Physiological and Morphological Assessment of the Saccule in Guinea Pigs After Noise Exposure

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Objective: To investigate whether the saccule exhibits temporary or permanent functional loss resembling threshold shifts in auditory brainstem response (ABR) of guinea pigs following noise exposure.

Design: Randomly bred guinea pigs were divided into 3 groups: A (short-term noise exposure, 30 minutes, n=15), B (long-term noise exposure, 40 hours, n=9), and C (no noise exposure, n=5).

Setting: University hospital.

Main Outcome Measures: All animals underwent vestibular-evoked myogenic potential (VEMP) and ABR tests. Chronological changes of VEMP and ABR responses following noise exposure were analyzed and compared. After audiovestibular function testing, animals were killed for morphological study with light and electron microscopy.

Results: In group A, temporary VEMP loss and ABR threshold shifts recovered 2 and 4 days, respectively, after short-term noise exposure, with an interval of 2 days earlier in the recovery of VEMPs than that of ABR thresholds. In contrast, in group B, 78% and 83% of the ears exhibited permanent VEMP loss and ABR threshold shifts, respectively, 10 days following long-term noise exposure. In group C, all animals showed normal VEMPs and ABRs throughout the study period. Light and electron microscopic studies confirmed that loss of VEMPs correlated with saccular lesion.

Conclusions: The saccule can exhibit temporary or permanent functional loss resembling hearing threshold shifts in guinea pigs following noise exposure. Recovery of VEMP precedes restoration of hearing threshold after damage from short-term noise exposure. Conversely, permanent VEMP loss after long-term noise exposure may reflect permanent hearing threshold shifts.


EXTREME NOISE CAN CLEARLY damage hair cells in the cochlea, leading to temporary or permanent threshold shifts in hearing. However, the effect of noise on the vestibular part remains poorly understood. Despite numerous documented cases of balance disorders from noise-induced hearing loss, vestibular symptoms resulting from acoustic trauma have not been studied thoroughly. Furthermore, excessive exposure to very loud music may also affect vestibular function. However, imbalance in noise-exposed workers or music-exposed young people has not been approved for compensation by insurance boards, possibly because vestibular dysfunction often recovers spontaneously via central compensation.

Phylogenetically, the saccule in the lower species such as amphibians and fish can act as an acoustic receptor. Hence, intense sound and vibration may produce vestibular reflexes, while the vestibular fibers can also respond to sound. Restated, loud noise damages the hair cells of the cochlea and may also affect the saccular macula.

Recently, the vestibular-evoked myogenic potential (VEMP) has been validated to originate from the saccule and is easily recorded via the contracting neck muscles using loud sound stimulation in humans and experimental animals. The use of experimental animals, such as guinea pigs, facilitates the study of the mechanism of saccular disorders by recording VEMPs and confirming cell damage by morphological assessment. Hence, this study investigated whether VEMPs exhibit temporary or permanent loss resembling threshold shifts of the auditory brainstem response (ABR) in guinea pigs following noise exposure by correlating the physiological results with morphological changes.
and use of laboratory animals by the Animal Research Committee during the study, each animal in group C underwent the same tests as after noise exposure on days 0, 3, 7, 10 and 30. Throughout the study, each animal in group C underwent the same tests as those in groups A and B.

This study was approved by the institutional review board and was conducted in accordance with the guidelines for the care and use of laboratory animals by the Animal Research Committee, College of Medicine, National Taiwan University, Taipei.

NOISE EXPOSURE

Each animal in the 2 noise exposure groups was placed in a cage and allowed to move freely. The noise level was measured with a sound level meter (model NA-24; Rion, Tokyo, Japan). Continuous broadband white noise at a mean (SD) intensity of 115 (5)-dB sound pressure level (SPL; duration, 0.1 milliseconds [ms]) was delivered via an earphone connected through a short tube inserted into the ear canal to produce permanent threshold shifts. Another 5 animals with no noise exposure (group C) served as a control group.

All animals in group A underwent ABR and VEMP tests during the pre–noise exposure period and after noise exposure on days 0, 1, 2, 3, 4, and 7. For animals in group B, ABR and VEMP tests were performed during the pre–noise exposure period and after noise exposure on days 0, 3, 7, 10 and 30. Throughout the study, each animal in group C underwent the same tests as those in groups A and B.

This study was approved by the institutional review board and was conducted in accordance with the guidelines for the care and use of laboratory animals by the Animal Research Committee, College of Medicine, National Taiwan University, Taipei.

ABR TEST

After the VEMP test, each animal underwent the ABR test after receiving an intraperitoneal pentobarbital sodium (35 mg/kg) injection. Active and reference needle electrodes were inserted in the vertex and ipsilateral retroauricular region, respectively, while a ground electrode was placed in the neck of the animal. Click stimuli (duration, 0.1 ms) were delivered via a plastic tube inserted into the ear canal to record the ABR (Smart EP2), monaurally. The repetition rate was 20/s, with a mean of 400 sweeps. The stimulus intensity began from 100-dB peak equivalent SPL, followed by 10-dB step decrements until waveforms I, III, and V disappeared, thus determining the ABR threshold.

MORPHOLOGICAL ASSESSMENT

Following deep anesthesia with intraperitoneal injection of pentobarbital sodium (50 mg/kg), the animals were transcardially perfused with isotonic sodium chloride solution, followed by a fixative containing glutaraldehyde, 2.5%, in 0.1 M phosphate buffer at pH 7.4. After complete fixation, the animals were decapitated, and the temporal bones were harvested and placed in the same fixative for 24 hours and decalcified with 10% EDTA containing glutaraldehyde, 2.5%, at pH 7.4 for 1 week. Tissue blocks were cut horizontally into 200-µm-thick slices with a vibratome and postfixed in 1% osmium tetroxide for 1 hour. The sections were dehydrated in ascending ethanol solution, infiltrated with propylene oxide, and finally embedded in Araldite-Epon mixture (Electron Microscopy Science; Fort Washington; Pennsylvania). Semi-thin (1-µm) sections were cut by a Leica Ultracut E ultramicrotome (Leica, Vienna, Austria) and stained with toluidine blue for light microscopic study. For electron microscopic study (Hitachi 7100; Hitachi, Tokyo, Japan), ultrathin sections (60-80 nm) of the specimen were cut and stained with 1% lead citrate.

STATISTICAL ANALYSIS

The abnormal percentages of VEMP results following short- or long-term noise exposure were compared by the McNemar test. Chronological changes in ABR threshold following short- or long-term noise exposure were compared against the paired t test. Moreover, abnormal percentages of VEMP and ABR results were compared by the McNemar test. Trends in recovery of VEMP responses and ABR thresholds were analyzed by Kaplan-Meier survival analysis. Finally, the log-rank test was used to compare recovery curves between VEMP and ABR. *P < .05 was considered to be statistically significant.

RESULTS

BEHAVIOR CHANGE AFTER NOISE EXPOSURE

During noise exposure, most animals appeared agitated, while other animals kept immobile at 1 corner of the cage, usually the corner farthest from the noise source. However, neither spontaneous nystagmus nor head tilt was observed in any animals after both short- and long-term noise exposure.

CLICK-EVOKED RESPONSES FOLLOWING GENERAL ANESTHESIA

All guinea pigs in group C showed VEMP responses before anesthesia. Following intraperitoneal administration of pentobarbital sodium (35 mg/kg), click-evoked
ABR was observed at the 0.5- to 4.0-ms latency, which was consistent across SPLs. However, no animals displayed evoked potentials on the neck at the 6- to 9-ms latency (Figure 1).

SHORT-TERM NOISE EXPOSURE

Fifteen animals (group A, 30 ears) were subjected to short-term noise exposure (30 minutes). The percentages for absent VEMPs during the pre-noise exposure period and after noise exposure on days 0, 1, 2, 3, 4, and 7 were 0%, 70%, 27%, 10%, 0%, 0%, and 0%, respectively, indicating absent VEMPs on post-noise exposure days 0 and 1 ($P<.01$, McNemar test). However, the percentage of normal VEMPs on post-noise exposure days 2 through 7 did not significantly differ from the same pre-noise exposure period ($P<.05$, McNemar test; Table). Figure 2 illustrates the absence of VEMPs in a guinea pig immediately following short-term noise exposure. On post-noise exposure day 2, VEMPs returned to normal.

The mean (SD) ABR threshold for the pre-noise exposure period was 42.5 (5.4)-dB SPL, thus an ABR threshold exceeding 53.3-dB SPL (equal to mean $+\text{2 SD}$) was defined as a threshold shift. Mean (SD) ABR thresholds on post-noise exposure days 0, 1, 2, 3, 4, and 7 were 71.2 (9.6)-, 58.5 (8.1)-, 54.0 (9.6)-, 46.7 (7.8)-, 45.8 (7.6)-, and 42.8 (6.0)-dB pe SPL, respectively, indicating temporary threshold shifts on post-noise exposure days 0 through 3 ($P<.05$, paired t test). Accordingly, percentages for ABR threshold shift in group A were 97%, 70%, 67%, 30%, 0%, and 0% on post-noise exposure days 0, 1, 2, 3, 4, and 7, respectively, indicating that recovery of ABR threshold oc-

Table. Comparison of Abnormal Percentages of VEMP and ABR in Guinea Pigs After Short- and Long-term Noise Exposure

<table>
<thead>
<tr>
<th>No. of Ears</th>
<th>VEMP, %</th>
<th>ABR, %</th>
<th>$P$ Value (McNemar Test)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>After Short-term Noise Exposure (Group A)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-noise exposure period</td>
<td>30</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Day 0</td>
<td>30</td>
<td>70$^a$</td>
<td>97$^a$</td>
</tr>
<tr>
<td>Day 1</td>
<td>30</td>
<td>27$^a$</td>
<td>70$^a$</td>
</tr>
<tr>
<td>Day 2</td>
<td>30</td>
<td>10</td>
<td>67$^a$</td>
</tr>
<tr>
<td>Day 3</td>
<td>30</td>
<td>0</td>
<td>30</td>
</tr>
<tr>
<td>Day 4</td>
<td>30</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Day 7</td>
<td>30</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>After Long-term Noise Exposure (Group B)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-noise exposure period</td>
<td>18</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Day 0</td>
<td>18</td>
<td>100$^b$</td>
<td>100$^b$</td>
</tr>
<tr>
<td>Day 3</td>
<td>18</td>
<td>94$^b$</td>
<td>94$^b$</td>
</tr>
<tr>
<td>Day 7</td>
<td>18</td>
<td>83$^b$</td>
<td>89$^b$</td>
</tr>
<tr>
<td>Day 10</td>
<td>18</td>
<td>78$^b$</td>
<td>83$^b$</td>
</tr>
<tr>
<td>Day 30</td>
<td>18</td>
<td>78$^b$</td>
<td>83$^b$</td>
</tr>
</tbody>
</table>

Abbreviations: ABR, Auditory brainstem response; NS, nonsignificant difference ($P>.05$); VEMP, vestibular-evoked myogenic potential.

$^a$ $P<.01$ when compared with the pre-noise exposure period (McNemar test).

$^b$ $P<.001$ when compared with the pre-noise exposure period (McNemar test).

Figure 1. Click-evoked myogenic potential test in a guinea pig after receiving general anesthesia reveals no evoked potentials at the 6- to 9-millisecond latency. A, Right side recording; B, left side recording (acoustic stimulation intensity from 130- to 70-dB peak equivalent sound pressure level). B (A) indicates bilateral stimulation with monaural recordings.

Figure 2. Absence of VEMPs in a guinea pig immediately following short-term noise exposure. On post-noise exposure day 2, VEMPs returned to normal.
curred on post–noise exposure day 4 (Table). Figure 3 shows a guinea pig with an ABR threshold of 75-dB pe SPL immediately after short-term noise exposure, which returned to a threshold of 45-dB pe SPL on post–noise exposure day 4.

Comparing the abnormal percentages between VEMP and ABR revealed statistically significant differences on post–noise exposure days 0 through 3 ($P < .05$, McNemar test). However, no statistically significant difference (100% vs 100%) was observed on post–noise exposure days 4 and 7 ($P \geq .05$, McNemar test; Table). Trends in recovery of VEMP responses and ABR thresholds demonstrated by a recovery curve from Kaplan-Meier survival analysis revealed that VEMP responses and ABR thresholds recovered within 2 and 4 days, respectively, indicating a significant difference ($P < .001$, log-rank test; Figure 4A).

**LONG-TERM NOISE EXPOSURE**

Absent percentages of VEMPs in group B (9 animals, 18 ears) during the pre–noise exposure period and on post–noise exposure days 0, 3, 7, 10, and 30, were 0%, 100%, 94%, 83%, 78%, and 78%, respectively, showing statistically significant differences between the pre–noise exposure period and conditions on post–noise exposure days 0 through 3 ($P < .001$, McNemar test; Table). In addition, mean (SD) ABR thresholds for the test animals after long-term noise exposure on post–noise exposure days 0, 3, 7, 10, and 30 were 91.1 (8.0)-, 75.0 (9.9)-, 68.1 (7.9)-, 65.0 (7.7)-, and 66.9 (7.1)-dB pe SPL, respectively, revealing permanent ABR threshold shifts on post–noise exposure days compared with the pre–noise exposure threshold ($P < .001$, paired $t$ test). Thus, abnormal percentages for ABR in group B were 100%, 94%, 89%, 83%, and 83% on post–noise exposure days 0, 3, 7, 10, and 30, respectively.

Comparing the abnormal percentages between VEMP and ABR in group B revealed no statistically significant differences on post–noise exposure days 0 through 30 ($P \geq .05$, McNemar test; Table). The recovery curves of group B produced by the Kaplan-Meier survival analysis method displayed a critical 10-day period for recovery of VEMP responses and ABR thresholds. Notably, the recovery curves of VEMP responses and ABR thresholds did not differ significantly ($P \geq .05$, log-rank test; Figure 4B). Beyond 10 days following long-term noise exposure, absent VEMPs and permanent ABR threshold shifts were observed.

In control group C, all animals showed normal VEMPs and ABRs throughout the study period.

**MORPHOLOGICAL ASSESSMENT IN ANIMALS**

**After Short-term Noise Exposure**

One week after short-term noise exposure, guinea pigs in group A with normal VEMPs were killed for morphological assessment. On light microscopic examination, hair and supporting cells of the saccular macula were in-
tact. Normal otolithic membrane with numerous small crystalline bodies (otoconia) was shown (Figure 5A).

Ultrastructurally, both flask-shaped type I and cylindrical type II hair cells of the saccular macula remained intact. One kinocilium and many stereocilia were observed on the top of the hair cells. Type I hair cells had a spherical nucleus and an afferent nerve chalice almost completely surrounding the entire cell. Type II hair cells had buttonlike attachments of both afferent and efferent nerve endings. The supporting cells, basement membrane and vestibular nerve fiber remained intact (Figure 6A).

After Long-term Noise Exposure

One month after long-term noise exposure, group B animals with absent VEMPs were killed for morphological assessment. On light microscopic examination, the cell bodies of the hair cells in the saccular macula showed signs of disruption and atrophy, eg, hair cells missing from the neuroepithelium or lucent areas between hair cells. The supporting cells, otolithic membrane, and otoconia were normal (Figure 5B).

Ultrastructurally, numerous vacuoles in the cytoplasm or loss of nucleus were found in many type I hair cells, but this was rarely seen in type II hair cells. The supporting cells, basement membrane and vestibular nerve fiber remained intact (Figure 6B).

Although temporary or permanent threshold shifts in hearing following extreme noise exposure is well known to occur in both humans and animals, relatively few studies have examined similar phenomena in the vestibular system, possibly because the semicircular canals are less sensitive to impulse noise, even at very high intensities. However, vestibulo-ocular reflex gain can be enhanced by sound when the labyrinth is opened, eg, superior semicircular canal dehiscence, a condition termed Tullio phenomenon. This designation is currently applied to patients in whom there is evidence of vestibular activation in response to acoustic stimulation, manifested as vertigo, imbalance, or oscillopsia. Note that the auditory sensitivity of the saccule is mirrored by the sensitivity of the semicircular canals to sound: canal dehiscence is the most obvious example.

Excluding the Tullio phenomenon, vestibular symptoms in patients with acute acoustic trauma may be related to functional impairment of the saccule. This is probably due to the saccule retaining an ancestral acoustic sensitivity in humans; anatomical proximity of the saccule to the footplate of stapes also suggests that acoustic trauma may be associated with saccular damage. Furthermore, the membrana limitans and trabecular meshwork act as a barrier, leading to differential sensitivity of cochlear and vestibular sensory cells in the presence of noxious substances. Although the cochlear duct develops from a comparatively large saccule, both cochlear and saccular partitions display different neuronal circuit routes, ie, the auditory brainstem system and sacculocollic reflex system, respectively. Hence, this study applied both ABR and VEMP tests to assess how noise effects the peripheral auditory and vestibular systems of guinea pigs.

Figure 3. Auditory brainstem response (ABR) from the right ear of a guinea pig at day 0 (A) and day 4 (B) after noise exposure. The ABR threshold is 75-dB peak equivalent (pe) sound pressure level on day 0 and 45-dB pe SPL on post–noise exposure day 4. R (A) indicates right ear stimulation with monaural recordings.
Unlike well-known ABR studies in experimental animals, the VEMP test in alert guinea pigs is a recent procedure. To verify that recorded potentials are not contaminated from ABR responses, animals have been anesthetized with pentobarbital sodium intraperitoneally. General anesthesia causes muscle relaxation, and relaxation of the neck muscles may abolish VEMPs entirely. In the present study, the fact that during acoustic stimulation no animals displayed evoked potentials from a neck muscle at the 6- to 9-ms latency after receiving general anesthesia (Figure 1) further supports the myogenic origin of these biphasic responses.

Following short-term (30 minutes) noise exposure, 70% of the ears exhibited temporary VEMP loss. However, VEMPs recovered in 90% of the ears within 2 days (Table and Figure 2). Likewise, temporary threshold shifts in ABR were observed in 97% of the ears immediately after exposure, which resolved within 4 days (Table and Figure 3). Recovery of VEMPs preceded ABR thresholds returning to normal by 2 days in guinea pigs following short-term noise exposure (Figure 4), indicating that a rest period of 48 to 96 hours following noise exposure is effective for restoring both cochlear and saccular function in guinea pigs.

Because a single discharge from a high-powered rifle can result in as much damage as 40 hours of continuous exposure at 90 dB (A-weighted), animals in group B were subjected to long-term (40 hours) noise exposure. Permanent VEMP loss and ABR threshold shifts were noted in 78% and 83% of the ears, respectively, 10 days following noise exposure (Table). Restated, beyond a critical 10-day period, most (>70%) ears exhibited irreversible changes in ABR thresholds and VEMPs (Figure 4). Yamashita et al reported that reactive oxygen species and reactive nitrogen species (as determined by 4-hydroxynonenal and nitrotyrosine immunoreactivity) increased from post–noise exposure days 3 to 7, with maximum expression at days 7 to 10; ABR threshold deficits and hair cell loss correspondingly plateaued at post–noise exposure days 7 to 10, which is compatible with our results. Because intense noise exposure leads to a prolonged set of biochemical processes that determine the final level of tissue damage, a critical 10-day period of recovery could be a window of opportunity to treat the acoustic trauma with, for example, antioxidants or steroids.

McCabe and Lawrence observed damage to the otolithic membrane and collapse of the saccule in a study of animals exposed to a noise intensity of 136- to 150-dB SPL for 20 minutes. However, in the present study, contours of the saccule and otolithic membrane remained

![Figure 4](https://archotol.jamanetwork.com/)

**Figure 4.** Recovery curves of vestibular-evoked myogenic potential (VEMP) responses and auditory brainstem response (ABR) thresholds after short-term (A) and long-term (B) noise exposure by the Kaplan-Meier survival analysis method. The interval between recovery of VEMP responses and ABR thresholds is 2 days (median) (A). A critical 10-day period is required for recovery of both VEMP responses and ABR thresholds; beyond 10 days, neither VEMP nor ABR exhibits further significant change (B).

![Figure 5](https://archotol.jamanetwork.com/)

**Figure 5.** Light microscopic photomicrographs of the saccule. A, Guinea pig with normal vestibular-evoked myogenic potentials (VEMPs) 1 week after short-term noise exposure. B, Guinea pig with absent VEMPs 1 month after long-term noise exposure. Note that the loss (black arrow) and disruption (white arrow) of hair cells in the saccular macula are only found in panel B but not in panel A. Otoconia (arrowheads) are normal in both A and B. OM indicates otolithic membrane. (Toluidine blue stain, scale bar=200 µm.)
intact after both short- and long-term noise exposure, possibly due to the lower noise intensity (mean [SD], 115 [5]-dB SPL) applied. Nevertheless, in long-term noise-exposed guinea pigs with absent VEMPs, numerous vacuole formations were observed in type I hair cells of the saccular macula (Figure 5B and Figure 6B). Curthoys et al reported that irregular primary otolithic afferent neurons of the guinea pigs are especially sensitive to bone conducted vibration. In addition, the physiological evidence showed that irregular afferents contact type I hair cells preferentially. Therefore, there are some grounds for arguing that VEMPs are probably reflecting type I hair cell activity.

Clinically, patients with acute acoustic trauma often express concern about hearing loss. Although distortion-product otoacoustic emission can test the viability of the outer hair cells, it fails to predict the hearing outcome when temporary threshold shifts greater than 70-dB hearing level or cochlear damage is confined to the inner hair cells. Alternatively, the VEMP test may provide another clue for assessing the hearing outcome, as evidenced by a recent report that absent or delayed VEMPs in patients after acute acoustic trauma may indicate poor prognosis with respect to hearing improvement. Thus, clinical findings support our experimental results indicating that permanent saccular functional loss following noise exposure may reflect permanent hearing threshold shifts.

Submitted for Publication: August 10, 2007; final revision received November 3, 2007; accepted January 22, 2008.

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Author Contributions: Dr Young had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Acquisition of data: Hsu, Lue, and Day. Analysis and interpretation of data: Wang and Young. Drafting of the manuscript: Hsu, Wang, and Lue. Critical revision of the manuscript for important intellectual content: Day and Young. Statistical analysis: Hsu and Wang. Administrative, technical, and material support: Lue and Day. Study supervision: Young.

Financial Disclosure: None reported.

Funding/Support: This study was supported by grant NSC 96-2341-B002-135-MY3 from the National Science Council, Taipei, Taiwan.

REFERENCES

4. Ylikoski J, Juntunen J, Matikainen E, et al. Subclinical vestibular pathology in


**Correction**

Error in Byline. In the Original Article by Otteson et al titled "Acute and Chronic Changes in the Subglottis Induced by Graded Carbon Dioxide Laser Injury in the Rabbit Airway," published in the July 2008 issue of the Archives (2008;134[7]:694-702), the academic degree of one of the authors is incorrect. The author’s name and degree are Gregory M. DiSilvio, BS.