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Vestibular Evoked Myogenic Potentials in Normal Mice and *Phex* Mice With Spontaneous Endolymphatic Hydrops

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Abstract

Objective and Background—Vestibular evoked myogenic potentials (VEMPs) have been recorded from the neck musculature and the cervical spinal cord in humans and a limited number of laboratory animals in response to loud sound. However, the mouse VEMP has yet to be described. Evaluation of the sacculocollic pathway via VEMPs in mice can set the stage for future evaluations of mutant mice that now play an important role in research regarding human auditory and vestibular dysfunction.

Materials and Methods—Sound-evoked potentials were recorded from the neck extensor muscles and the cervical spinal cord in normal adult mice and in circling *Phex*^{Hyp-Duk/y} mice with known vestibular abnormalities, including endolymphatic hydrops (ELH).

Results—Biphasic potentials were recorded from all normal animals. The mean threshold of the VEMP response in normal adult mice was 60 dB normal hearing level with a mean peak latency of 6.25 ± 0.46 and 7.95 ± 0.42 milliseconds for p1 and n1 peaks, respectively. At the maximum sound intensity used (100 dB normal hearing level), 4 of 5 *Phex* mice did not exhibit VEMP responses, and 1 showed an elevated threshold, but normal response, with regard to peak latency and amplitude. The histologic findings in all of these *Phex* mice were consistent with distended membranous labyrinth, displaced Reissner membrane, ganglion cell loss, and ELH.

Conclusion—This is the first report of VEMP recordings in mice and the first report of abnormal VEMPs in a mouse model with ELH. The characteristics of these potentials such as higher response threshold in comparison to auditory brainstem response, myogenic nature of the response, and latency correlation with the cervical recording (accessory nerve nucleus) were similar to those of VEMPs in humans, guinea pigs, cats, and rats, suggesting that the mouse may be used as an animal model in the study of VEMPs. The simplicity and reliability of these recordings make the VEMP a uniquely informative test for assessing vestibular function, and these results suggest that they may be informative in mice with various mutations. However, further investigation is necessary.

Keywords

Endolymphatic hydrops; Mouse; Vestibular evoked myogenic potential

In nonmammals such as fish, the saccule has been found to be sensitive not only to linear acceleration but also to auditory stimuli (1). Vestibular sensitivity to sound has also been reported in deaf mice (2), squirrel monkeys (3), guinea pigs (4), and cats (5,6). Recently, short-latency p13-n23 potentials from the sternocleidomastoid muscle (SCM) have been recorded

in mammals in response to loud sound stimuli and have been termed vestibular evoked myogenic potentials (VEMPs) (7–9). Animal studies and patient data suggest a saccular origin of the VEMP (10–12) and a close relationship between the presence of a VEMP and the functional integrity of the inferior vestibular nerve. These responses have shown frequency selectivity similar to those demonstrated in the acoustically responsive saccular afferents in cats (13–15). There are few reports on animal models of the VEMP (16–19).

Although the mouse is the most commonly used animal model in biomedical research, the mouse VEMP has not been studied. Evaluating the sacculocollic pathway via VEMPs in normal mice can set the stage for future evaluations of mutant mice with vestibular and auditory dysfunction.

Phex, a phosphate-regulating gene with homologies to endopeptidases on the X chromosome, is mutated in X-linked hypophosphatemia in humans and mice. Mice carrying the *Phex* mutation in a particular background exhibit variable expressions of deafness, circling behavior, and abnormalities in other systems (20). Labyrinthine cross-sectional histology from male *Phex* mice reveals variable degrees and severity of endolymphatic hydrops (ELH), membranous labyrinth distention, degeneration of the spiral ganglion, and normal endolymphatic duct patency (20,21).

The purpose of this study is twofold 1) to record VEMPs for the first time in normal mice and explore recording feasibility, reproducibility, and characteristics via recordings from neck extensor muscles and the cervical spinal cord at the level of the spinal accessory nerve nucleus (C2-3) and 2) to evaluate vestibular abnormalities in *Phex* mice with spontaneous ELH using VEMPs.

Materials and Methods

The Institutional Animal Care and Use Committee has approved this research protocol. Ten normal male mice C57BL/6J aged 2 to 3 months (which were individually bred under controlled conditions of light, temperature, and humidity), and 5 age-matched male mutant mice were enrolled in the study. All normal animals had a positive Preyer reflex. Mice expressing the *Phex*^{Hyp-Duk} allele, a spontaneous mutation in the BALB/cAnBomUrd background (BALB/cUrd; stock no. 003905) were obtained from The Jackson Laboratory. To produce *Phex* males, BALB/cUrd *Phex*^{Hyp-Duk} carrier females (+/*Phex*^{Hyp-Duk}) were bred to BALB/cUrd *Phex* wild-type (+/Y) mice. Male *Phex* mice with circling behavior and expressing the mutation were selected in this study.

Two experiments were conducted on each mouse strain. The first experiment entailed VEMP recordings from the neck muscles of wakeful mice after their auditory brainstem responses (ABRs) were recorded. The second experiment consisted of the recording of potentials from the cervical spinal cord at the level of C2-3 corresponding to the spinal accessory nerve nucleus (22).

Animal Preparation

Animals were anesthetized by intraperitoneal injection with Avertin (tribromoethanol stabilized in tertiary amyl hydrate) given at a dose of 5 mg tribromoethanol per 10 g of body weight. Body temperature was maintained at 37°C to 38°C by placing animals on a homeothermic heating pad (Harvard apparatus, Holliston, MA, USA), and all testing was conducted in a sound-attenuated chamber. Levels of anesthesia were monitored in each mouse by visual inspection of respiratory rate and level of electromyographic (EMG) activity. External auditory canals and middle ears of all animals were examined directly with an

otoscope before test sessions and with surgical and dissection microscopy after killing to authenticate freedom from middle ear effusion at the conclusion of the experiments.

ABR Recording

Auditory brainstem responses from all animals were recorded after induction of anesthesia. Platinum-needle electrodes were inserted into the skin over the cranial vertex (noninverting), behind the opening of the left external auditory meatus (inverting), and over the dorsum of the neck (ground). Alternating polarity click stimuli (0.1 ms in duration) were presented through high-frequency transducers (23) connected via a short tube (10 mm) inserted into the ear canals. Click stimuli were applied to the right ear, and a white noise, (set 30 dB lower than those of the clicks) was applied to the left ear (23). Analysis time for each trace was 10 ms. Responses to 500 clicks were band-pass filtered (20–3,000 Hz) and averaged on the Intelligent Hearing Systems Smart EP (Miami, FL). Auditory brainstem response threshold was obtained for each animal by reducing the stimulus intensity from 100 dB peak equivalent sound pressure level (peSPL) in 10-dB steps and finally repeated in 5-dB steps until the lowest intensity that could evoke a reproducible ABR pattern was detected. The ABR threshold was defined as 0 dB nHL (normal hearing level) for the next experiments. Standard sound calibration and maintenance of machine and speakers' output were controlled by the manufacturing company. Baseline noise level was determined by recording several traces, whereas each animal was connected to the recording computer without sound presentation. The grand average of the recordings was used as a reference baseline noise level. This noise level was used to define onset and peak latency of ABR and VEMP waveforms.

VEMP Recordings

After completion of the ABR recordings, and while the animals were still under general anesthesia, VEMP recordings were initiated with simultaneous recording of the EMG potentials. At this stage of recording, under anesthesia, EMG activity was 1 standard deviation (SD) below the baseline noise level. Vestibular evoked myogenic potential recordings started and continued until animals were almost fully awake and had strong EMG potentials. The rationale for early start of the VEMP recording, while animals were sleeping, was to assess relationship between EMG activity level and VEMP amplitudes. A custom-made holder was used to restrain the body of the mouse while leaving its head and legs free (Fig. 1). During recordings, each animal was fixed in the prone position with its head elevated and in place between earphones and suspension wire. The animal's neck was hyperextended and stabilized with suspension wire. Combativeness or significant movement was interpreted as an animal's discomfort that required adjustment of the apparatus or, in a few instances, termination of the experiment session. In contrast to our expectations, continued recordings while keeping the awake animal in the acoustic chamber and darkness were possible, and animals tolerated the procedure well in most cases. This technique of keeping an animal in a holder with neck extension and successfully recording evoked potentials has previously been reported in the literature (17,19). Potentials were recorded from the neck extensors (semispinalis capitis muscles) at the level of C3. A platinum-needle electrode coated with ink was inserted into the neck extensors, whereas a reference electrode was placed on the occipital area at the midline. A ground electrode was placed on the back. Proper placement of the electrode and muscle anatomy was studied earlier on mice using a mouse atlas (The Anatomy of the Laboratory Mouse. Margaret J. Cook; M.R.C. Laboratory Animals Centre, Carshalton, Surrey, England, Academic Press, 1965). Placement of the electrode in this study was confirmed by recording EMG and by postmortem study of the dye position at the neck muscle. Electromyographic signals were amplified and band-pass filtered between 30 and 1,500 Hz. Monaural acoustic stimulation (click stimuli, 0.1 ms) was delivered, and ipsilateral responses were recorded. The stimulation rate was 5.1 clicks per second. The analysis time for each response was 50 milliseconds, and 200 responses were averaged for each run. Each animal underwent serial

VEMP testing beginning with an initial stimulus intensity of 100 dB nHL, followed by 10-dB-step decrements. Response threshold was determined by the lowest intensity at which the waveform was present. Multiple consecutive runs (3–5 runs) were performed to verify reproducibility. Thereafter, the latencies of response onset, peak latencies, and peak-to-peak amplitudes were measured. The response onset was defined as the point at which a recorded potential was 1 SD above the baseline noise level and yielded a reproducible positive peak.

Cervical Reflex Recordings

Under deep intraperitoneal anesthesia and aseptic conditions, with the mouse fixed to a surgical platform using 4 extremity restraints, the dorsal neck skin was incised, and the muscles were retracted to expose the dorsal spinal column. The dorsal lamina of the C2-3 vertebrae was removed, and the dura was excised to expose the spinal cord and spinal accessory nucleus. A concentric-needle electrode (0.4 mm in diameter) was then attached to a manipulator and inserted into the anterior horn of the spinal cord at the C2-C3 level. The electrode was inserted while confirming responses to sound stimuli at a site 1.5 to 2.5 mm lateral to the midline. This site was determined to be the position of the anterior horn of the spinal cord based on preliminary measurements made in 2 normal mice.

Anatomic Analysis

For pathologic studies, the inner ears of *Phex^{Hyp-Duk}/Y* mice were examined after conclusion of the recordings. Five *Phex^{Hyp-Duk}/Y* and 2 control (+/Y) mice were processed for histologic analysis. Under deep anesthesia, mice received a lethal dose of Avertin. The animals were then decapitated, and the temporal bones were removed after opening of the bullae. In all cases, the inner ear tissues were fixed by perilymphatic perfusion with phosphate-buffered 2.5% glutaraldehyde through the round window after removing the stapes and puncturing the round window membrane. The dissected temporal bone was then immersed in fixative for 24 h at 4°C and rinsed in 0.1 mol/L sodium phosphate buffer (pH 7.4) and processed as noted elsewhere. Distention of Reissner membrane in the cochlear duct and/or membranous labyrinth was deemed indicative of the presence of ELH in the affected ear and was consistent with findings in a larger study of the *Phex* mouse (21).

Statistical Analysis

The relationship between stimulus intensity and latency was analyzed by 1-way analysis of variance (ANOVA). The relationship between stimulus intensity and response amplitude was compared by ANOVA. The Student's *t* test was used to compare the thresholds of the VEMP and ABR responses. Values were deemed statistically significant at the level of $p < 0.05$.

Results

Auditory brainstem response peak latencies and thresholds were determined before recording the VEMPs and the cervical potentials (Fig. 2). Intense sound stimuli evoked biphasic potentials in all of the normal mice and in 1 of 5 *Phex* mice.

Normal Mouse

VEMPs

Latency of the response: The myogenic potentials were analyzed in detail across different stimulus intensities. Sound-evoked potentials were evoked only at high-stimulus intensities (100–60 dB nHL) in all normal mice (Fig. 3). No potentials were discerned in any mouse at or less than 60 dB nHL. The onset and peak latency of positive response peaks did not show any statistically significant relationship with stimulus intensity variation over the stimulus range of 100 to 60 dB nHL ($p > 0.05$; 1-way ANOVA test; Fig. 4). The onset latency of the recorded

positive peaks was 4.78 ± 0.61 milliseconds in response to clicks presented at an intensity of 90 dB nHL (Table 1). The latency of the positive peaks (p1) and negative peak (n1) was 6.25 ± 0.46 and 7.95 ± 0.42 milliseconds in response to clicks presented at 90 dB nHL. There was no statistically significant relationship between the onset and mean latencies of the positive and negative peaks and the stimulus intensities used (90–70 dB nHL; $p > 0.05$; 1-way ANOVA test; Table 1). The bandwidth of the response peaks (p1 and n1) was quite broad especially at the lower sound intensities, which made determination of the peak's maximum point somewhat challenging.

Amplitude of the response: The amplitudes of the negative and positive peaks changed considerably with different EMG activity levels related to stage of wakefulness and EMG activity level (Fig. 5). There was a linear correlation between EMG activity level and VEMP amplitude ($R^2 = 0.83$). The rejection for EMG levels criteria was set at 50 μ V to reject potentials with large amplitude from larger muscles such as masseter or leg muscles. The peak-to-peak amplitudes of p1-n1 peaks was 13.2 ± 3.8 μ V, which was calculated from recordings taken only from fully awake animals showing maximum EMG activity (Table 1). In contrast to response latencies, there was a statistically significant difference in the response amplitudes when the intensity dropped from 90 to 70 dB nHL in normal mice (Fig. 6).

Threshold of the response: The click-evoked VEMP mean threshold was significantly (60 dB) greater than the click-evoked ABR ($p < 0.05$; Student's *t* test). Figure 3 shows a typical response recorded from the needle electrodes in the neck muscles of a normal mouse in response to click stimuli. The sound intensity of the stimulus decreases from 90 dB nHL in 10-dB decrements. Responses consisted of biphasic negative and positive peaks. Responses fell below the noise level as click intensity fell to less than 60 dB nHL. However, ABR responses from the same animal could be discerned as low as 30 dB peSPL (Fig. 1).

Myogenic origin of the response: After ABR recording under general anesthesia, the VEMP electrode montage was applied to the mouse, and VEMP recording was initiated. Simultaneous EMG activity and respiration rates were used to monitor sleep stages. No VEMP was evoked during the waking period when there was no strong EMG activity (EMG activity at/below 1 SD of baseline noise level; Fig. 5). Figure 7 shows variation in EMG activity level during the waking-up period of a mouse. Measurable VEMPs began to emerge as EMG activity increased and the animals awakened from general anesthesia. The amplitude of recorded potentials consistently increased as the animals emerged from anesthesia and as EMG activity increased (Fig. 5). Maximum VEMP and EMG amplitudes were recorded when each animal was fully awake and active. Recording was continued with cervical recordings under deep anesthesia until a lethal dose of Avertin was administered to terminate the experiments, which revealed an absence of VEMP upon death.

Potentials Recorded From Cervical Spinal Cords: Under deep anesthesia, mice exhibited a monophasic response to acoustic stimuli from the anterior horn of the spinal cord at the level of the spinal accessory nerve nucleus (22). Figure 8 shows a typical response from the surface of the cervical spinal cord at the C2-3 level in response to a 90-dB nHL sound stimulus. The latency of the cervical response (4.7 ± 0.35 ms) is shorter than that of muscle (p1 of the VEMP; 6.25 ± 0.46 ms) by 1.35 ms, whereas the response threshold to acoustic stimuli was 60 dB nHL in both cases (Table 1). Cervical spinal cord responses were not affected by the level of anesthesia, indicating a neuronal origin of the response.

Phex Mice

VEMP and ABR Studies—Five mutant mice underwent ABR and VEMP testing, followed by histologic analysis of their temporal bones. All 5 mice showed elevated ABR thresholds

with normal waveform morphology and interpeak latencies (data not shown). Only 1 mutant mouse showed a reproducible muscle and spinal cord response to loud sound stimuli (Fig. 9). The VEMP response peak latencies were 5.2 and 7.2 milliseconds for p1 and n1, respectively, at the maximum stimulus intensity level of 100 dB nHL (Table 2), which was within the range recorded for normal mice. Mean response amplitude was 12.2 μ V, again within the normal range for mouse VEMP amplitude (Table 2). However, the response threshold for the *Phex* mouse VEMP response (100 dB nHL) was much higher than that for normal mice (60 dB nHL; $p < 0.05$; Student's *t* test; Table 2).

Histologic Studies—Light microscopic studies of *Phex* mouse inner ears revealed a severe hydropic picture in 4 of 5 mice tested in this study. Only 1 mouse, with VEMP responses of elevated threshold but normal latencies and amplitude, revealed a moderately membranous labyrinth shown in Figure 10. Histologic examination demonstrated mild ELH as evidenced by moderate distension of the Reissner membrane. Four other mutants showed severe hydrops in all parts of the membranous labyrinth, and cochlear duct sections showed displaced Reissner membranes. In those mutants, the Reissner membrane seemed to be ruptured in the basal-most part of the first turn. In some cases, the membranous wall of the saccule was also ruptured because of hydrops (data not shown). For a full description of this mutant model, please see the report from Megerian et al. (21).

Discussion

The VEMP is a useful clinical test of vestibular function in humans and is becoming more frequently used. This is the first study to report recordings of the VEMP response in a mouse model. We recorded this response from the neck muscles and demonstrated its dependency on muscle tonic contraction and vestibulocollic sound activation by recording from the cervical spinal cord. The mouse VEMPs had similar characteristics to human VEMPs with regard to the following: 1) the response threshold was higher (60 dB nHL) than the ABR threshold; 2) the response amplitude was linearly related to EMG activity level of the neck muscles; the maximum VEMP amplitudes were recorded during maximum tonic muscle contraction; 3) the adaptational phenomenon encountered in startle and other postauricular muscle responses was not observed during wakefulness (24); and 4) the responses had higher thresholds or were absent in the *Phex* mouse model with balance dysfunction and spontaneous inner ear hydrops shown by behavioral pattern and inner ear histology.

Vestibular sensitivity to sound and, more recently, VEMP recordings in mammals have been reported (3,16,17,19,25). Neural connections and pathways underlying this sacculocollic reflex were studied by Uchino et al. (26) and Kushiro et al. (27). Electric stimulation of the saccular nerve evokes inhibitory postsynaptic potentials in the ipsilateral SCM motoneurons and excitatory postsynaptic potentials to bilateral neck extensors. The modulation of EMG activity resulting from saccular nerve stimulation has also been documented in humans (28). Basta et al. (29) have recorded VEMPs after direct electric stimulation of the human inferior vestibular nerve and reported ipsilateral potentials at a mean latency of 9.1 ± 2.2 and 13.2 ± 2.3 milliseconds for the positive and negative peaks, respectively. No contralateral SCM response was reported. Electric stimulation of the superior vestibular nerve did not result in any EMG response of the SCM.

Yang and Young (19) recorded VEMPs from the neck muscles of awake guinea pigs in response to loud sounds. At 100-dB peSPL click stimulation, they reported mean latencies of positive and negative peaks of 7.24 ± 0.49 and 9.15 ± 0.47 milliseconds, respectively. This study and subsequent research from the same laboratory reported a decrease in VEMPs and electronystagmographic (caloric stimulation) responses from guinea pigs treated with gentamicin, whereas ABR responses remained grossly intact (17). Sakakura et al. (16) recorded VEMPs

from decerebrated and freely moving rats with onset and positive peak latencies of 4.6 ± 0.4 and 6.8 ± 0.9 milliseconds, respectively. The thresholds of the myogenic potentials were reported to be 45 to 60 dB greater than the threshold of the ABR. The potentials we recorded from neck muscle of awake mice showed mean peak latencies of 6.25 and 7.95 milliseconds for p1 and n1 peaks, respectively. These values are shorter than the guinea pig but longer than rat VEMP peak latencies.

Neck muscle tension and the level of EMG activity play a critical role in recording VEMPs in humans. Relaxation of the neck muscles may abolish VEMPs entirely (30,31). Direct evidence that muscles generate the VEMP was recently published (28). The amplitude of the response has direct relationship to the level of EMG activity of the muscles and amplitude increases as a function of EMG activity level, as well as stimulus intensity (32,33). Recently, VEMPs recorded from an awake animal model also confirmed the EMG activity dependence of the responses (16). In these studies, the myogenic origin of the responses was tested with application of intraperitoneal injection of a muscle relaxant that abolished VEMP but not ABR. In our tests of the relationship between VEMP amplitude and EMG activity level, we anesthetized the animals by intraperitoneal Avertin administration. No animals showed any reproducible potential within the latency range of 5 to 10 milliseconds, whereas ABR potentials were present during the period at which the latency was less than 5 milliseconds (Fig. 2). To ensure sufficient muscular contraction during wakeful periods (Fig. 7), animals were held in a position of neck hyperextension using a special holder with a head sling throughout the test (Fig. 1). Vestibular evoked myogenic potential and EMG were simultaneously recorded during periods of emergence from anesthesia (Fig. 5) and showed a direct correlation between the VEMP amplitudes and EMG activity level of the neck muscles (Fig. 5; $R^2 = 0.83$). Maximum response amplitudes were recorded only after an animal was fully awake and active, demonstrating the necessity of strong EMG activity and tonic muscle contraction for VEMP recording (Fig. 5).

Masaki et al. (34) reported sound-evoked potentials on the anterior horn of the spinal cord at the levels of C3-C6 in 17 cats. They reported potentials with a peak latency of 4.89 to 5.10 milliseconds only at the C3 level. The potentials disappeared after destruction of the medial vestibulospinal tract, whereas ABRs remained intact. In our study, we recorded a negative response at the level of the spinal accessory nerve in response to loud clicks (Fig. 9). The response threshold was much higher than the ABR. The positive peak latency was shorter for cervical recording than for muscular recording. The response latency was also shorter than the muscle responses (VEMPs) recorded from the same animal. The latency differences are speculated to be the result of the longer pathway, longer conduction time, and muscle activation required for muscle potentials in comparison to spinal cord potentials. In contrast to VEMPs (which could be recorded from the neck muscle only from wakeful animals), the spinal cord responses persisted under general anesthesia induced by intraperitoneal Avertin. These findings suggest a vestibulocollic pathway underlying the recorded responses.

Several types of responses to strong acoustic stimulation can be recorded from the necks of animals: the startle neck reflex (with latency of 5–8 ms in rats) (35,36); the ABR; and the p1 peak of the middle-latency evoked potential (with 13-ms latency in rats) (36,37). In guinea pigs, sound-evoked potentials have been recorded in the vestibular nerve, and the vestibular nuclei latencies were 1 and 3 milliseconds, respectively. At the level of the spinal accessory nerve nuclei, the response onset and peak latency were 4 to 5 and 6 to 7 milliseconds, respectively (38,39). The latencies of inhibitory postsynaptic potentials at the level of the ipsilateral neck extensor motor neurons in rats, in response to electric stimulation of the saccular nerve, is 3.1 to 4.0 milliseconds (26,27). The peak latency of 4.7 ± 0.35 milliseconds for spinal cord recordings in this study is in agreement with the previously mentioned reports. In our study, EMG level dependency, potentials at the level of the spinal cord under general

anesthesia, and lack of the adaptational phenomenon encountered in startle and other postauricular muscle responses rule out the possibility that these responses were artifactual.

A short-latency-negative wave of possible vestibular origin has been reported in normal-hearing subjects, in the otherwise flat ABR recordings of some profoundly deaf children, and deaf adults (40–44). This negativity occurs at approximately 3 milliseconds in response to loud clicks. In adult patients, the presence of an N3 is related to the presence of normal vestibular function and VEMPs. The VEMP and N3 thresholds reported seem to be well correlated (40, 44). Although both the VEMP and the N3 potential seem to originate from the sacculus, the characteristics of these 2 responses are not identical, indicating that additional factors might be involved in the generation of the N3 potential (40,42,43). Nong et al. (42) studied 20 patients with bilateral profound hearing loss aged 6 to 62 years, including 16 cochlear implant recipients. The authors showed that approximately one third of the ears with profound hearing loss and absence of N3 response had VEMPs, pointing to difference in generator or sensitivity of the tests. Subsequent studies supported the similar conclusion (45,46). Therefore, these different techniques should be regarded as complementary in evaluating saccular function and not identical.

The authors do not believe that recorded potentials in this study are a composite “N3”-VEMP. The negativity before P1 of our recording likely corresponds to a negative peak after the last wave of the ABR responses, which may be equivalent to negative peak after wave V of human ABR, as observed in other studies (21).

Considering the latency of N3 and p13 in humans, one can expect that the latency of the N3 potentials in mice may be very much shorter than 6 to 7 milliseconds recorded for P1 peak in mice. However, the negativity after ABR and before VEMPs in this study is approximately 4 milliseconds at 90 dB nHL, which is even longer than the latency of human N3 potentials. Nonetheless, these data presented here are meant to stimulate further research in characterizing the mouse VEMP and related physiologic parameters, and it is anticipated that additional research will help better characterize these important issues.

The Phex mouse is a unique animal model with a distinct circling behavioral phenotype suggesting balance and vestibular compromise. Histologic studies suggested evidence of ELH, ganglion cell loss, normal endolymphatic duct, and normal organ of Corti (20,21). We used this animal model to test the usefulness of recorded VEMP responses in evaluating a mutant model with known vestibular pathology. Vestibular evoked myogenic potential responses from neck muscles and the spinal cord were absent in 4 of 5 affected animals with circling behavior, elevated ABR threshold, and histologic evidence of well-developed ELH (Reissner membrane almost touching the bony labyrinth). Only 1 mouse with moderate hydrops (displaced Reissner membrane and mild loss of ganglion cells) had VEMPs with an elevated response threshold on both muscle and spinal cord recordings (Fig. 9). These findings were interpreted, at least in part, to be the result of ELH, which has been shown in humans to cause abnormal VEMPs (47,48). It is important to mention that the absence of VEMP responses in Phex mice may be 1 manifestation of a more global pattern of vestibular loss in these mice. In addition, this study does not intend to establish that the loss of VEMP is a specific feature of ELH. Rather, it is likely that the loss of VEMP mirrors an overall loss of vestibular function in these animals.

Recently, the mouse vestibulo-ocular reflex (VOR; stabilizes the head in space by excitation of neck muscles that oppose head rotation) and vestibulocollic reflex (VCR; stabilizes the head position in space during motion of the body) have been characterized so that genetic manipulations of the vestibular system can be examined (49,50). The VCR is usually recorded from mice (restrained at the neck and free to rotate their heads) by using an electromagnetic technique often used to measure eye movements. The VCR is a complex behavioral response

that is likely to be dependent on attentional mechanisms, intention, and sensory inputs from the vestibular and visual systems. If it is to be used successfully as an assay of vestibular function, the relative influence of these factors on the strength of VCR responses to rotation must be established. It has been suggested that, although the horizontal semicircular canals and the otolithic organs perform different functions, they do not operate independently of each other, but are intimately coupled in their function. The general conclusion has been that the canals are responsible for VOR in response to rapid or high-frequency rotation, whereas the otolithic organs provide signals to control low-frequency counter-rotations of the eyes. Incomplete understanding of interactions of the semicircular canals and otolithic organs at the present time and the limited capability for assessing independent function of each end-organ type is a limitation of these tests. In contrast, VEMP is a simple test to perform and interpret. It does not require special equipment or personnel with additional training. Vestibular evoked myogenic potential has the capability of testing the function of the unilateral sacculus independent of semicircular canals, sacculocollic pathway, and lower brainstem integrity (10–12). Adding this test to the battery of other vestibular tests battery in mice such as caloric, VOR, and VCR will allow assessment of the functional integrity of mutant mouse strains in greater detail.

The VEMP is a test of sacculocollic pathway integrity. It holds great promise for diagnosing and monitoring several neurotologic disorders in humans but remains an evolving field both in basic science and in clinical applications. Basic science research and development of animal models to explore its pathway and receptor(s) will lead to a better understanding of the response and its generators. Mutant mouse models with a variety of audiovestibular abnormalities and human correlates will likely be augmented by the application of preliminary VEMP studies as outlined in this article.

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FIG. 1.

Schematic drawing of the mouse positioning during VEMP recording, including muscle orientation and recording sites. Muscle response was performed first and was followed by cervical recordings. The drawing simply illustrates all of the recording sites and montage together for convenience.

FIG. 2. Serial ABR responses recorded from a normal mouse. The acoustic threshold for this mouse was 35 dB peSPL by click stimulation. Recording time was 25 ms (truncated to 10 ms in this figure).

FIG. 3.

Click-evoked myogenic potentials in a normal mouse. Myogenic potentials evoked by monaural click stimulation with unilateral recording in a mouse. It reveals positive peak (p1) and negative peak (n1) elicited by 90- to 60-dB nHL acoustic stimulation. However, no response was recorded at 60-dB (last tracings) click stimulation. Multiple responses have been superimposed for each condition to demonstrate reproducibility of VEMP in each individual recording. *Filled triangle* represents onset of response defined by point of emergence of the response from baseline noise level.

FIG. 4.

The average and 1 SD of p1 and n1 peaks latencies of VEMPs at different stimulus strengths. The peak latencies lengthened nonsignificantly as a result of weaker stimulation.

FIG. 5.

The relationship between average muscle tonus and p1-n1 amplitude is plotted. Stimulus intensity is 90 dB nHL. For each stimulus, the VEMP amplitude increased as a function of EMG level. The figure revealed significant correlation between the 2 parameters.

FIG. 6. The average and 1 SD on p1-n1 peak amplitude of VEMPs at different stimulus intensities. The amplitude of the response decreases as stimulus intensity drops to lower intensities.

FIG. 7. Electromyographic activity of a mouse during awakening and gaining more robust EMG. A small amount of sporadic activity (A), followed by more regular activity (B), but still low amplitude. Active animal with regular and robust muscle activity (C).

FIG. 8.

Vestibular evoked myogenic potentials recorded from a *Phex* mouse with elevated threshold for ABR and histologic evidence of mild to moderate ELH. The response threshold is higher than ones recorded from normal mice. The latency and amplitude of VEMP was within the range recorded from normal animals. *Filled triangle* represents onset of response defined by point of emergence of the response from baseline noise level.

FIG. 9.

Sound stimuli-evoked potentials recorded from a normal mouse on the surface of the spinal cord at the level of accessory spinal nerve nucleus (C2-3). The latency of the response is earlier than the muscle responses because of a shorter neuronal circuit. The response latency was consistent across the stimuli intensities.

FIG. 10.
Light microscopic photograph of *Phex* mouse vestibule with elevated VEMP response threshold stained with hematoxylin and eosin. Moderate distention of saccular membranous labyrinth resulting from inner ear hydrops.

TABLE 1
Tabulated values for the onset and mean latencies and amplitudes of evoked potential peaks at 90-dB nHL sound intensity

	Muscle potentials	Spinal cord potentials
Onset latency	4.78 ± 0.61 ms	—
p1 Peak latency	6.25 ± 0.46 ms	4.7 ± 0.35 ms
n1 Peak latency	7.95 ± 0.42 ms	—
p1-n1 Amplitude	13.2 ± 3.8 μ V	—
Threshold	60 dB nHL	60 dB nHL

Note the earlier latencies for spinal cord recordings, and also note for higher threshold (60-dB nHL) for the VEMPs in comparison to ABR.

ABR indicates auditory brainstem response; nHL, normal hearing level; VEMP, vestibular evoked myogenic potential.

TABLE 2
Peak latencies, amplitude, and thresholds of VEMPs recorded from neck muscles in both normal and mutant mice

	p1 Latency	n1 Latency	Amplitude	Threshold
Normal mouse	6.25 ± 0.46 ms	7.95 ± 0.42 ms	13.2 ± 3.8 μV	60 dB nHL
<i>Phex</i> mouse	5.8 ms	7.2 ms	12.2 μV	100 dB nHL

Note higher threshold but same amplitude and latency for *Phex* mouse with ELH in comparison to normal mice. nHL indicates normal hearing level.