Original Article

The Effects of Acoustic White Noise on the Rat Central Auditory System During the Fetal and Critical Neonatal Periods: A Stereological Study

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Abstract

Aim: To evaluate the effects of long-term, moderate level noise exposure during crucial periods of rat infants on stereological parameters of medial geniculate body (MGB) and auditory cortex. **Materials and Methods:** Twenty-four male offspring of 12 pregnant rats were divided into four groups: fetal-to-critical period group, which were exposed to noise from the last 10 days of fetal life till postnatal day (PND) 29; fetal period group that exposed to noise during the last 10 days of fetal life; critical period group, exposed to noise from PND 15 till PND 29, and control group. White noise at 90 dB for 2 h per day was used. **Statistical Analysis Used:** Variance for variables was performed using Proc GLM followed by mean comparison by Duncan's multiple range test. **Results:** Numerical density of neurons in MGB of fetal-to-critical period group was lower than control group. Similar results were seen in numerical density of neurons in layers IV and VI of auditory cortex. Furthermore, no significant difference was observed in the volume of auditory cortex among groups, and only MGB volume in fetal-to-critical period group was higher than other groups. Estimated total number of neurons in MGB was not significantly different among groups. **Conclusion:** It seems necessary to prevent long-term moderate level noise exposure during fetal-to-critical neonatal period.

Keywords: Auditory cortex; medial geniculate body; stereological parameters; white noise

Introduction

Along with industrial development, acoustic noise pollution from a variety of sources is considered as a serious health hazard.[1] It is fully accepted that acoustic noise with enough intensity and duration could lead to temporary and permanent hearing threshold level shifts, although its mechanisms are not well understood.[2] Noise-induced hearing impairment is fundamentally associated with spiral ganglion cell degeneration and/or cochlear outer hair cell loss; however, all of the central auditory centers such as cochlear nucleus, superior olivary complex, trapezoid body, inferior colliculus, medial geniculate body (MGB) and auditory cortex are affected.[3] These damages in adults can occur when ambient noise exceeds a safe intensity. The acceptable environment noise is 90 dB for no more than 8 h of exposure, 95 dB for 4 h, and 100 dB for 2 h.[4]

In human base studies, several experiments demonstrate acoustic noise can cause hearing loss in adults;[5] however, very little is known about the effects of noise exposure during fetal and neonatal life on hearing impairment.[6] In rats, audition begins in postnatal day (PND) 15, and it develops during the following two to three weeks, which is considered the critical period to form adult-like organization in the

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auditory cortex. It has been reported that exposure to specific acoustic inputs during critical period could easily be distorted into auditory cortex.[7] Although most studies have investigated effects of intense noise on hearing, few of them evaluated impacts of moderate level noise on auditory function.[8] Furthermore, based on previous study, long-term exposure to moderate level noise during the critical period can cause impairment of auditory object exploration behavior.[9] Nonetheless, whole aspects of long-term, moderate level noise impact on the central auditory system is not clear.

Given the fact that, MGB and auditory cortex are involved in the higher order processing of auditory information, long-term exposure to moderate level acoustic noise during the critical period of their development, may lead to tonotopic reorganization. Since volume and numerical density are two parameters which define the structure, any factors which affect these putative parameters can ultimately change the organization. Hence, in the present study we hypothesized that long-term moderate level noise exposure during crucial periods such as fetal and critical neonatal periods could change the volume and numerical density of neurons in the auditory cortex and MGB *via* alteration of stereological parameters of these areas.

Subject and Methods

Experimental design

In the present study, 24 male offspring of 12 pregnant rats were divided into four groups: fetal-to-critical period group, which were exposed to noise from the last 10 days of fetal life till PND 29 [$n = 6$, Figure 1a]; fetal period group, exposed to noise just during the last 10 days of fetal life [$n = 6$, Figure 1b]; critical period group, exposed to noise from PND 15 till PND 29 [$n = 6$, Figure 1c] and control group which had never been exposed to noise. The rats were randomly selected and housed in the Center of Comparative and Experimental Medicine of Shiraz University of Medical Sciences, Shiraz, Iran under controlled temperature (22°C), humidity (~40%) and lighting (12:12 h light–dark cycle) conditions, with free access to food and water. The ambient noise level in the animal colony was around 57 dB. All procedures on the rats were approved and performed according to the recommendations of the Animal Care Committee of Shiraz University of Medical Sciences. It should be noted that, following the parturition, the number of infants of all 12 dams was adjusted to eight (four males and four females). All infants were weighed weekly till PND 29.

Exposure to noise

For noise exposure, animals were kept in their home cages (42 cm × 26 cm × 15 cm) which were located in the 1 m × 1 m × 2 m chamber. TrueRTA software (real time audio spectrum analyzer, made by John Murphy, USA) was used to produce white noise as previously described.[10] For sound amplification, we used an active two-way monitor (Japan's victor company, JVC, SP-XG950V, 50 W, Japan) located 50 cm above the animal cages. Noise intensity was measured

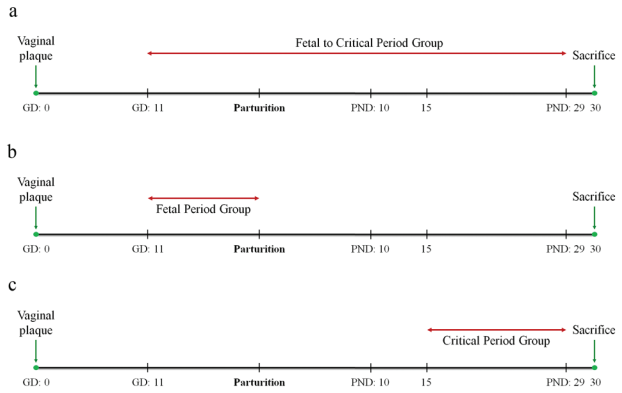

Figure 1: Schematic view of noise exposure protocol in fetal-to-critical period group (a), fetal period group (b), and critical period group (c). GD = gestation day, PND = postnatal day

by sound analyzer real time (TES 1358, Taiwan) each day prior to animal exposure, by placing the analyzer in the animal cages at several locations, and taking an average of the different readings. Animals were exposed to white noise at 90–93 dB for 2 h per day during the experiment. Control animals were placed in the chamber box as noise-exposed rats and for the same period of time, but without being exposed to noise.

Perfusions and tissue preparation

At PND 30, male offspring were anesthetized and perfused transcardially with 0.9% saline solution followed by 10% buffered formalin. The brains were immediately removed and post-fixed in the same fixative solution overnight and immersed in 30% sucrose in phosphate-buffered saline for 48 h and then frozen and stored at −80°C until further processing. Frozen brains were sectioned (30 µm) serially and coronally using a cryostat (SLEE Medical GmbH, Mainz, Germany). The sections were transferred to a 12-well plate containing cryoprotectant solution and stored at −20°C until staining. All sections from the third and eighth wells were mounted on a slide and stained with cresyl violet. Stained sections were compared with "The Rat Brain in Stereotaxic Coordinates" atlas[11] to identify the primary auditory cortex (Bregma −3.24 to −6.84) and MGB (Bregma −4.80 to −6.60).

Stereological studies

Volume of the MGB and auditory cortex was estimated by the point-counting method, using the Cavalieri's principle. Briefly, a grid of points were laid over the image of the section on the monitor of a computer, and the points falling on the both areas were counted. The reference volume (V_{ref}) of the MGB or auditory cortex was determined by applying the following formula: $V_{ref} = \Sigma P \times a(p) \times d$.[12] A total of 200–300 points per brain were counted on each area. We used the ImageJ software and Stereology Tools macros (provided by Dr. Aleksandr Mironov from the University of Manchester) for generated grids.

The numerical density (N_v) of neurons in entire MGB, as well as the numerical density of neurons in layers I, II, IV, and VI of

auditory cortex were estimated using the $N_v = \Sigma Q/[\Sigma P \times a(f) \times h]$ as described previously.[13] For counting the neurons in auditory cortex, the counting frame was defined 30 μm under cortical surface for layer I and next 30 μm beneath layer I for layer II, 600 μm above corpus callosum for layer IV and 30 μm above corpus callosum for layer VI. The counting frame area was 400 μm^2.

The total number of (N) neurons in the MGB was calculated by multiplying the numerical density of the MGB by the volume of the MGB ($N = V_{ref} \times N_v$).

Statistical analysis

Data on pups weight, estimated volume, numerical density and total neurons were subjected to the test of normality. Variance for variables was performed using Proc GLM (statistical analysis software, SAS; 2002) followed by mean comparison by Duncan's multiple range test. The mean ± SEM are reported in the text, and $P < 0.05$ was considered statistically significant.

Results

The body weight of offspring from PND 1 till PND 29 is represented in Figure 2. It was revealed that infants which were exposed to noise during fetal period (fetal period and fetal-to-critical period groups) had lower body weight than the other groups (control and critical period groups) from PND 8 till PND 29 ($P < 0.05$). There is no significant difference in body weight between control and critical period groups at PND 22 and PND 29 ($P > 0.05$).

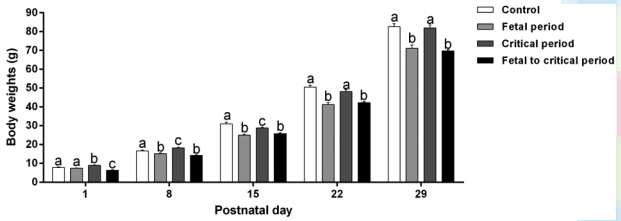

Figure 2: Mean ± SEM of male offspring's body weights (g) from postnatal day 1 till 29. Different superscript letters indicate significant differences among different groups in the same postnatal day ($P < 0.05$)

Estimated volumes of the MGB and auditory cortex have been indicated in Figure 3. MGB volume in fetal-to-critical period group was higher than the other groups [Figure 3a, $P = 0.005$]. On the other hand, no significant difference in auditory cortex volume was detected among groups [Figure 3b, $P = 0.071$].

To clarify the general morphological features of medial geniculate body, cresyl violet staining of MGB of all experimental groups are illustrated in Figure 4a–d. Numerical density of neurons in MGB of fetal-to-critical period group was lower than control group [Figure 4e, $P = 0.034$]. Similar results were seen in numerical density of neurons in layer IV [Figure 5c, $P = 0.0002$] and VI [Figure 5d, $P = 0.004$] auditory cortex of two above-mentioned groups.

Estimated total number of neurons in MGB between experimental groups reflected in Figure 4f, which no significant difference based on this parameter was observed among groups ($P = 0.87$).

Discussion

Excessive noise is the most ordinary environmental and occupational health hazard. Noise-induced hearing loss is the second most common sensorineural hearing deficit, after age-related hearing loss.[14,15] Nonetheless, a complete understanding of the noise impact on the central auditory

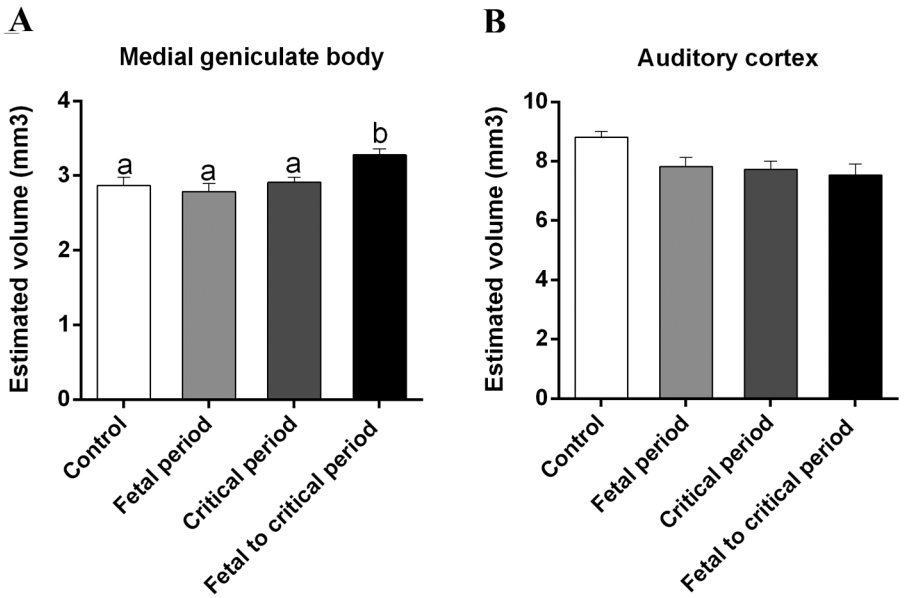

Figure 3: Mean ± SEM of estimated volume of medial geniculate body (a) and auditory cortex (b). Different superscript letters indicate significant differences among groups ($P < 0.05$)

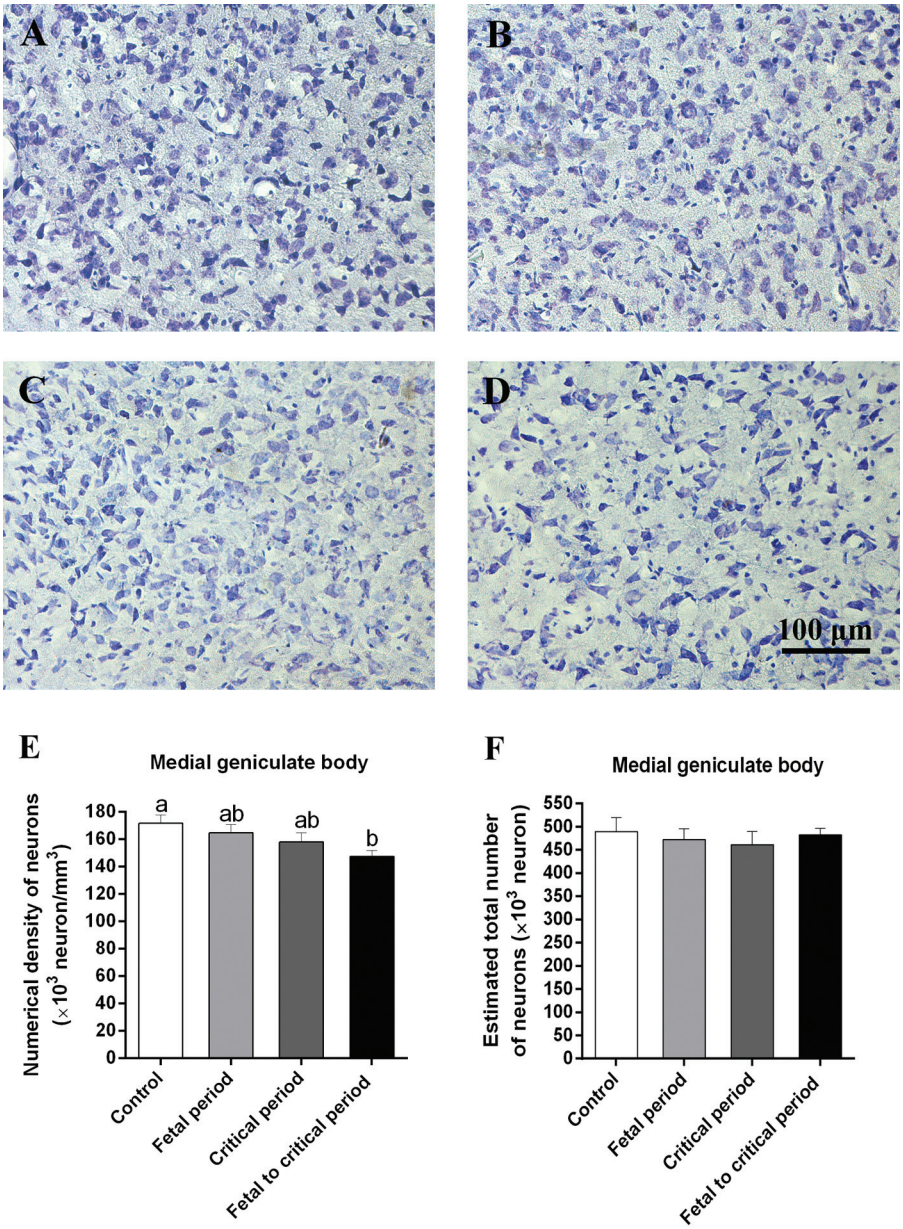

Figure 4: The representative micrographs stained by cresyl violet revealed numerical density of neurons in the medial geniculate body in control group (a), critical period group (b), fetal period group (c), and fetal-to-critical period group (d). Mean ± SEM of estimated numerical density of neurons in entire medial geniculate body (e) and mean ± SEM of estimated total number of neurons in medial geniculate body (f). Different superscript letters indicate significant differences among groups ($P < 0.05$)

system is not clear. In the present study, we evaluated effects of long-term moderate level noise exposure during crucial periods of rat infants on stereological parameters of MGB and auditory cortex.

For estimating volume and numerical density of the MGB, we considered all three subdivisions of this structure (medial, lateral, and ventral parts) as a single unit since Nissl staining alone is inadequate for defining the accurate location of borders between adjacent subregions,[16] and the precise nuclear borders in the MGB can only be achieved when different methods are applied.[17] In the present study, MGB volume in fetal-to-critical period group was significantly higher than other groups. On the other hand, although volume of the auditory cortex in control group was higher than others, no significant difference was detected among groups. It has been shown that, auditory stimuli higher or equal to 90 dB caused audiogenic stress which is associated with significant corticosterone release and intensity-dependent increase in c-fos mRNA induction in a number of auditory structures as well as some areas contained mostly in the forebrain.[18] In this context, MGB plays a fundamental role since rats sustaining complete medial geniculate lesions block corticosterone release normally induced by loud noise as well as blockade of c-fos mRNA induction in several audiogenic stress responsive regions.[19] Noticeably, the

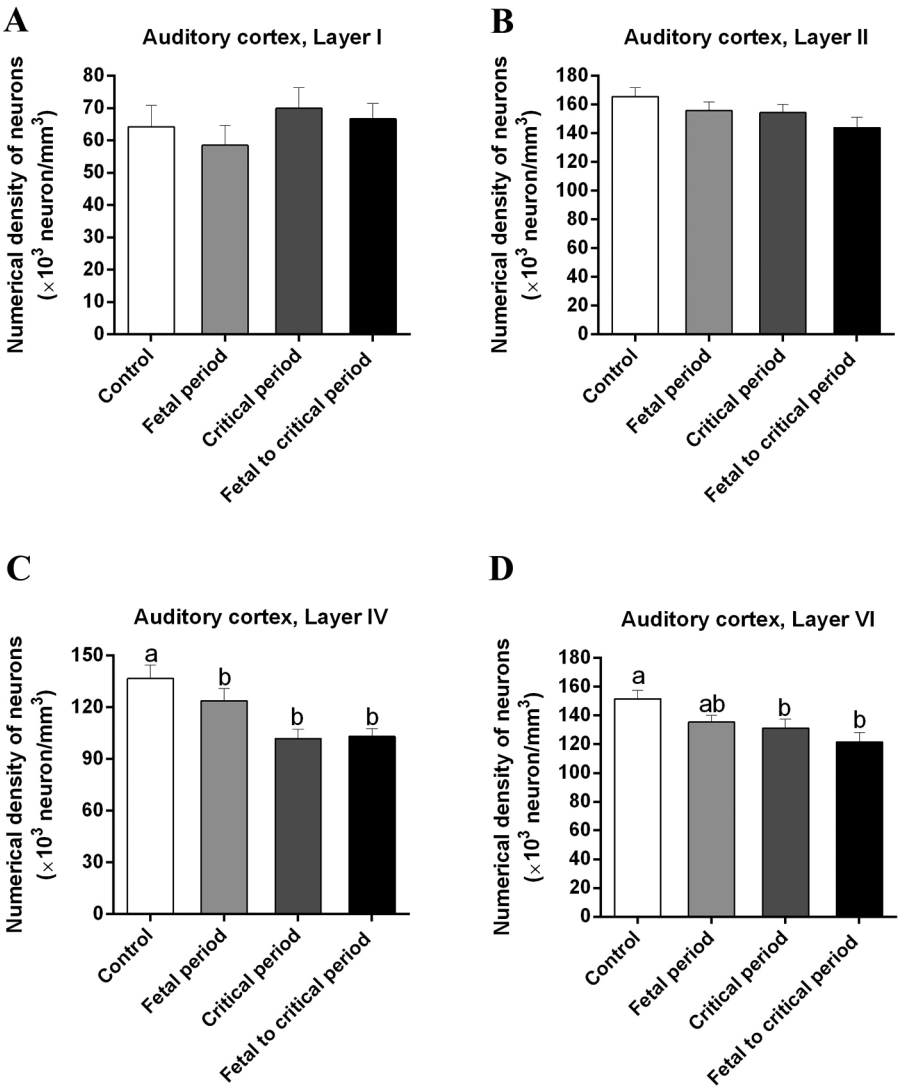

Figure 5: Mean ± SEM of estimated numerical density of neurons in layers I (a), II (b), IV (c), and VI (d) auditory cortex. Different superscript letters indicate significant differences among groups ($P < 0.05$)

effects of the MGB lesions are specific to audiogenic stress as no corticosterone decline was observed in MGB lesioned rats in response to restraint or ether stress.[19] In contrast, auditory cortex lesions did not demonstrate such impacts on adrenocorticotropic hormone or corticosterone release,[20] which reflect the prominent role of MGB to rely audiogenic stress information to the hypothalamus to release corticotropin-releasing hormone.[19]

Since in the present study, we applied 90 dB acoustic white noise, which can cause audiogenic stress, increased MGB volume in fetal-to-critical period group may imply the inflammation. Decreased numerical density of neurons in the MGB without changing in total number of neurons and increasing the volume of MGB could also indicate that inflammation may increase space among neurons.[13] It has been reported that exposure of White Leghorn chickens fertilized eggs to loud noise from embryonic day 10 until hatching could lead to increase glial cell number as well as glia to neuron ratio in nucleus magnocellularis and nucleus laminaris, two brainstem auditory nuclei.[21] Furthermore, dendritic atrophy was observed in inflammation,[22] and it has been proved earlier that psychosocial stress can induce dendritic atrophy,[23] and it was revealed that chronic stress can lead to dendritic atrophy in MGB.[24] Accordingly, this increased volume of MGB may be attributed as the consequence of inflammatory responses to audiogenic stress. Nonetheless, further validation studies are required to evaluate this finding.

Numerical density of neurons in MGB of fetal-to-critical period group was lower than control group. Similar results were seen in numerical density of neurons in layers IV and VI auditory cortex. Regarding the fact that different thalamic nuclei send projections to specific layers of the cortex, lesions in any of these nuclei could lead to lamina-specific degeneration.[25] Since medial part of MGB projects to layers I and VI and ventral part of MGB projects to layers III and IV,[26] therefore, decreased numerical density of MGB neurons in fetal-to-critical period group compare to control

group may be related to lower numerical density of layers IV and VI of auditory cortex in aforementioned group. In addition, numerical density of neurons in layer I was not affected by noise in spite of projections of medial part of MGB to this layer. It has been reported that, these projections from medial part of MGB do not extremely affect the function of layer I because this lamina is mainly responsible of processing intracortical information.[3] It should be noted that, although the same trend as layers IV and VI was observed in layer II, this reduction was not statistically significant [Figure 5b]. Consistent with our finding, Basta *et al.*[3] reported that intense acute noise exposure (115 dB for 3 h) can induce cell death in the mouse MGB (all three subdivisions) and primary auditory cortex (layers IV–VI).

Therefore, decreased numerical density of neurons in the MGB of fetal-to-critical period group may imply inflammation or cell death or both; however, this study did not show significant decrease of total number of neurons in the MGB of aforementioned group which may be assumed that inflammation could be the cause, although this query needs to be elucidated by more studies.

In the present study, weekly bodyweight of experimental pups was also evaluated. For as much as number of infants of each dam affected their growth, at PND 1, number of offspring of all 12 dams was adjusted to eight (four male and four female). Measuring body weight at four time points during PND 8 till PND 29 revealed infants of dams which were exposed to noise during second half of their pregnancy (fetal period and fetal-to-critical period groups) had lower body weight than other two groups. It seems that the lower growth is the result of the stress that dams experienced due to noise exposure during pregnancy as previously reported in rat[27] and human.[28] Subsequently, in stressed females, alteration of both physiological and maternal care can lead to the lower growth of the infants.[29] On the other hand, it has been reported that noise exposure after birth can reduce level of growth hormone and cause pups growth retardation[30] while no significant difference in body weight between control and critical period groups at PND 22 and PND 29 was observed in our study. This may be related to duration or intensity of noise exposure.

In conclusion, the data presented here demonstrate that long-term, moderate level noise exposure during the fetal-to-critical neonatal periods could exert different effects on higher central auditory system. These results to some extent are consistent with similar researches that recruit acute, high-level noise exposure. Therefore, it seems necessary to prevent long-term moderate level noise exposure during these crucial periods.

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Conflicts of interest

There are no conflicts of interest.

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