Original Article

Effects of Noise Exposure on the Auditory Function of Ovariectomized Rats with Estrogen Deficiency

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OBJECTIVE: The benefits of estrogen for the auditory function of women depend on a number of factors. In this study, we aimed to examine the impact of noise trauma on the auditory function of ovariectomized rats with estrogen deficiency.

MATERIALS and METHODS: Twenty-eight young, female Sprague-Dawley rats were assigned to three groups (OVX+N, OVX-N, Sham+N). Rats in the OVX+N group and the OVX-N group underwent bilateral ovariectomy (OVX); the OVX+N group alone was also exposed to white noise (N) of 115 dB SPL for 8 hours a day over 14 days. The Sham+N group consisted of rats with intact ovaries that were exposed to the same noise. The auditory function of all rats was measured before treatment and after noise exposure by the signal-to-noise ratio (SNR) of distortion-product otoacoustic emissions (DPOAE) and the threshold of auditory-evoked brainstem response (ABR).

RESULTS: The Sham+N group (intact ovaries, noise-exposed) had worse auditory function than the OVX-N group (ovariectomy, no noise). The OVX+N group had decreased SNRs of DPOAE and increased ABR thresholds relative to the Sham+N group.

CONCLUSION: Noise exposure may cause greater damage to auditory function when estrogen levels are low in females.

KEYWORDS: Ovariectomy, estrogen, auditory function, rats, noise exposure

INTRODUCTION

Noise-induced hearing loss is unavoidable, as people are often exposed to noise from music concerts, transportation systems, construction sites, audio systems, and sporting events. The widespread use of earbuds with cell phones and personal audio devices has become a contemporary hazard to hearing. Buds inserted in ear canals can reduce ambient noise, encouraging listeners to increase the volume. As the buds are close to the eardrums, the loudness of incoming sounds can increase by 7–9 dB. The Occupational Safety and Health Administration (OSHA) recommends a maximum permissible noise limit of 8 hours a day at an intensity of 90 dBA [1]. The output of an iPod can easily surpass 100 dBA at full volume and 90 dBA at 80% volume [2]. The World Health Organization (WHO) warns that over one billion teenagers and young adults are at risk of noise-induced hearing loss due to recreational sounds [3]. The aim of the present study was to investigate if noise exposure can cause more damage to the auditory function of females when their estrogen levels are low.

Estrogen levels in women may change during their menstrual cycle and pregnancy [4], after menopause [5], due to Turner's syndrome [6], or after oophorectomy [7]. It is well known that estrogen is a primary sex hormone and a neurotransmitter in the brain [8]. In the auditory system, changes in estrogen levels may affect the cochlea [9] and the central auditory pathway [10-12]. In addition, there is evidence that estrogen can protect and maintain auditory integrity [13].

Women with low estrogen levels may experience symptoms of menopause and other detrimental effects, such as brain aging [14, 15]. In addition, estrogen deficiency may affect the hearing function of women [16, 17]. In the laboratory, bilateral ovariectomy in female rats may be an appropriate approach to simulate the effects of estrogen deficiency on hearing function [18, 19]. For example, significant changes in auditory brainstem responses, measured with wave latencies, have been demonstrated for ovariectomized rats compared to intact-ovary rats [20]. All these clinical and laboratory studies seem to suggest that estrogen has direct benefits for auditory function. However, the relationship between estrogen and auditory function is complex, as other factors may affect estrogen deficiency. For example, hearing loss may be related to different stages of menopause [21]. The higher prevalence of hearing loss in women may also result from the interaction of estrogen deficiency with age [22], genetic origin [23], and ototoxic drugs [24]. Taken together, it is reasonable to infer that auditory function will be at risk when women with estrogen deficiency experience additional challenges.

Noise exposure is another challenging factor that may interact with estrogen. The auditory system is fragile and vulnerable to damage upon exposure to noise. Clinically, there is no published data on the overall effects of noise exposure on the auditory function of women with estrogen deficiency. However, the benefits of estrogen have been demonstrated in animals that are exposed to noise. For example, Meltser et al. [25] reported that estrogen-receptor beta (ERβ) can protect the auditory system from acoustic trauma in male and female mice. Wang et al. [26] also demonstrated the protective role of estrogen in auditory function by injecting estradiol into male guinea pigs that were exposed to noise.

To study the effects of noise exposure on the auditory function of estrogen-deficient females, we conducted an experiment with young, female rats. Estrogen deficiency was achieved by bilateral ovariectomy. After the ovariectomized rats recovered from the surgery, they were exposed to loud noise. The noise-induced hearing damage was measured by comparing the auditory function of the ovariectomized rats to that of intact-ovary rats that received the same dose of noise exposure.

MATERIALS and METHODS

Animals and Grouping
Twenty-eight female Sprague-Dawley rats were used. The rats were 2 months old and weighed 180 to 200 grams. All rats were supplied by Liantong Lihua (Beijing, China) and were housed in an animal house during the study. The rats were fed a standard laboratory diet. All surgeries, experiments, and early euthanasia (if needed) for the test animals were approved by the ethical committee of Zhejiang Animal Research, China. The rats were randomly divided into three groups (OVX+N, OVX-N, Sham+N). Ten rats in the OVX+N group underwent bilateral ovariectomy (OVX) and were then exposed to noise (N). Eight rats in the OVX-N group received OVX but were not exposed to noise. This group served as a control group. Ten rats in the third group underwent sham surgery with intact ovaries and were later exposed to noise (Sham+N).

Surgery
All rats were anesthetized on a table with intraperitoneal injection of 10% chloral hydrate ketamine (Qingdao; 3 mL/kg body weight) for surgery. The rats were monitored daily to ensure that they were warm during anesthesia and remained healthy. No rats died or became ill before the end of the study. For the OVX+N and OVX-N groups, the abdominal skin and peritoneum of each rat were cut open. The ovarian arteries were ligated and the ovaries were excised bilaterally. Finally, the muscle wall and skin were sutured. For the Sham+N group, each rat also underwent the same surgical procedure, except the ovarian arteries and ovaries were kept intact.

Noise Exposure
Forty-two days (i.e., 6 weeks) after surgery, the rats in the OVX+N and Sham+N groups were exposed to free-field white noise (equal power across frequencies) inside an electrostatic-screening sound chamber (A204S; Tuoenkang, China) for 8 hours a day over 14 days. The white noise was delivered by an audio signal generator (AWA1650; Hoytech, China) at 115 dB SPL via two loudspeakers (D1080; Hivi, China). The sound pressure level was monitored to be within ±5 dB with a sound analyzer (220; Bruel & Kjaer, Naerum, Denmark) at several positions of the animal cages.

Recording of Auditory Function
The auditory function of all rats in the three groups was tested in a sound chamber before the experiment (pre-surgery, pre-noise). The auditory function of the rats was measured again after the OVX+N and Sham+N groups were exposed to noise (post-noise). All rats were sedated with intraperitoneal injections of 10% chloral hydrate ketamine (3 mL/kg body weight) for the auditory measurements. The ear canals were examined to ensure there were no obstructions. Approximately one hour per animal was required to complete the auditory test battery in one session.

The auditory test battery included the SNR of DPOAE with a DPOAE machine (Eclipse; Interacoustics, Middlefart, Denmark) and the ABR threshold with an ABR machine (Audera; Grason-Stadler, Minnesota, USA). DPOAEs are acoustic responses when the cochlea is stimulated simultaneously by two pure tones (f1, f2). The two sinusoids were set to a frequency ratio of f2/f1=1.22, with intensity levels of L_1 at 65 dB SPL and L_2 at 55 dB SPL. Stimulus frequencies of f2=2, 4, 6, and 8 kHz were selected for greater accuracy [27]. SNR was reported in this study instead of DPOAE amplitude, as they were nearly equivalent [28]. SNR is the estimated dB difference between the OAE energy at 2f1–f2 and the energy in adjacent background noise bins. All stimuli were delivered to the ear canals (sealed with putty) through the OAE probe transducer. A decrease in SNR indicates a deteriorated peripheral (cochlear) response.

ABR is a far-field electrical recording of neural activity during the processing of sounds in the brain via electrodes placed on the scalp. The recording is a series of vertex positive waves (I–V). In this study, the hearing thresholds were measured when the rats were stimulated with clicks (alternating monopolar electrical pulses, 100 μs duration, 39.1/s repetition rate) and 2, 4, 6, and 8 kHz tone bursts (Blackman filtered). The ABR thresholds were determined by reducing the stimulus level in 5 dB steps until wave II could not be identified. An increase in the threshold indicates decreased auditory function in the central auditory system. The ABR measurements were conducted with four stainless-steel needle electrodes placed subcutaneously at the vertex (non-inverting electrode), the back of the neck (ground electrode), and the right and left mastoids (inverting electrodes). ER-3A earphones were inserted into the ear canals (sealed with putty) of the rats. The ABR responses were filtered with a band pass of 100 Hz–3000 Hz. Individual responses of one ear at a time were averaged over 500 repetitions until the results could be replicated.

RESULTS
For each stimulus condition, one-way repeated-measures analysis of variance (ANOVA) was used to examine the within-subjects factor of measurement time (pre-noise, post-noise) and the between-subjects factor of group (OVX-N, Sham+N, OVX+N) on the auditory function of the subjects. For simplicity, the measurement time before the experiment (pre-surgery, pre-noise) is labeled "pre-noise". The SPSS 19.0 (IBM, Armonk, USA) statistical package was employed.

SNRs of DPOAE
Figure 1 shows the mean SNRs for the three treatment groups measured pre-noise (circles, dotted line) and post-noise (triangles, solid line) with stimulus frequencies of 2, 4, 6, and 8 kHz. A lower SNR indicates a weaker evoked cochlear response.
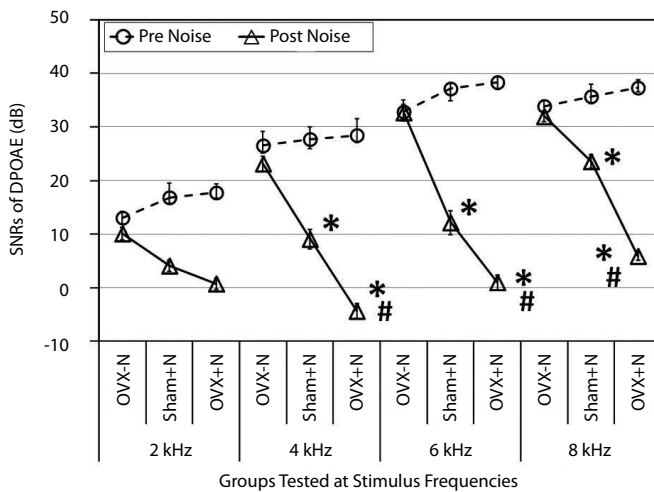
Figure 1. SNRs of DPOAE obtained pre-noise (circles) and post-noise (triangles) for 3 treatment groups with stimulus frequencies of 2, 4, 6, and 8 kHz. Error bars are one standard deviation. An asterisk (*) indicates that the SNR of a group is significantly lower than that of the OVX-N group, while a number sign (#) shows that the SNR of the OVX+N group is significantly lower than that of the Sham+N group.
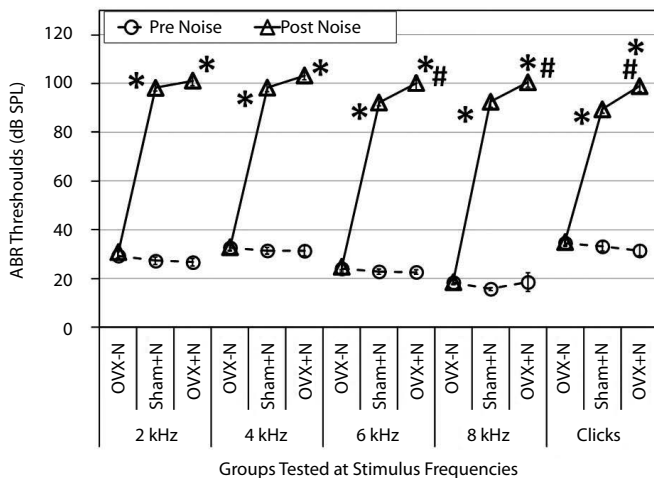OVX: ovariectomy; N: noise exposure

Figure 2. ABR thresholds obtained pre-noise (circles) and post-noise (triangles) for 3 treatment groups with stimuli of clicks and frequencies of 2, 4, 6, and 8 kHz. Error bars are one standard deviation. An asterisk (*) indicates that the threshold of a group is significantly higher than that of the OVX-N group, while a number sign (#) shows that the threshold of the OVX+N group is significantly higher than that of the Sham+N group.
OVX: ovariectomy; N: noise exposure

As shown in Figure 1, the pre-noise SNRs are similar for the three groups across the stimulus frequencies. This is expected, as all rats pre-noise had not yet undergone surgery or been exposed to noise. When the post-noise and pre-noise SNRs are compared, the OVX-N group (no noise) shows a small decrease in SNR due to the effects of ovariectomy alone. However, the SNR of the Sham+N group (intact ovaries) decreased by more than 10 dB after noise exposure. In addition, the SNRs of the OVX-+N group were 3 dB, 13 dB, 11 dB, and 13 dB lower than those of the Sham+N group at 2 kHz, 4 kHz, 6 kHz, and 8 kHz, respectively.

The results of one-way repeated-measures ANOVA for the SNRs indicate that the factor of measurement time is significant for 2

kHz $(F(1,25)=136.6, \eta^2=0.8)$, 4 kHz $(F(1,25)=260.8, \eta^2=0.9)$, 6 kHz $(F(1,25)=637.1, \eta^2=0.9)$, and 8 kHz $(F(1,25)=564.4, \eta^2=0.9)$, $p<0.001$, power=1.0.

The ANOVA results also show that the group effect is significant for 4 kHz $(F(1,25)=33.7, \eta^2=0.7)$, 6 kHz $(F(1,25)=67.7, \eta^2=0.8)$, and 8 kHz $(F(1,25)=69.5, \eta^2=0.8)$, $p<0.01$, power=1.0. The SNR at 2 kHz is not significantly different among the treatment groups, $p>0.05$. Post-hoc analysis indicates that the OVX+N group has significantly lower SNRs than both the OVX-N and Sham+N groups for 4, 6, and 8 kHz; also, the Sham+N group also has a significantly lower SNR than the OVX-N group. Therefore, as noise alone causes more damage to the peripheral auditory system than ovariectomy alone (i.e., Sham+N relative to OVX-N), the auditory function of ovariectomized rats is more vulnerable to noise than that of ovary-intact rats (i.e., OVX+N relative to Sham+N).

ABR Thresholds
Figure 2 plots the mean ABR thresholds obtained at two measurement times (pre-noise, post-noise) with different stimulus frequencies for the three treatment groups. An increased threshold indicates decreased function in the central auditory pathway. The pre-noise thresholds are similar between the three groups across all stimulus frequencies. In general, the post-noise thresholds are approximately 50–80 dB SPL worse than the pre-noise thresholds for the OVX+N and Sham+N groups. The effects of ovariectomy alone on the threshold are small, as shown in the OVX-N group. When comparing the OVX+N group to the Sham+N group post-noise, the threshold of the OVX+N group is approximately 10 dB SPL higher than that of the Sham+N group for clicks and for the 6 kHz and 8 kHz test stimuli.

The ANOVA results for the ABR thresholds indicate that the factor of measurement time is significant for all test stimuli: 2 kHz $(F(1,25)=3428.2)$, 4 kHz $(F(1,25)=2838.7)$, 6 kHz $(F(1,25)=3729.6)$, 8 kHz $(F(1,25)=3775.1)$, and clicks $(F(1,25)=1603.1)$, $\eta^2=0.9$, $p<0.001$, power=1.0.

The statistical results also show that the group effects on the ABR threshold are significant for all test stimuli: 2 kHz $(F(1,25)=423.4)$, 4 kHz $(F(1,25)=415.5)$, 6 kHz $(F(1,25)=506.8)$, 8 kHz $(F(1,25)=510.9)$, and clicks $(F(1,25)=358.3)$, $\eta^2=0.9$, $p<0.001$, power=1.0. Post-hoc analysis indicates that the Sham+N and OVX+N groups have significantly higher thresholds than the OVX-N group for all test stimuli, $p<0.05$. In addition, the OVX+N group has significantly higher thresholds than the Sham+N group for 6 kHz, 8 kHz, and clicks, $p<0.05$. This finding suggests that the exposure of ovariectomized rats to noise can increase the ABR threshold more than that of ovary-intact rats at higher frequencies (6 kHz and above).

DISCUSSION
The current results show that the Sham+N group (intact ovaries, noise exposed) has significantly lower SNRs of DPOAE than the OVX-N group (ovariectomy, no noise). This finding indicates that loud noise caused more damage to the auditory function of rats than ovariectomy alone. In addition, the SNR of the OVX+N group shows a significant decrease of 11–13 dB relative to the Sham+N group when tested at stimulus frequencies of 4, 6, and 8 kHz. This finding suggests that noise can cause more damage to the peripheral auditory

pathway in females with estrogen deficiency than in those without. The ABR measurements show that noise alone (Sham+N group) also causes a significant increase in the ABR threshold relative to ovariectomy alone (OVX-N group). The OVX+N group has a significant ABR threshold elevation of approximately 10 dB relative to the Sham+N group at 6 kHz, 8 kHz, and clicks. The ABR findings also suggest that noise can cause more damage to the central auditory pathway in females with estrogen deficiency than in those without.

Apparently, noise causes a greater decrease in DPOAE (Figure 1) than elevation of the ABR threshold (Figure 2). Noise primarily affects the inner cells of the cochlea, causing damage to the peripheral auditory pathway, while estrogen deficiency primarily prevents neurons from processing sounds in the central auditory pathway. In this study, the change in the central auditory system due to ovariectomy alone is small. This may explain the fact that when noise interacts with estrogen deficiency, the damage in the peripheral auditory system is more observable, as shown by the DPOAE results.

For women with estrogen deficiency, hearing loss can be observed at high frequencies (3–8 kHz) [22]. The current study also shows that noise can interact with estrogen deficiency to cause greater hearing damage at high stimulus frequencies (4 to 8 kHz in DPOAE and 6–8 kHz in ABR).

Limited data are available on the effects of noise on patients with estrogen deficiency. The current results, which show auditory damage caused by noise in ovariectomized rats, may support the findings of Meltser et al. [25], which demonstrate that the estrogen receptor ERβ protects against noise trauma in mice. However, the estrogen deficiency in the current study was achieved by performing bilateral ovariectomy on female rats, while Meltser et al. [25] used the technique of knocking out certain estrogen receptors in male and female mice. The noise-induced effects on auditory function shown in the current study represent an overall result when the production of estrogen in the ovaries is disrupted. In comparison, Meltser et al. [25] focused on noise damage on auditory function when a specific estrogen receptor was absent. The other difference between two studies involves the dose of noise exposure. Meltser et al. [25] used broadband noise for 45 minutes at 100 dB SPL. This relatively small amount of noise exposure can cause a temporary threshold shift. In contrast, the current study applied a relatively high dose of 115 dB SPL white noise for 8 hours a day over 14 days. The noise damage to the auditory function in this study is more severe. Taken together, the findings of both studies suggest that the auditory function of females with estrogen deficiency is vulnerable to noise, whether the noise dose is low or high.

As the effects of estrogen deficiency on hearing function can be complex, it is difficult for researchers to control factors that may interact with estrogen. Therefore, it is not surprising to find reports that suggest that estrogen deficiency has no significant effects on auditory function [29-31]. In the current study, the effect of ovariectomy alone (OVX-N) on auditory function is also small and insignificant (relative to pre-ovariectomy). Using a relatively high dose of noise in this experiment may be appropriate, as noise can be a primary factor interacting with estrogen deficiency to cause hearing deterioration.

CONCLUSION

The current data demonstrates that the auditory function of ovariectomized rats has increased susceptibility to excessive noise exposure. This finding for ovariectomized rats may simulate the clinical scenario for women with low estrogen levels (e.g., menstrual cycle, pregnancy, induced menopause). As a precaution to noise trauma, whether recreational or occupational, women are more at risk of noise-induced hearing loss than men when the production of female estrogen decreases.

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