A two-way ANOVA showed no significant effects of polarity ($P = 0.1$) and afferent location ($P = 0.34$) on latency difference and no significant interaction between the two factors ($P = 0.13$).

A correlation analysis was performed to examine the relationship between latency and CPE for sound-sensitive afferents. For the SO, IO, AC, and HC, CPE was significantly inversely correlated with latency, i.e., the larger the response, the shorter the latency. For rarefaction clicks, the $R$ values were $0.42 (P < 0.001)$, $0.49 (P < 0.001)$, $0.53 (P < 0.001)$, and $0.47 (P < 0.005)$ for the SO, IO, AC, and HC, respectively. For condensation clicks, the $R$ values were $0.52 (P < 0.001)$, $0.34 (P = 0.065)$, $0.58 (P < 0.001)$, and $0.64 (P < 0.001)$ for the SO, IO, AC, and HC, respectively.

**DISCUSSION**

The present study addresses a fundamental issue regarding sound activation of the vestibular system, i.e., the extent to which air-conducted clicks activate different vestibular end organs. This is the first study that has quantitatively analyzed
vestibular afferent responses to the clicks used in clinical VEMP testing. The main findings are that clicks activate irregular afferents significantly more frequently than regular or intermediate afferents. For irregular afferents, clicks excited those from the otoliths (81%) significantly more frequently than those from canals (43%). These results call into question which vestibular organs are responsible for clinical cVEMP responses.

Comparison with previous studies. There are several single-unit studies that examined sound activation of vestibular afferents in animals with intact labyrinths, but different findings were reported on the extent to which sound activates different vestibular end organs. On one hand, Young et al. (1977) and Carey et al. (2004) reported that long-duration tones activated both the canals and the otoliths. Young et al. (1977) reported that phase-locking and tonic increases in the firing rate to long-duration tones were seen in units from all five end organs of monkeys, but lower threshold was observed in saccular afferents. Carey et al. (2004) showed that tonic responses were evoked by long-duration tones in 20% of AC units and in 58% of HC units in chinchillas with intact labyrinth, but phasic responses could be elicited in only 7% of AC units and in none of the HC units. On the other hand, the Curthoys group reported that sound primarily activated the sacculus (Curthoys et al. 2006; Murofushi et al. 1995; Murofushi and Curthoys 1997; for review, see Curthoys 2010) and utricle (Curthoys and Vulovic 2011). Because the studies of the Curthoys group were designed to examine the neural basis for VEMP testing and employed clinical VEMP stimuli (i.e., short air-conducted clicks and brief bone-conducted clicks and tone bursts), their works have been most often cited to support the cVEMP as a test of otolith function. These pioneer studies by the Curthoys group have played an important role in the development of VEMP testing. McCue and Guinan (1994, 1995, 1997) reported that sound primarily activated saccule in cats. Their studies are often cited to support the saccule theory, even though they only recorded from the inferior vestibular nerve.

In the present study, we examined click-evoked responses of vestibular afferents using the same surgical procedures and click stimulation parameters (0.1 ms, 80 dB SL re ABR threshold) as those in studies by Murofushi et al. (1995) and Murofushi and Curthoys (1997). In contrast to their results, we found that air-conducted clicks activate afferents from both otoliths and canals. The discrepancies between our results and theirs could be due to the following reasons. The first reason is the method used to identify the origin of a vestibular afferent. In Curthoys’s studies, an afferent was classified as otolithic if it was responsive to static tilts. In the present study, we used the pitch and roll combinations to bring each canal into the plane of earth-horizontal rotation and tested whether the afferent was modulated during sinusoidal earth-horizontal rotations; and we also tested the afferent’s responses during sinusoidal pitch rotations (dynamic vertical head tilt). Units were considered to be otolithic if they were modulated during dynamic vertical head tilts and not during earth-horizontal rotations (also see METHODS). The second reason is the method used to detect and define sound-sensitive neurons. In Curthoys’s studies, a sound-sensitive afferent was defined by detecting an “audible change” in firing rate that was associated with repeated presentations of sound stimulation (Curthoys et al. 2006; Murofushi et al. 1995; Murofushi and Curthoys 1997). The method may only detect the strong sound-sensitive afferents. As we showed in the present study, vestibular afferent sound sensitivity forms a continuum ranging from no response to strong response, and our quantitative analysis provided a detailed picture of relative sound sensitivity among different vestibular end organs. Young et al. (1977) showed that some vestibular afferents exhibited phase-locking at stimulus levels well below the levels that can cause an audibly detectable change in firing rate. As a population, however, these “non-audible detectable” sound-sensitive afferents in animals with intact labyrinths, but different findings were reported on the extent to which sound activates different vestibular end organs. On one hand, Young et al. (1977) and Carey et al. (2004) reported that long-duration tones activated both the canals and the otoliths. Young et al. (1977) reported that phase-locking and tonic increases in the firing rate to long-duration tones were seen in units from all five end organs of monkeys, but lower threshold was observed in saccular afferents. Carey et al. (2004) showed that tonic responses were evoked by long-duration tones in 20% of AC units and in 58% of HC units in chinchillas with intact labyrinth, but phasic responses could be elicited in only 7% of AC units and in none of the HC units. On the other hand, the Curthoys group reported that sound primarily activated the sacculus (Curthoys et al. 2006; Murofushi et al. 1995; Murofushi and Curthoys 1997; for review, see Curthoys 2010) and utricle (Curthoys and Vulovic 2011). Because the studies of the Curthoys group were designed to examine the neural basis for VEMP testing and employed clinical VEMP stimuli (i.e., short air-conducted clicks and brief bone-conducted clicks and tone bursts), their works have been most often cited to support the cVEMP as a test of otolith function. These pioneer studies by the Curthoys group have played an important role in the development of VEMP testing. McCue and Guinan (1994, 1995, 1997) reported that sound primarily activated saccule in cats. Their studies are often cited to support the saccule theory, even though they only recorded from the inferior vestibular nerve.

In the present study, we examined click-evoked responses of vestibular afferents using the same surgical procedures and click stimulation parameters (0.1 ms, 80 dB SL re ABR threshold) as those in studies by Murofushi et al. (1995) and Murofushi and Curthoys (1997). In contrast to their results, we found that air-conducted clicks activate afferents from both otoliths and canals. The discrepancies between our results and theirs could be due to the following reasons. The first reason is the method used to identify the origin of a vestibular afferent. In Curthoys’s studies, an afferent was classified as otolithic if it was responsive to static tilts. In the present study, we used the pitch and roll combinations to bring each canal into the plane of earth-horizontal rotation and tested whether the afferent was modulated during sinusoidal earth-horizontal rotations; and we also tested the afferent’s responses during sinusoidal pitch rotations (dynamic vertical head tilt). Units were considered to be otolithic if they were modulated during dynamic vertical head tilts and not during earth-horizontal rotations (also see METHODS). The second reason is the method used to detect and define sound-sensitive neurons. In Curthoys’s studies, a sound-sensitive afferent was defined by detecting an “audible change” in firing rate that was associated with repeated presentations of sound stimulation (Curthoys et al. 2006; Murofushi et al. 1995; Murofushi and Curthoys 1997). The method may only detect the strong sound-sensitive afferents. As we showed in the present study, vestibular afferent sound sensitivity forms a continuum ranging from no response to strong response, and our quantitative analysis provided a detailed picture of relative sound sensitivity among different vestibular end organs. Young et al. (1977) showed that some vestibular afferents exhibited phase-locking at stimulus levels well below the levels that can cause an audibly detectable change in firing rate. As a population, however, these “non-audible detectable” sound-sensitive afferents in animals with intact labyrinths, but different findings were reported on the extent to which sound activates different vestibular end organs. On one hand, Young et al. (1977) and Carey et al. (2004) reported that long-duration tones activated both the canals and the otoliths. Young et al. (1977) reported that phase-locking and tonic increases in the firing rate to long-duration tones were seen in units from all five end organs of monkeys, but lower threshold was observed in saccular afferents. Carey et al. (2004) showed that tonic responses were evoked by long-duration tones in 20% of AC units and in 58% of HC units in chinchillas with intact labyrinth, but phasic responses could be elicited in only 7% of AC units and in none of the HC units. On the other hand, the Curthoys group reported that sound primarily activated the sacculus (Curthoys et al. 2006; Murofushi et al. 1995; Murofushi and Curthoys 1997; for review, see Curthoys 2010) and utricle (Curthoys and Vulovic 2011). Because the studies of the Curthoys group were designed to examine the neural basis for VEMP testing and employed clinical VEMP stimuli (i.e., short air-conducted clicks and brief bone-conducted clicks and tone bursts), their works have been most often cited to support the cVEMP as a test of otolith function. These pioneer studies by the Curthoys group have played an important role in the development of VEMP testing. McCue and Guinan (1994, 1995, 1997) reported that sound primarily activated saccule in cats. Their studies are often cited to support the saccule theory, even though they only recorded from the inferior vestibular nerve.

In the present study, we examined click-evoked responses of vestibular afferents using the same surgical procedures and click stimulation parameters (0.1 ms, 80 dB SL re ABR threshold) as those in studies by Murofushi et al. (1995) and Murofushi and Curthoys (1997). In contrast to their results, we found that air-conducted clicks activate afferents from both otoliths and canals. The discrepancies between our results and theirs could be due to the following reasons. The first reason is the method used to identify the origin of a vestibular afferent. In Curthoys’s studies, an afferent was classified as otolithic if it was responsive to static tilts. In the present study, we used the pitch and roll combinations to bring each canal into the plane of earth-horizontal rotation and tested whether the afferent was modulated during sinusoidal earth-horizontal rotations; and we also tested the afferent’s responses during sinusoidal pitch rotations (dynamic vertical head tilt). Units were considered to be otolithic if they were modulated during dynamic vertical head tilts and not during earth-horizontal rotations (also see METHODS). The second reason is the method used to detect and define sound-sensitive neurons. In Curthoys’s studies, a sound-sensitive afferent was defined by detecting an “audible change” in firing rate that was associated with repeated presentations of sound stimulation (Curthoys et al. 2006; Murofushi et al. 1995; Murofushi and Curthoys 1997). The method may only detect the strong sound-sensitive afferents. As we showed in the present study, vestibular afferent sound sensitivity forms a continuum ranging from no response to strong response, and our quantitative analysis provided a detailed picture of relative sound sensitivity among different vestibular end organs. Young et al. (1977) showed that some vestibular afferents exhibited phase-locking at stimulus levels well below the levels that can cause an audibly detectable change in firing rate. As a population, however, these “non-audible detectable” sound-sensitive
afferents may contribute to the generation of VEMPs and should be taken into consideration.

Sound stimulation and transmission. In the present study, the intensity of sound stimuli is referred to the threshold of the ABR of individual animals. This approach was first adopted by the Curthoys group to ensure that the intensities in animal studies are comparable to the intensities used in clinical VEMP testing (for review, see Curthoys 2010). To selectively activate the saccule, clinical VEMP testing often uses air-conducted sound that is within 15–20 dB of the VEMP threshold (for review, see Colebatch 2010; Rosengren et al. 2010). Because the normal human VEMP threshold is ~70 dB above the ABR threshold (Murofushi et al. 1995), the intensity of clinical VEMP testing is about 85–90 dB above the ABR threshold. In terms of normal hearing level (NHL), clicks with 0.1-ms duration and 100 dB NHL (0 dB NHL = 45 dB peak sound pressure level (pSPL)) are used in human cVEMP testing, which are equivalent to 145 dB pSPL (Halmagyi and Colebatch 1995). Thus the intensity in our study (80 dB above the ABR threshold, i.e., ~130 dB pSPL) is comparable to that used clinically as well as to that used by Murofushi et al. (1995; 0.1 ms, 60–80 dB SL re ABR threshold) and Murofushi and Curthoys (1997; 0.1 ms, 60–90 dB SL re ABR threshold). In the present study, only a single intensity was tested. The vestibular threshold to clicks in a rat has not been determined.

The anatomic structures of rat middle and inner ears are similar to those of human middle ear (Albin et al. 1986; Burda and Voldrick 1980; Hellstrom et al. 1982; Hofstetter et al. 2001; Judkins and Li 1977; Komárek et al. 2000; Muller 1991; Pinilla et al. 2001). The range of hearing in the rat is ~250–80,000 Hz at 70 dB (Heffner et al. 1994; Kelly and Masterton 1977). In contrast, a human can hear sounds from ~16 to 20,000 Hz. In general, the rat middle ear is much more efficient transmitting sound power to the cochlea at high frequencies relative to the human, whereas the human middle ear is much more efficient at low frequencies relative to the rat. Although the middle and inner ear structures are quite similar between the two species and the rat is a valuable animal model to understand human auditory and vestibular neurophysiology, independent studies in humans are crucial to develop clinical VEMP tests.

Sound sensitivity of regular and irregular vestibular afferents. Discharge regularity measured by CV* is characteristic of each afferent and is a useful marker to classify afferents into separate populations (for review, see Goldberg 2000). Afferents that differ in CV* also differ in many other properties, such as sensitivity to sensory inputs, efferent activation, and externally applied galvanic currents. Earlier studies noted a correlation between discharge regularity and sound sensitivity (Carey et al. 2004; Murofushi and Curthoys 1997; Young et al. 1977). However, the correlation between discharge regularity and sound sensitivity was not systematically examined for different vestibular end organs. In the present study, we addressed this issue by plotting CPE as a function of CV* for a large population of afferents from different end organs. Of the 504 regular units, only 0.2% were sensitive to air-conducted clicks. Of the 40 intermediate units, only 5% were sound sensitive. Of the 380 irregular units, however, 58% were sound sensitive. The percentages of sound-sensitive irregular afferents were 83, 74, 59, 47, and 7% for afferents from the SO, IO, AC, HC, and PC, respectively.

Sound-sensitive canal afferents. The present study provides new insights into the neural basis of the cVEMP and its clinical interpretations. It is generally believed that air-conducted clicks predominantly activate the saccule, and therefore the click-evoked cVEMP assesses saccule function (for review, see Colebatch 2001, 2010; Curthoys 2010; Halmagyi et al. 2005; Rosengren et al. 2010; Zhou and Cox 2004). Human studies reported preservation of the cVEMP in vestibular neuritis patients who lost caloric responses, which are believed to strongly support a dominant effect of saccule (Chen et al. 2000; Halmagyi and Colebatch 1995; Kim et al. 2008; Murofushi et al. 1996). However, there is evidence suggesting that sound also activates other vestibular end organs. Young et al. (1977) demonstrated that long-duration tones activate the afferents that innervate all five vestibular end organs, but saccule was relatively more sound sensitive. Carey et al. (2004) further showed in intact chinchillas that afferents from AC and HC exhibited few or none phasic response but exhibited slow tonic responses (20% of the AC afferents and 58% of the HC afferents) to long-duration tones. A recent study by Curthoys and Vulovic (2011) showed that air- or bone-conducted long-duration tones activate irregular afferents from both saccule and utricle. The present study provided a quantitative analysis of vestibular afferent responses to air-conducted clicks that are used in clinical VEMP testing and demonstrated that clicks activated both the otoliths and the canals. The findings in alert behaving monkeys (Xu et al. 2009; Zhou et al. 2004, 2005, 2007) are consistent with these results. Since the SCM motoneurons receive inputs from both the canals and the otoliths (for reviews, see Uchino et al. 2005; Wilson and Schor 1999), the canal contribution to cVEMP may need to be revisited.

Different sound sensitivities of the vestibular end organs. In addition to showing different sound sensitivities between regular and irregular afferents, our results also reveal different sound sensitivities among different vestibular end organs. The two otoliths (SO and IO) are more sensitive to clicks than the canals (AC, HC, and PC). It should be noted that we did not perform the tests to further assign the SO units into the utricle and the saccule. As shown in Fig. 2 (top left), the SO and IO afferents exhibited different sound sensitivity distributions. Whereas the IO afferents were clustered in the high CPE range, the SO afferents scattered over a larger range. Future studies need to address relative sound sensitivity between the saccule and the utricle. Among the three canals, the sound sensitivity order is AC > HC > PC. Although clicks evoked responses in AC and HC irregular afferents, they evoked little response in the PC, indicating that PC afferents do not significantly contribute to sound-evoked vestibular responses. The result is in agreement with McCue and Guinan (1994), who recorded from the inferior vestibular nerve and found that sound-sensitive afferents only arose from the saccule. Although the underlying mechanisms remain unknown, the differences in sound sensitivity may provide clues to find the sound parameters that preferentially activate specific vestibular end organs (Zhu et al. 2010).

This study has used a sensitive method to provide evidence of more widespread activation of vestibular afferents by clicks than previously reported. The strongest excitations were shown for otolith afferents, but some canal afferents were also excited. The threshold of excitation was not measured, and it is not known whether this animal model shows a vestibular threshold to sound activation similar to that of humans. Although clinical
evidence supports a predominant effect of saccular activation in mediating the cVEMP, the present findings raise the question of more widespread vestibular afferent excitation by clicks.

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

REFERENCES


