Published in final edited form as:

J Neurol Sci. 2011 July 15; 306(1-2): 29–37. doi:10.1016/j.jns.2011.04.003.

Assessment of Auditory Brainstem Function in Lead-Exposed Children Using Stapedius Muscle Reflexes

S.A. Counter, D.M.Sc., Ph.D.^{1,2}, Leo H. Buchanan, Ph.D.^{3,4}, Fernando Ortega, M.D., Ph.D.⁵, Jeannette van der Velde, MPH, MSc⁶, and Erik Borg, M.D., Ph.D.⁷

¹Department of Neurology, Harvard Medical School/The Biological Laboratories, Cambridge, Massachusetts, USA

²Department of Neurophysiology, Massachusetts General Hospital, Boston, Massachusetts, USA

³Department of Pediatrics, University of Massachusetts Medical School/Eunice Kennedy Shriver Center, Waltham, MA, USA

⁴Department of Otolaryngology, Harvard University Health Services, Cambridge, MA USA

⁵Integrated Community Development Department, College of Health Sciences, Universidad San Francisco de Quito, Quito, Ecuador

⁶Department of Laboratory Medicine, Children's Hospital Boston, Boston, Massachusetts, USA

⁷Audiological Research Center (Ahlsens), University Hospital of Örebro, Sweden

Abstract

The purpose of this study was to investigate the neurological integrity and physiological status of the auditory brainstem tracts and nuclei in children with chronic lead (Pb) exposure using non-invasive acoustic stapedius reflex (ASR) measurements of afferent and efferent-neuromuscular auditory function. Following audiological examinations, uncrossed (ipsilateral) and crossed (contralateral) brainstem ASR responses were evoked by pure tone (500, 1000, and 2000 Hz), and broadband noise (bandwidth: 125-4000 Hz) stimulus activators. The ASR threshold (ASRT), amplitude growth, and decay/fatigue were measured by conventional clinical middle ear immittance methods in a group of Andean children (age range: 2-18 years) with a history of chronic environmental Pb exposure from occupational Pb glazing. Blood lead (PbB) levels of the study group (n = 117) ranged from 4.0 to 83.7 µg/dL with a mean PbB level of 33.5 µg/dL (SD: 23.6; median: 33.0; CDC III Classification). The PbB distribution data indicated that 77.8% (n = 91) of the children had PbB levels greater than the CDC action line of 10 µg/dL. Repeatable, normal ASRTs were elicited for ipsilateral (mean: < 90 dB HL) and contralateral (mean: < 97 dB HL) stimulation for each acoustic activator. Spearman Rho correlation analysis indicated no significant association between PbB level and ipsilateral or contralateral ASRT for any of the stimulus activators. The ASR amplitude growth results showed typical growth functions with no Pb-associated aberrations. No statistical association was found between ASR decay/adaptation (ASRD) and PbB level for any of the stimulus activators. The results of stapedius muscle reflex testing using several stimulus activators showed no significant relationship between PbB level and

© 2010 Published by Elsevier B.V.

Address correspondence to: Dr. S. Allen Counter, Department of Neurology, Harvard Medical School, The Biological Laboratories, 16 Divinity Avenue, Cambridge, MA 02138 USA, Telephone: (617) 495-1527, FAX: 781 642-0238, allen_counter@harvard.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

the physiological integrity of the auditory brainstem mediated ASR responses in children with chronic Pb exposure and elevated PbB levels.

Keywords

middle ear muscle; stapedius; acoustic reflex; auditory nerve; facial nerve; impedance; immittance; lead poisoning; brainstem; auditory nervous system

1. Introduction

Pediatric lead poisoning (Pb) has been associated with a range of neurodevelopmental disabilities in children [1-7]. Neurocognitive deficits have been reported in children at low Pb exposure levels [5,8,9], with signs of intellectual impairment below a blood lead (PbB) level of 10 µg/dL (Centers for Disease Control and Prevention's [CDC] action line). Pb exposure has been found to induce focal central nervous system damage, primarily in the frontal lobes and hippocampus, as well as sensory and motor impairment [10,11]. Of particular interest to this study, some investigators have reported Pb-induced hearing disorders in children, and have suggested that the cognitive deficits observed in Pb-exposed children may be related to auditory impairment [2,12,13]. However, the locus of the reported Pb-induced auditory sensory-neural damage has not been unequivocally identified in the peripheral or central auditory systems.

Using auditory brainstem evoked responses (ABR) in human subjects, some investigators have localized Pb-induced injury to the auditory brainstem pathways [14-19]. Similarly, experimental studies in Pb-exposed animals have reported neural damage at the level of the auditory brainstem [20-22]. For example, recent experimental studies in animals that were systematically exposed to Pb have shown specific areas of neuronal damage in the superior olivary complex, an integral component of the bilateral ascending auditory tracts and nuclei in the brainstem [20,23]

Our previous studies of the effects of chronic Pb exposure on the peripheral and central auditory systems of children with high PbB levels, using non-invasive measures of inner ear (pure-tone behavioral thresholds, and otoacoustic emissions) and auditory brainstem function, have shown no unequivocal evidence of Pb-induced injury to the cochlea, 8th cranial nerve, and auditory brainstem ascending tracts, which includes the putative origins of the summated auditory brainstem evoked potentials: cochlear nucleus, superior olivary complex, and inferior colliculus [24-26]. Further, the absence of electrophysiological ABR evidence of anomalies of the brainstem auditory pathways in chronically Pb-exposed children tested in our earlier studies may indicate that the ABR technique lacks the sensitivity to consistently detect clinical or subclinical Pb-induced neuronal damage in the brainstem.

Although the ABR test has served as the primary clinical tool for examining the status of the auditory brainstem neuronal tracts and nuclei, another noninvasive physiological method used to examine the auditory brainstem integrity is the acoustic stapedius reflex (ASR) test. The ABR and the ASR involve elements of the same ascending auditory pathways, including the 8th cranial nerve, and in the brainstem, the cochlear nucleus and superior olivary complex. The superior olivary complex has been reported to be a brainstem target of Pb exposure in experimental mammals [21,23]

The mammalian ASR represents a multi-synaptic brainstem chain that involves both sensory and motor functions. The acoustically-activated sensory afferent bipolar neurons of the

auditory nerve synapse in the cochlear nucleus, which sends neuronal projections to both the ipsilateral (uncrossed) and contralateral (crossed) superior olives. The superior olive sends axonal projections to neurons in and around the nucleus of the 7th cranial nerve. The motoneurons of the facial nerve send projections through their stapelial branches to innervate the stapedius muscles bilaterally. Similar to the bilateral pupillary reflex contraction of the iris sphincter muscle of the eye in response to a unilateral bright light stimulus, the bilateral pathway of the acoustic stapedius reflex enables contraction of the muscles in both the right and left middle ear cavities when only one ear is stimulated. The facial nerve motoneurons conduct action potentials to terminals that form cholinergic neuromuscular synapses with the stapedius muscles, completing the auditory brainstem stapedius reflex arc [27,28].

The motor activated component of this synaptic arc involves the contraction of the stapedius muscle, the smallest human skeletal muscle, and movement of its ligament, resulting in the abduction of the stapes footplate from the oval window of the inner ear, thereby protecting the cochlear receptors from intense acoustic stimuli. The ASR is biologically activated by loud ambient sounds, and internal noises such as vocalization, chewing and crying. During contraction of the stapedius muscles, low frequency sounds are attenuated, thereby improving auditory discrimination in noisy environments [29]. The contraction of the stapedius muscle results in an increase in the impedance of the middle ear system, which can be measured non-invasively with an electro-acoustic impedance/admittance or immittance measurement system. It may be inferred from some human and experimental animal studies that prolonged or chronic Pb exposure that results in elevated PbB levels will impair auditory brainstem neuronal and synaptic functions that mediate the ipsilateral and contralateral ASR.

The acoustic stapedius reflex test procedure is a well-established clinical tool that has been used to examine patients with a spectrum of neurological disorders, [30,31] including myasthenia gravis, and multiple sclerosis, as well as neuro-otological diagnosis of brainstem lesions, auditory neuropathy spectrum disorder, Bell's palsy, acoustic neuromas, and hydranencephaly [29,32-36]. It has also been used as an objective physiological measure of the hearing status of infants, and patients who are unable to give conventional behavioral responses to auditory stimulation [37]. To our knowledge, there have been no other systematic clinical studies of brainstem auditory function in children with chronic Pb exposure using the non-invasive ASR immittance technique to examine the sensory-motor activity of the stapedius reflex arc.

As part of our comprehensive auditory clinical test battery, we conducted diagnostic acoustic immittance measurements on Pb-exposed children, using standard clinical procedures to evoke the ASR. The ASR recordings from a group of Pb-exposed children were analyzed in the current study to determine the effects of chronic Pb poisoning on the auditory brainstem neurons and neuromuscular function of the facial nerve terminals and the stapedius muscle. We hypothesized that Pb-induced insult in any part of the brainstem stapedius arc would be reflected in elevated ASR thresholds (ASRT), decreased ASR amplitude growth, and/or rapid stapedius muscle contraction decay/fatigue, as indicated by ASR response decay times.

2. Participants and Methods

Study Area

This field study was conducted in two rural ceramic production and Pb-glazing Andean villages of La Victoria and Racar, Ecuador. La Victoria is a village in Pujilí County, Cotopaxi Province, and is located about 125 km south of Quito, Ecuador at an altitude of

approximately 2,850 meters in the Andes Mountains. The village of Racar is located approximately 15 km west of the southern Ecuadorian city of Cuenca in Azuay Province at an altitude of approximately 2500 meter above sea level. The inhabitants of both villages are exposed to Pb from a ceramics Pb-glazing cottage industry that produces artisan crafts and roof tiles. The inhabitants of the study area are of low socioeconomic and educational status. The population of the study area is largely homogeneous, and most families engage in Pb-glazing occupational practices, and have similar pathways of environmental Pb exposure. The Pb-glazing process and areas of Pb contamination in the milieu of the study area have been described in detail elsewhere [38]

Study Participants

The ASR was examined with one or more stimulus activators in 117 children. The participants ranged in age from 2-18 years (mean age: 9.9 yrs.; SD: 3.2), and included 60 females (mean age: 9.9 yrs.; SD: 3.3; age range: 2-18), and 57 males (mean age: 9.9 yrs; SD: 3.1; age range: 3-18). The children in both study areas were accompanied by their parents, guardians, or teachers of the local schools for testing. Informed consent was obtained from the parents/guardians of the children who participated in the study. This study was conducted under the auspices of Universidad San Francisco de Quito, and was approved by the Comité de Bioética (Human Studies Committee) of the Universidad San Francisco de Quito.

Blood Lead Analysis

Blood samples were collected by trained Ecuadorian medical personnel at the local infirmary or

in our field clinic. Following a thorough cleaning of the skin using isopropanol swabs, 2-4 mL of blood were drawn from the antecubital vein of each participant and stored in 4 mL-Vacutainer tubes with Li-heparin. All blood samples were stored in a refrigerated container and later analyzed for Pb concentrations by graphite furnace atomic absorption spectrometry (GFAAS) with Zeeman background correction at Boston Children's Hospital Chemistry Laboratory, or by inductively coupled plasma mass spectrometry (ICP-MS) at the Channing Trace Metals Laboratory of the Harvard School of Public Health. Previous investigations by the authors have shown that these two PbB analysis procedures are comparable [39].

The PbB test results for the children were summarized by grouping them in accordance with the

classification system of the Centers for Disease Control and Prevention (CDC, 1991) [40], which $\mu\text{g/dL}$: abnormally elevated PbB level; frequent re-screening and community Pb prevention include the following Classifications: I ($< 10 \mu\text{g/dL}$: not considered Pb-poisoned), IIA (10-14.9 activities recommended), IIB (15-19.9 $\mu\text{g/dL}$: nutritional, educational and environmental interventions and frequent screening recommended), III (20-44.9 $\mu\text{g/dL}$: environmental evaluation and remediation, and medical evaluation recommended; pharmacological treatment may be indicated), IV (45-69.9 $\mu\text{g/dL}$: environmental and medical interventions, including chelation therapy recommended), and V ($> 70 \mu\text{g/dL}$: medical emergency; immediate medical and environmental management recommended). The CDC Classification I is currently used as an international guideline for monitoring childhood Pb poisoning.

Parents and guardians of the participants were advised of the current PbB levels of their children, and given instructions on ways to avoid or minimize Pb exposure. Children were referred to Ecuadorian physicians for medical treatment where appropriate.

Auditory Test Procedures

Each participant was given a conventional audiologic examination, which included otoscopy, pure-tone threshold measurements, and tympanometry, to examine the status of the middle system, and to rule out hearing loss before conducting ASR tests. All ASR test data were obtained concurrently with the collection of blood samples from the study participants, thus precluding any inadvertent investigator bias. The physiological contraction of the stapedius muscle may be measured directly by electromyography [41,42], or indirectly as impedance or admittance changes recorded with an electroacoustic immittance system [29,42]. In the current study, the ASR was recorded indirectly with the Grason-Stadler GSI 33 and the Grason-Stadler TympStar immittance systems using a probe tone frequency of 226 Hz. The probe was hermetically sealed in the external auditory meatus of the ear under test, and tympanometry was performed to determine the status of the tympanic membrane and the middle ear system. The ASR responses were measured ipsilaterally and contralaterally in subsets (Tables 1 and 2) of the total study group using pure-tone stimulus activators at sinusoidal frequencies of 500, 1000, and 2000 Hz, and broadband noise (BBN) (bandwidth = 125-4000 Hz). Unlike in a controlled clinical environment, in field investigations, there are inherent uncontrollable variables and constraints, such as participants' availability, work and school schedules, and time limitations. Because of some of these constraints, ipsilateral and contralateral ASR recordings for the stimulus activators of 500, 1000, 2000 and broadband noise used in our standard clinical battery could not be obtained on each participant in the study group.

Initial ASR screening was conducted at 1000 Hz (114 participants) and at 2000 Hz (117 participants) since these frequencies show better ASRT than the lower or higher frequencies [43,44,45]. The ipsilateral ASR was measured with the activator stimulus and the immittance measuring probe in the same ear to record the reflex response from the uncrossed reflex arc. The contralateral ASR was elicited with the probe in one ear, and the stimulus activator delivered by an insert earphone to the contralateral ear in order to measure the crossed brainstem reflex arc. The ASRT, amplitude growth, and acoustic stapedius reflex decay (ASRD) were analyzed for all activator stimuli. The ASRD was defined as the time in seconds at which the stapedius muscles relaxes to 50% of its initial peak contraction (as indicated by the amplitude of the recorded deflection) over a 10-second period. Thus, a reduction in response amplitude of 50% or greater within a 10-second period (i.e., ASR half-life of < 10 seconds) was considered abnormal decay or abnormal muscle adaptation. Stimulus presentations of increasing intensity increments of 5 dB were used to evoke and confirm ASRT responses, and to measure amplitude growth over a 15-dB range above threshold (sensation level [SL]) in order to evaluate the dynamic range or magnitude of the stapedius muscle contraction. The ASRT was defined as the lowest stimulus intensity level (measured in clinical test units as hearing level [HL] in dB) at which a reproducible ASR deflection from baseline (representing a minimum of 0.1 cm³ change in immittance). Ipsilateral and contralateral ASRD were determined at 500, 1000, and 2000 Hz, and for BBN.

Statistical Analysis

Means, standard deviations, medians, and ranges were computed for PbB level, ASRT, ASR amplitude growth, and ASRD. Since many of the variables had skewed distributions, nonparametric statistical tests, as well as parametric tests, were used for data analysis. The associations of PbB level with ASRT, and PbB level with ASRD rate were probed with the

Spearman correlation coefficient. The Mann-Whitney *U* test was used to evaluate the difference between the growth function for the ipsilateral and contralateral conditions. An *a priori* alpha level of < 0.05 was accepted as statistically significant.

3. Results

Blood Lead (PbB) Levels

The PbB levels for the pool of 117 participants in the Pb-contaminated Ecuadorian study sites ranged from 4.0 to 83.7 $\mu\text{g/dL}$, with a mean PbB level of 33.5 $\mu\text{g/dL}$ (SD: 23.6; median: 33.0; CDC Classification III). The PbB distribution data indicated that 77.8% ($n = 91$) of the children had PbB levels greater than the CDC action line of 10 $\mu\text{g/dL}$. Twenty-nine percent of the children ($n = 34$) had elevated PbB levels in the CDC IV Classification (45–69.9 $\mu\text{g/dL}$), and 6% ($n = 7$) were in the CDC V Classification ($> 70 \mu\text{g/dL}$), both of which call for medical intervention.

Acoustic Stapedius Reflex

Ipsilateral and Contralateral ASR thresholds—The means, standard deviations, and percentiles of the ASRT for the four ipsilateral and contralateral reflex activators (500, 1000, 2000 Hz, and BBN) are presented in Tables 1 and 2. The mean ASRT for the ipsilateral condition ranged from a high of 90 dB for the 500 Hz stimulus activator to a low of 71 dB for the BBN activator. The mean ASRT for the contralateral condition ranged from a high of 97 dB at 500 Hz to a low of 79 dB for BBN. Figure 1 shows the means of the ipsilateral (A) and contralateral (B) ASRTs for the study group compared to thresholds obtained from a non-Pb exposed population in a normative study of young adults [45]. The dotted lines in Figure 1 A, B represent +2 standard deviations for the normative or reference group. As can be seen from Figure 1, the mean ipsilateral and contralateral ASRTs at each activator stimulus for the Pb-exposed children in the study group are similar to the normative data obtained on the normal-hearing, non-Pb exposed population reported by Wiley, et al. [45]. Paired *t*-test analysis showed that the ipsilateral ASRTs for 500 Hz ($t = 5.204$, $p = < 0.0001$), 1000 Hz ($t = 3.485$, $p = 0.001$), and BBN ($t = 4.623$, $p = < 0.0001$) stimulus activators were elicited at a significantly lower HL than the contralateral ASRTs. The 2.4 dB difference between the ipsilateral and contralateral ASRTs at 2000 Hz was not statistically significant ($t = 1.604$, $p = 0.115$). Spearman Rho correlation analyses presented in Tables 3 and 4 indicate that there was no significant relationship between PbB level and the ipsilateral or contralateral ASRT for the pure-tone and noise activators.

Ipsilateral and Contralateral ASR Amplitude Growth—Figure 2 A, B, C, D compares the growth functions for the ipsilateral and contralateral ASR amplitudes at threshold (0 dB SL), at 5 dB SL, 10 dB SL, and at 15 dB SL for the stimulus activators of 500, 1000, and 2000 Hz and for BBN. Statistical analyses using the Mann-Whitney *U* test revealed the mean ASR ipsilateral growth magnitude to be significantly steeper than the contralateral growth magnitude for the 500 Hz stimulus activator at 10 dB SL (tied $Z = -1.951$, tied $p = 0.051$) and 15 dB SL (tied $Z = -2.310$, $p = 0.020$). For 1000 Hz, the ipsilateral growth magnitude was significantly greater than the contralateral growth magnitude at 15 dB SL (tied $Z = -2.554$, tied $p = 0.010$) only. At 2000 Hz, the ipsilateral growth magnitude was significantly steeper than the contralateral growth magnitude at 10 dB SL (tied $Z = -2.275$, tied $p = 0.023$) and at 15 dB SL (tied $Z = -2.858$, tied $p = 0.004$). The BBN stimulus activator showed similar ASR amplitude growth functions for the ipsilateral and contralateral ASR conditions, with no statistically significant differences at any of the SLs. In an effort to further probe the growth function between the uncrossed and crossed ASR, the ipsilateral/contralateral ratios at peak amplitude (15 dB SL) were calculated. The ASR ipsilateral/contralateral amplitude growth ratios at 15 dB SL were 1.66

(SD: 1.09) for 500, 2.13 (SD: 2.13) for 1000, 1.73 (SD: 1.39) for 2000, and 2.07 (SD: 2.15) for BBN. The amplitude growth ratio values at peak SL (15 dB) was found to have no statistically significant association with PbB levels.

Ipsilateral and Contralateral ASR Decay—The mean ipsilateral ASRD times for the 500 Hz stimulus activator were 8.1, 9.1, and 9.3 seconds for 5, 10, and 15 dB SL, respectively. The median ASRD for 500 Hz was 10 seconds for each of the three SLs. The mean ipsilateral ASRD times at 1000 Hz were 6.4, 8.7, and 8.8 seconds for 5, 10, and 15 dB SL, respectively. The median ASRD times for 1000 Hz were 8, 10, and 10 seconds for 5, 10, and 15 dB SL, respectively. For 2000 Hz, the mean ipsilateral ASRD times were 5.4, 6.5, and 5.7 seconds at 5, 10, and 15 dB SL, respectively (median: 4.5, 5.5, and 6.0 for 5, 10 and 15 dB SL, respectively indicating decay or muscle fatigue at less than 10 seconds. Ipsilateral ASRD was not recorded for BBN. The mean contralateral or crossed brainstem reflex arc ASRD times at 500 Hz were 8.3, 9.3, and 9.3 seconds for 5, 10, and 15 dB SL, respectively. The median contralateral ASRD for 500 Hz was 10 seconds for each of the three SLs. The mean contralateral ASRD times at 1000 Hz were 8.7, 9.1, and 9.0 seconds for 5, 10, and 15 dB SL, respectively. The median contralateral ASRD for 1000 Hz was 10 seconds for each SL. Similar to the ipsilateral ASRD times at 2000, the mean contralateral ASRD times at 2000 Hz showed values of 7.3, 7.3, and 7.4 seconds for 5, 10 and 15 dB SL, respectively (median = 9, 8, and 8 seconds for 5, 10 and 15 dB SL, respectively), indicating decay or muscle fatigue at less than 10 seconds. For the BBN activator, the mean contralateral ASRD times were 8.7, 8.9, and 8.9 for 5, 10, and 15 dB SL, respectively. The median ASRD time was 10 second for each SL. Paired *t*-tests analysis of ASRD rates as a function of SL of the stimulus activators revealed statistically significant differences among SLs only for the ipsilateral 1000 Hz (5 vs. 10 dB SL, and 5 vs. 15 dB SL) and the contralateral 2000 Hz (5 vs. 10 dB SL) conditions. A Spearman rho correlation analysis showed no significant association between PbB level and ipsilateral or contralateral ASRD for the stimulus activators of 500, 1000, 2000 Hz and BBN at any of the three SLs (5, 10, 15 dB).

Case Illustrations

Case Illustrations 1 and 2

The ASR recordings in Figure 3 A, B illustrate the contralateral or crossed brain ASRT and the ASRD morphology in response to a 1000 Hz stimulus activator for an 8-year-old male with a low PbB level of 5 $\mu\text{g/dL}$ (CDC I Classification), and the recordings in Figure 3 C, D show the responses to a BBN stimulus activator for a 13-year-old female with an elevated PbB level of 57 $\mu\text{g/dL}$ (CDC IV Classification). The 1000 Hz pure-tone stimulus activator shown in Figure 3A for the 8-year-old child with a PbB of 5 $\mu\text{g/dL}$ elicited a contralateral ASRT response within the normal range at 95 HL (0.02 cm^3), with amplitude growth up to 105 dB HL (0.06 cm^3 change in acoustic immittance), and no further increase in response amplitude at 15 dB SL. The contralateral ASRD (Figure 3B) recording at 1000 Hz showed a robust deflection from baseline with no reduction in ASR amplitude at 10 dB and 15 dB SL. For the 13-year-old participant with an elevated PbB level of 57 $\mu\text{g/dL}$, the BBN stimulus activator shown in Figure 3C evoked a contralateral ASRT response at 75 dB HL, with a systematic increase in ASR amplitude growth up to 95 dB HL. Consistent with previous research and clinical experience, the ASRT for the BBN stimulus activator in the current study is 20 dB lower than that of the tonal activator (shown in Figure 3A), the participant's high PbB level of 57 $\mu\text{g/dL}$ notwithstanding. The ASRD recordings in Figure 3D at both 10 dB SL (85 dB HL) and 15 dB SL (90 dB HL) show a normal sustained stapedius contraction at peak amplitude with no significant amplitude reduction until stimulus offset at 10 second, at which time the stapedius muscle relaxes, and the immittance value returns to baseline.

Case Illustration 3

The ASR recordings in a 5-year-old male participant with a high PbB level of 65 $\mu\text{g/dL}$ (CDC IV Classification) are illustrated in Figure 4. The recordings demonstrate ipsilateral or uncrossed (Figure 4 A, B), and contralateral or crossed (Figure 4C, D) ASRTs and ASRD at 1000 Hz. The 1000 Hz stimulus activator in Figure 4A elicited an unequivocal ipsilateral ASRT response at 80 dB HL (0.02 cm^3), and a contralateral ASRT (Figure 4C) at 90 dB HL, both within the normal range. Both the ipsilateral and contralateral recordings show amplitude growth with an increase in intensity up to 15 dB SL (ipsilateral) and 10 dB SL (contralateral). The ipsilateral (Figure 4B) and contralateral (Figure 4D) ASRD recordings at 10 dB SL (90 and 100 dB HL, respectively) revealed normal, sustained stapedius muscle contractions at peak amplitude without significant amplitude reduction until stimulus offset at 10 second, at which time the stapedius muscle relaxes, and the immittance value returns to baseline.

Case Illustration 4

Figure 5 illustrates contralateral ASR recordings for 1000 Hz (Figure 5A, B) and 2000 Hz (Figure 5C, D) stimulus activators for an 11-year-old female with a higher PbB level of 71 $\mu\text{g/dL}$ (CDC V Classification). The contralateral ASR was elicited within the normal range at 95 dB HL (0.02 cm^3) for the 1000 Hz tone activator (Figure 5A), and at 100 dB HL (0.06 cm^3) for the 2000 Hz stimulus activator (Figure 5C). The contralateral ASRD recordings for 1000 Hz (Figure 5 B) at 5 dB SL (100 dB HL) and 10 dB SL (105 dB HL) both showed normal sustained stapedius contraction at peak amplitude without significant amplitude reduction until stimulus offset at 10 second, at which time the stapedius muscle relaxes, and the immittance value returns to baseline. The ASRD recordings for 2000 Hz (Figure 5 D) showed a sustained stapedius muscle contraction at threshold (100 dB HL) and at 5 dB SL (105 dB HL) over a 10-second period, indicating a sustained stapedius muscle contraction with no significant ASR adaptation or decay. In summary, this study participant with a PbB level of 71 $\mu\text{g/dL}$ showed normal ASR functioning, implying that the 8th cranial nerve and the auditory brainstem neurons and synapses involved in ASR mediation are unimpaired by her high PbB level.

Case Illustrations 5 and 6

The ASR and ASRD responses to a contralateral 2000 Hz stimulus activator from a 9-year old female with an extremely elevated PbB level of 83 $\mu\text{g/dL}$ (CDC V Classification) are illustrated in Figure 6A and B. The contralateral ASRT recordings in Figure 6A illustrate a threshold deflection at 95 dB HL (0.02 cm^3) with increases in the amplitude of the recording over a range of 10 dB SL. The ASRD recordings in Figure 6B show normal sustained muscle contractions over a 10-second period at 10 and 15 dB SLs. Figure 6C, D shows the contralateral or crossed ASRT and ASRD recordings from another child (an 11-year-old male) with extreme plumbism, presenting with a PbB level of 84 $\mu\text{g/dL}$ (CDC V Classification). The 1000 Hz stimulus activator in Figure 6C elicited an ASRT response at 90 dB HL (0.02 cm^3) with an increase in amplitude growth up to 100 dB HL (0.07 cm^3). The extremely elevated PbB level notwithstanding, the ASRD recordings at both 10 dB SL (100 dB HL) and 15 dB SL (105 dB HL) revealed a normal sustained stapedius contraction at peak amplitude with no amplitude reduction until stimulus offset at 10 second, at which time the stapedius muscle relaxes, and the immittance value returns to baseline. The normal ASR results from these two children suggest that the inner ear, 8th cranial nerve and the auditory brainstem neurons and synapses involved in ASR mediation are unimpaired, despite the children's extremely high PbB level.

4. Discussion

Pb exposure in children has been associated with a spectrum of adverse neurologic, nephrologic and hematologic outcomes. Clinical neurologic studies and investigations on experimental animals have reported that Pb exposure results in sensory-neural injury in the auditory brainstem. Some electrophysiological ABR investigations in human subjects and histological studies in experimental animals have suggested generalized Pb induced brainstem damage, including lesions in the superior olivary complex, a neuronal center that is important for auditory temporal processing and bilateral mediation of the acoustic stapedius reflex [14-16,18-20,23,27]. However, other electrophysiological studies using ABR as an assay have found no consistent association between Pb exposure and impairment of the brainstem auditory tracts and nuclei [24,26,46,47]. Oto-neurological involvement, such as that seen clinically in facial nerve injury, including Bell's palsy, as well as 8th nerve and brainstem tumors, myasthenia gravis, and multiple sclerosis, results in injury or neuronal damage in the brainstem ASR arc and abnormal ASR responses.

In the present study, as part of a clinical hearing assessment battery, we used the ASR procedure, a reliable, non-invasive electrophysiological technique, to examine the intricate brainstem mediated ASR in a group of Pb-exposed children living in a highly Pb-contaminated environment. The participants in the study group were found to have significantly elevated PbB levels (mean: 33.5 µg/dL), indicating that the average child in the study area was Pb intoxicated, with a PbB level in the CDC III Classification. Moreover, it was observed that 36% of the children had PbB levels in the CDC IV and V Classifications, that call for immediate medical intervention. Although the children were found to have severely elevated PbB levels, the findings of the current study suggested that the sensory and motor neural elements of the brainstem mediated ASR were generally unaffected by the Pb intoxication levels of the participants. Ipsilateral and contralateral ASRTs in the Pb-exposed children in the study group were in the range of approximate 70-95 dB HL, and consistent with threshold values observed in normal hearing non- Pb-exposed research and clinical populations. Although the number of children/ears tested at each stimulus activator frequency varied, the *n* was sufficient to support a reliable statistical analysis of the data. The essentially linear ASR amplitude growth above the threshold in the Pb-exposed study group showed a normal stimulus-response pattern, in which the ipsilateral ASR amplitude growth function was steeper than the contralateral amplitude growth for the 500, 1000 and 2000 Hz stimulus activators. This finding, coupled with the fact that the ipsilateral ASRTs were elicited at lower HLs than the contralateral ASRTs in the Pb-exposed children, indicates that the ipsilateral ASR is more sensitive than the contralateral ASR, consistent with observations in the normal general population. As has been shown in other studies, the ASR amplitude growth function for the BBN was shallower than that for the tonal stimulus activators [36,48,49,50]. The ipsilateral/contralateral amplitude growth ratios observed in this study were consistent with the response pattern seen in normal non-Pb exposed populations [50,51]. These findings suggest that the neurons activated in the ASR from the 8th nerve through the brainstem continued to increase in number and exhibited normal temporal and spatial processing as the intensity of the stimulus activators was increased.

Of particular note were the sustained contractions of the stapedius muscle in response to prolonged acoustic stimulation, measured indirectly as a change in acoustic immittance of the ear over a standard clinical 10-second time period, and referred to as ASRD. In demyelinating diseases, such as multiple sclerosis, as well as in diseases of the neuromuscular synapse, such as myasthenia gravis, or in cases of 8th nerve or brainstem acoustic neuroma, associated with a reduction in the neuron pool, the magnitude and duration of the peak ASR is rapidly reduced [29]. The immittance recordings of the ASR in the present study generally revealed robust deflections from baseline to peak that maintained

maximum amplitude from the onset of the acoustic activator to stimulus offset at 10 seconds, without indications of fatigue or adaptation. Physiologically, the protracted deflections in the ASR recordings reflected normal neuronal conduction, sustained release of the cholinergic neurotransmitter substance from the pre-synaptic motoneuron terminals of the stapedial branch of the facial nerve, and prolonged contractile protein activity in the fibers of the stapedius muscle. The mean decay/fatigue rates in the present study for the 500 and 1000 Hz, and BBN stimulus activators were within the normal range, and as the case illustrations show, participants with extreme plumbism exhibited vigorous, prolonged stapedius muscle contractions with no decay or adaptation for these stimulus activators. On the other hand, some degree of decay or adaptation was observed for the 2000 Hz stimulus activator for both ipsilateral and contralateral stimulation, suggesting abnormalities in the ASR at this frequency. However, these observations were not associated with elevated PbB levels. Additional studies are needed to determine if the ASRD observed at 2000 Hz in this Pb exposed population is a reflection of brainstem impairment, or represents the more rapid ASR adaptation (shorter half-life time) observed in some non-Pb-exposed subjects at 2000 Hz.

To our knowledge, this is the first study to use the ASR to assess the integrity of the brainstem neurons, synaptic chain, and neuro-motor function in Pb poisoned children. The findings of this study suggest no measurable impairment of the ascending and descending neuronal components of the brainstem ASR arc, including the first order neurons of the 8th cranial nerve that synapse in the cochlear nucleus, the superior olive, the motor neurons in and around the facial nerve nucleus, as well as the neuronal elements of the stapedius branch of the 7th cranial nerve, which innervates the stapedius muscle. The Pb-exposed children showed unexpectedly vigorous ASR responses from threshold level intensities to suprathreshold levels, with normal ASR amplitude growth, and generally normal sustained muscle activity.

In conclusion, while some studies have reported impairment of the auditory brainstem in Pb-exposed populations, the results of the present study showed no significant relationship between PbB level and the auditory brainstem mediated ASR responses in children with chronic Pb exposure and elevated PbB levels. These findings suggest that the neuronal elements, tracts and nuclei of the auditory brainstem, and the peripheral neuromuscular structures innervated by efferent brainstem auditory motoneurons are not inevitably impaired by acute or chronic Pb exposure. The results of this study are consistent with our previous findings, using behavioral and physiological auditory procedures, which showed no association between PbB level and auditory sensory-neural function.

Acknowledgments

We appreciate the support of Ing. Luis Parodi V., Dr. Miguel Falconi Puig, Dr. Gonzalo Mantilla, and the Universidad San Francisco de Quito. We are grateful to Dr. Jeremy Bloxham, Harvard University Biological Laboratories, and University of Massachusetts Medical School/Shriver Center for support. We thank Anthony B. Jacobs for excellent technical assistance; Dr. Nader Rafai, and the Boston Children's Hospital Department of Laboratory Medicine for laboratory support.

References

- [1]. el Khateeb I, Abdul Razzak B, Moosa A. Auditory brainstem responses (ABR) in children with neurological disorders. *Brain Dev.* 1988; 10:243–8. [PubMed: 3218703]
- [2]. Anderson, AC.; Pueschel, SM.; Linakis, JG. Pathophysiology of lead poisoning. In: Pueschel, SM.; Linakis, JG.; Anderson, AC., editors. *Lead poisoning in childhood*. Paul H. Brookes Publishing Co. Inc.; Baltimore: 1996. p. 75-96.

- [3]. Banks EC, Ferretti LE, Shucard DW. Effects of low level lead exposure on cognitive function in children: A review of behavioral, neuropsychological and biological evidence. *Neurotoxicology*. 1997; 18:237–282. [PubMed: 9216005]
- [4]. Shannon, MW. Etiology of childhood lead poisoning. In: Puschel, SM.; Linakis, JG.; Anderson AC, AC., editors. *Lead poisoning in childhood*. Paul H. Brookes Publishing Co. Inc.; Baltimore: 1996. p. 75-96.
- [5]. Canfield RL, Henderson CR, Cory-Slechta DA, Cox C, Jusko TA, Lanphear BP. Intellectual impairment in children with blood lead concentrations below 10 µg per deciliter. *N Engl J Med*. 2003; 348:1517–26. [PubMed: 12700371]
- [6]. Agency for Toxic Substances and Disease Registry (ATSDR). Draft Toxicological Profile for Lead. U.S. Department of Health and Human Services, Public Health Service; 2005.
- [7]. Surkan PJ, Zhang A, Trachtenberg F, Daniel DB, McKinlay S, Bellinger DC. Neuropsychological function in children with blood lead levels <10 mg/dL. *Neurotoxicology*. 2007; 28:1170–77. [PubMed: 17868887]
- [8]. Lanphear BP, Dietrich K, Auinger P, Cox C. Cognitive deficits associated with blood lead concentrations <10 microg/dL in US children and adolescents. *Public Health Rep*. 2000; 115:521–29. [PubMed: 11354334]
- [9]. Lanphear BP, Hornung R, Khoury J, et al. Low-level environmental lead exposure and children's intellectual function: an international pooled analysis. *Environ Health Perspect*. 2005; 113:894–99. [PubMed: 16002379]
- [10]. Finkelstein Y, Markowitz ME, Rosen JF. Low-level lead-induced neurotoxicity in children: An update on central nervous system effects. *Brain Res Rev*. 1998; 27:168–76. [PubMed: 9622620]
- [11]. Meng X-M, Zhu DM, Ruan DY, She JQ, Luo L. Effects of chronic lead exposure on 1H MRS of hippocampus and frontal lobes in children. *Neurology*. 2005; 64:1644–47. [PubMed: 15883337]
- [12]. Schwartz J, Otto D. Blood lead, hearing thresholds and neurobehavioral development in children and youth. *Arch Environ Health*. 1987; 42:153–60. [PubMed: 3606213]
- [13]. Otto DA, Fox DA. Auditory and visual dysfunction following lead exposure. *Neurotoxicology*. 1993; 14:191–207. [PubMed: 8247393]
- [14]. Holdstein Y, Pratt H, Goldsher M, Rosen G, Shenav R, Linn S, et al. Auditory brainstem evoked potentials in asymptomatic lead-exposed subjects. *J Laryngol Otol*. 1986; 100:1031–36. [PubMed: 3760684]
- [15]. Rothenberg SJ, Poblano A, Morales S. Prenatal and perinatal low level lead exposure alters brainstem auditory evoked responses in infants. *Neurotoxicology*. 1994; 15:695–9. [PubMed: 7854608]
- [16]. Rothenberg SJ, Poblano A, Schnaas L. Brainstem auditory evoked response at five years and prenatal and postnatal blood lead. *Neurotoxicol Teratol*. 2000; 22:503–10. [PubMed: 10974588]
- [17]. Osman K, Pawlas K, Schütz A, Gazdzik M, Sokal JA, Vahter M. Lead exposure and hearing effects in children in Katowice, Poland. *Environ Re*. 1999; 80:1–8.
- [18]. Bleecker ML, Ford DP, Lindgren KN, Scheetz K, Tiburzi MJ. Association of chronic and current measures of lead exposure with different components of brainstem auditory evoked potentials. *Neurotoxicology*. 2003; 24:625–31. [PubMed: 12900075]
- [19]. Zou C, Zhao Z, Tang L, Chen Z, Du L. The effect of lead on brainstem auditory evoked potentials in children. *Chin Med J (Engl)*. 2003; 116:565–8. [PubMed: 12875723]
- [20]. Lurie DI, Brooks DM, Gray LC. The effect of lead on the avian auditory brainstem. *Neurotoxicology*. 2006; 27:108–17. [PubMed: 16162360]
- [21]. Jones LG, Prins J, Park S, Walton JP, Luebke AE, Lurie DI. Lead exposure during development results in increased neurofilament phosphorylation, neuritic beading, and temporal processing deficits within the murine auditory brainstem. *J Comp Neurol*. 2008; 506:1003–17. [PubMed: 18085597]
- [22]. Prins JM, Brooks DM, Thompson CM, Lurie DI. Chronic low-level Pb exposure during development decreases the expression of the voltage dependent anion channel in auditory neurons of the brainstem. *Neurotoxicology*. 2010; 31:662–73. [PubMed: 20797405]
- [23]. Fortune T, Lurie DI. Chronic low-level lead exposure affects the monoaminergic system in the mouse superior olivary complex. *J Comp Neurol*. 2009; 513:542–58. [PubMed: 19226511]

- [24]. Counter SA, Buchanan LH, Ortega F, Laurell G. Normal auditory brainstem and cochlear function in extreme pediatric plumbism. *J Neurol Sci.* 1997; 152:85–92. [PubMed: 9395129]
- [25]. Buchanan LH, Counter SA, Ortega F, Laurell G. Distortion product oto-acoustic emissions in Andean children and adults with chronic lead intoxication. *Acta Otolaryngol.* 1999; 119:652–8. [PubMed: 10586997]
- [26]. Counter SA. Brainstem neural conduction biomarkers in lead-exposed children of Andean lead-glaze workers. *J Occup Environ Med.* 2002; 44:855–64. [PubMed: 12227678]
- [27]. Borg E. On the neuronal organization of the acoustic middle ear reflex. A physiological and anatomical study. *Brain Res.* 1973; 49:101–23. [PubMed: 4349006]
- [28]. Borg E, Counter SA, Rydqvist B. Contraction properties and functional morphology of the avian stapedius muscle. *Acta Oto-Laryngol.* 1979; 88:20–6.
- [29]. Borg E, Counter SA. The middle ear muscles. *Sci Am.* 1989; 260:74–80. [PubMed: 2643827]
- [30]. Jerger S, Jerger J. Auditory findings in brain stem disorders. *Arch Otolaryngol.* 1974; 99:342–50. [PubMed: 4832615]
- [31]. Johns ME, Ruth RA, Jahrsdoerfer RA, Cantrell RW. Stapedius muscle function tests in the diagnosis of neuro-muscular disorders. *Otolaryngol Head Neck Surg.* 1979; 87:261–5. [PubMed: 503498]
- [32]. Lehrer JF, Poole DC. Abnormalities of the stapedius reflex in patients with vertigo. *Am J Otol.* 1981; 3:96–103. [PubMed: 7304727]
- [33]. Ardiç FN, Topaloğlu I, Oncel S, Ardiç F, Uzun MZ. Does the stapes reflex remain the same after Bell's palsy? *Am J Otol.* 1997; 18:761–5. [PubMed: 9391674]
- [34]. Tóth L, Lampé I, Diószeghy P, Répássy G. The diagnostic value of stapedius reflex and stapedius reflex exhaustion in myasthenia gravis. *Electromyogr Clin Neurophysiol.* 2000; 40:17–20. [PubMed: 10782353]
- [35]. Berlin CI, Hood LJ, Morlet T, et al. Multi-site diagnosis and management of 260 patients with auditory neuropathy/dys-synchrony (auditory neuropathy spectrum disorder). *Int J Audiol.* 2010; 49:30–43. [PubMed: 20053155]
- [36]. Counter SA. Brainstem mediation of the stapedius muscle reflex in hydraencephaly. *Acta Otolaryngol.* 2007; 127:498–504.
- [37]. Mazlan R, Kei J, Hickson L. Test-retest reliability of the acoustic stapedial reflex test in healthy neonates. *Ear Hear.* 2009; 30:295–301. [PubMed: 19322092]
- [38]. Counter SA, Buchanan LH, Ortega F, Amarisiriwardena C, Hu H. Environmental lead contamination and pediatric lead intoxication in an Ecuadorian Andean village. *Int J Occup Environ Health.* 2000; 6:169–76. [PubMed: 10926719]
- [39]. Counter SA, Buchanan LH, Laurell G, Ortega F. Field screening of blood lead levels in remote Andean villages. *Neurotoxicology.* 1998; 19:671–8.
- [40]. Centers for Disease Control and Prevention (CDC). Preventing Lead Poisoning in Young Children: A Statement by the U.S. Department of Health and Human Services. U.S. Government Printing Office; Atlanta: Oct. 1991
- [41]. Borg E, Zakrisson J-E. The activity of the stapedius in man during vocalization. *Acta Otolaryngol.* 1975; 79:325–33. [PubMed: 1155042]
- [42]. Zakrisson JE, Borg E, Blom S. The acoustic impedance change as a measure of stapedius muscle activity in man. *Acta Otolaryngol.* 1974; 78:357–64. [PubMed: 4451084]
- [43]. Anderson H, Wedenberg E. Audiometric identification of normal hearing carriers of genes for deafness. *Acta Otolaryngol.* 1968; 65:535–54.
- [44]. Osterhammel D, Osterhammel P. Age and sex variations for the normal stapedial reflex thresholds and tympanometric compliance values. *Scan Audiol.* 1979; 8:153–58.
- [45]. Wiley TL, Oviatt DL, Block MG. Acoustic-immittance measures in normal ears. *J Speech Hear Res.* 1987; 30:161–170. [PubMed: 3599948]
- [46]. Wong V, Ng T, Young C. Electrophysiologic study in acute lead poisoning. *Ped Neurol.* 1991; 7:133–36.
- [47]. Yokoyama K, Araki S, Yamashita K, et al. Subclinical cerebellar anterior lobe, vestibulo-cerebellar and spinocerebellar afferent effects on young female lead workers in China:

- Computerized posturography with sway frequency analysis and brainstem auditory evoked potentials. *Industrial Health*. 2002; 40:245–53. [PubMed: 12141372]
- [48]. Silman S, Popelka GR, Gelfand S. Effect of sensorineural hearing loss on acoustic stapedius reflex growth functions. *J Acoust Soc Amer*. 1978; 64:1406–11. [PubMed: 744841]
- [49]. Wilson RH, McBride LM. Threshold and growth of the acoustic reflex. *J Acoust Soc Amer*. 1978; 63:147–54. [PubMed: 632406]
- [50]. Hall JW. Acoustic reflex amplitude: II. Effects of age-related auditory dysfunction. *Audiol*. 1982; 21:386–99.
- [51]. Hall JW. Quantification of the relationship between crossed and uncrossed acoustic reflex amplitudes. *Ear Hear*. 1982; 3:296–300. [PubMed: 7152152]

Figure 1.

Mean acoustic stapedius reflex thresholds (ASRT) in dB hearing level (HL) for four acoustic stapedius reflex (ASR) stimulus activators (500, 1000, 2000 Hz, and broadband noise [BBN]) for lead (Pb) exposed children living in rural Pb-glazing villages in the Andes Mountains of Ecuador. The results for ipsilateral stimulation are shown in graph A, and those for contralateral stimulation in graph B. For both the ipsilateral and contralateral conditions, mean ASRTs for the Pb-exposed children are compared to normative data (Reference Group) obtained on young adults with no known Pb exposure by Wiley, et al. (1987). The broken lines in the graphs represent the + 2 standard deviation range for the Reference Group.

Figure 2.

Ipsilateral and contralateral acoustic stapedius reflex (ASR) amplitude growth functions for four stimulus activators (500, 1000, 2000 Hz, and broadband noise [BBN]) as a function of stimulus activator intensity level (0, 5, 10, and 15 dB sensation level [SL]) for lead-exposed Ecuadorian Andean children. The 0 dB SL indicates the ASR amplitude at threshold, and the 5, 10 and 15 dB SLs show the ASR growth at corresponding levels above the reflex threshold. The reflex growth amplitude as plotted on the y -axis is recorded in immittance compliance units (cm^3).

Figure 3.

(Case Illustrations 1 and 2). Figure 3 A, B shows the recordings of contralateral acoustic stapedius reflex threshold (ASRT) and acoustic reflex decay (ASRD) in response to a 1000 Hz stimulus activator for an 8-year-old male with a low blood lead exposure level of 5 µg/dL (CDC I Classification). Figure 3C, D shows the ASRT and ASRD to a broadband noise stimulus activator for a 13-year-old female with a blood lead exposure level 10 times higher (57 µg/dL: CDC Classification IV). Both participants live in Pb-contaminated communities in the Andes Mountains of Ecuador.

Figure 4. (Case Illustration 3). Recordings of ipsilateral and contralateral acoustic stapedius reflex thresholds (ASRT) and acoustic stapedius reflex decay (ASRD) at 1000 Hz for a 5-year-old male with a high blood lead (Pb) level of 65 µg/dL (CDC Classification IV) from Pb-glazing of ceramics in an Andean Ecuadorian community. The ASRT responses are shown in Figure 4 A,B, and the ASRD recordings are shown in Figure 4 B,D.

Figure 5. (Case Illustration 4). Recordings of contralateral acoustic stapedius reflex thresholds (A and C) and reflex decay (B and D) at 1000 and 2000 Hz for an 11-year-old female with a high blood lead level of 71 $\mu\text{g/dL}$ (CDC Classification V) from lead-glazing of ceramics in an Andean Ecuadorian community.

Figure 6.

(Case Illustrations 5 and 6). Recordings of contralateral acoustic stapedius reflex thresholds and reflex decay for two Pb-exposed children (aged 9 and 11 years) with extremely elevated blood lead (PbB) levels of 83 µg/dL (A and B) and 84 µg/dL (C and D), respectively from Pb-glazing of ceramics in an Andean Ecuadorian community. Both participants' PbB levels place them in the CDC V Classification.

Table 1

Means and percentiles for ipsilateral acoustic stapedius reflex thresholds (dB Hearing Level) for pure-tone (500, 1000, 2000 Hz), and broadband noise stimulus activators in lead-exposed Andean children. Standard deviation values are shown in parentheses; n refers to the number of ears tested in each subset.

	Ipsilateral Stimulus Activators			
	500 Hz (n = 45)	1000 Hz (n = 52)	2000 Hz (n = 49)	Broadband Noise (n = 44)
Mean ASR Threshold	90.4 (6.6)	86.3 (6.7)	88.4 (6.6)	71.1 (9.3)
10 th Percentile	80.0	75.0	80.0	60.0
25 th Percentile	85.0	85.0	85.0	65.0
50 th Percentile	90.0	85.0	90.0	70.0
75 th Percentile	95.0	90.0	90.0	75.0
90 th Percentile	100.0	95.0	95.0	85.0

Table 2

Mean and percentiles for contralateral acoustic stapedius reflex thresholds (dB Hearing Level) for pure-tone (500, 1000, 2000 Hz), and broadband noise stimulus activators in lead-exposed Andean children. Standard deviation values are shown in parentheses; n refers to the number of ears tested in each subset.

	Contralateral Stimulus Activators			
	500 Hz (n = 51)	1000 Hz (n = 145)	2000 Hz (n = 150)	Broadband Noise (n = 62)
Mean ASR Threshold	97.0 (7.1)	91.6 (6.8)	90.8 (7.8)	79.0 (9.7)
10 th Percentile	90.0	85.0	80.0	65.0
25 th Percentile	90.0	85.0	85.0	75.0
50 th Percentile	95.0	90.0	90.0	80.0
75 th Percentile	100	95.0	95.0	85.0
90 th Percentile	107.0	100.0	100.0	90.0

Table 3

Spearman rho correlation coefficients for blood lead (PbB) level and ipsilateral acoustic stapedius reflex thresholds (I-ASRT) of Pb-exposed Andean children for 500, 1000, 2000 Hz, and broadband noise (BBN) stimulus activators. The values for ρ are corrected for ties, and the p -values are tied p -values. NS = non-significant

Comparison Variables	ρ	P	Significance
PbB ($\mu\text{g/dL}$), I-ASRT at 500 Hz	-0.003	0.984	NS
PbB ($\mu\text{g/dL}$), I-ASRT at 1000 Hz	-0.112	0.426	NS
PbB ($\mu\text{g/dL}$), I-ASRT at 2000 Hz	-0.063	0.664	NS
PbB ($\mu\text{g/dL}$), I-BBN	-0.055	0.721	NS

Table 4

Spearman rho correlation coefficients for blood lead (PbB) level and contralateral acoustic reflex thresholds (C-ASRT) of Pb-exposed Andean children for 500, 1000, 2000 Hz, and broadband noise (BBN) stimulus activators. The values for ρ are corrected for ties, and the p -values are tied p -values. NS = non-significant

Comparison Variables	ρ	P	Significance
PbB ($\mu\text{g/dL}$), C-ASRT at 500 Hz	0.118	0.403	NS
PbB ($\mu\text{g/dL}$), C-ASRT at 1000 Hz	-0.082	0.326	NS
PbB ($\mu\text{g/dL}$), C-ASRT at 2000 Hz	0.133	0.105	NS
PbB ($\mu\text{g/dL}$), C-BBN	0.037	0.771	NS