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In Utero and Postnatal Exposure of Wistar Rats to Low Frequency/High Intensity Noise Depletes the Tracheal Epithelium of Ciliated Cells

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Abstract. Chronic exposure of men or rodents to low frequency/high intensity (LFHI) noise causes a number of systemic changes that make up the so-called vibroacoustic disease (VAD), a disorder that includes alterations of the respiratory system, namely, of its epithelial layer. We have investigated here the susceptibility of the tracheal epithelium of Wistar rats to in utero and postnatal exposure to LFHI noise by comparing its ultrastructure with that of the tracheal epithelium of control rats and of animals exposed to LFHI noise only after reaching adulthood (8 weeks of age). Scanning electron microscopy (SEM) of the inner surface of rat trachea was used to determine the relative areas covered by ciliated and non-ciliated cells. In rats that were exposed in utero and postnatally to LFHI noise, we observed that out of 100 μm^2 of tracheal epithelium only 31 $\pm$ 14 μm^2 were covered by cilia, whereas in control rats; ciliated cells occupied an average of 60 $\pm$ 18 μm^2 out of 100 μm^2 of the epithelium; this difference between the two groups was statistically significant ($p < 0.05$). In rats that were exposed to LFHI noise only after reaching adulthood, cilia covered 55 $\pm$ 22 μm^2 out of 100 μm^2 of the luminal surface of the trachea, a value that, although lower than that of controls, was not found to be statistically different. We conclude that (1) the tracheal ciliated cells are damaged by exposure of rats to LFHI noise if the animals are kept under this environmental aggression during in utero and postnatal periods; (2) tracheal ciliated cells from adult rats are more resistant to the deleterious effects of LFHI noise than pleura or lung alveolar cells that were shown before to undergo marked changes upon chronic exposure of rats to LFHI noise. These

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findings suggest a note of caution regarding pregnant women and young children: they should be prevented from areas where LFHI noise occurs, namely, in aircraft and textile industries where this type of environmental hazard is often present.

Key words: Scanning electron microscopy—Ciliated cells—Morphometry—Noise—Trachea

Introduction

Low frequency/high intensity (LFHI) noise (≤500 Hz, ≥90 dB) is a frequent feature of the working environment of modern man, particularly in highly industrialized areas and in some particular industries (such as airplane and textile plants). Chronic exposure of humans to LFHI noise may result in important alterations in the physiology of several organs and systems, namely nervous, respiratory, and vascular tissues, as a number of publications from others and us have documented [2, 4, 6–8, 10, 17]. These systemic alterations caused by exposure to LFHI noise make up the so-called vibroacoustic disease (VAD) of man [3].

A few epidemiological studies have suggested that in utero exposure to noise and vibration may be associated with pathology of newborn children [1, 9, 20]. We have devoted particular attention to alterations of the respiratory system associated with VAD [11, 13, 14]. We have continued our investigations by searching for whether the rat respiratory epithelium will be damaged by in utero and postnatal exposure of animals to LFHI noise.

For that, we have studied by scanning electron microscopy (SEM) the luminal surface of the trachea of Wistar rats submitted to LFHI noise and we have compared the ultrastructural data with that obtained in control rats. Our data show that there is a significant decrease in the area of the tracheal epithelium that is covered by cilia in rats exposed in utero and at the postnatal period to LFHI noise. This new information suggests that pregnant women and children should be excluded from environments where LFHI noise occurs in order to prevent the development of VAD.

Materials and Methods

Animals and Experimental Groups

We have used 16 male Wistar rats in this experimental study. The animals were obtained from a local breeder (Gulbenkian Institute of Science, Oeiras, Portugal). All animals were kept in standard animal house conditions, had unrestricted access to food (commercial chow) and water, and were treated in accordance with the European Union laws on animal protection (86/609/EC). The animals were divided into 3 experimental groups: (1) rats not exposed to LFHI noise (controls); (2) rats exposed to LFHI noise both in utero (i.e., since fertilization) and after they were born until reaching adulthood (for a total of 2000 hours of LFHI noise) according to an occupationally simulated time schedule (8 hours/day; 5 days/week with weekends in silence; (3) rats

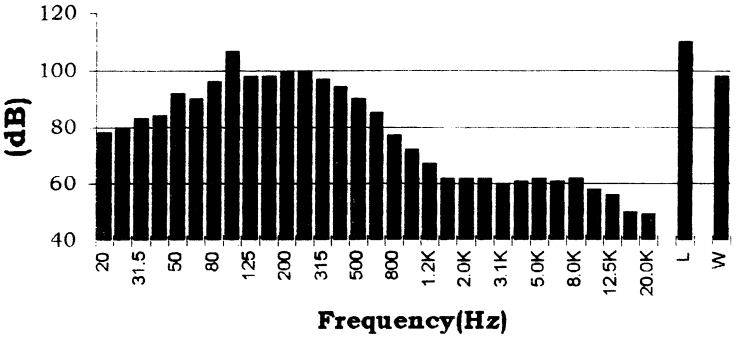

Fig. 1. Spectral analysis of the low frequency/high intensity noise that was present in the environment of the rats used in this study. Linear (L) and A-weighted (W) noise levels and spectral analysis to which the animals are exposed.

exposed, after reaching adulthood (that is, since they were 8 weeks old) to a total of 2000 hours of LFHI noise, according to the same occupationally simulated time schedule (8 hours/day, 5 days/week with weekends in silence).

Noise Exposure

A signal was generated by an analogue noise generator that was amplified and frequency filtered. Fig. 1 illustrates the overall linear and A-weighted noise levels, as well as the spectral analysis of the excitation signal collected at the position near the rat test group inside the experimental chamber. A digital real time analyzer (B&K 2144, Denmark) recorded the noise. The sound energy was highly concentrated in the lower frequency bands due to the influence of the low-pass filter. In the frequency bands ranging from 50 to 500 Hz, the noise levels exceeded 90 dB. The overall levels were registered above 109 dB, with the A-weighted levels being around 98 dB [11, 13, 14]. This environment is acoustically similar to that of patients suffering from VAD that we have previously studied at an airplane repair plant.

Scanning Electron Microscopy (SEM)

Rats of the three experimental groups were sacrificed by a lethal intravenous injection of sodium-pentobarbital (40 mg/kg) and tracheal samples were obtained and processed for SEM. The trachea of each rat was divided into halves along the saggital line. The samples were then placed in a solution of 3% glutaraldehyde in 0.1 M phosphate buffer, pH 7.2 and washed in several changes of 5% sucrose in 0.1 M phosphate buffer, pH 7.2. The samples were dehydrated, critical point-dried, and coated with gold-palladium. Examination with the electron microscope (JEOL JSM-35C, Japan) was performed at an accelerating voltage of 10 kV [13].

Statistical Analysis

Numerical data are presented as average ± standard deviation. Differences between the three experimental groups were evaluated by using Student's *t*-test; differences with $p < 0.05$ were considered statistically significant [12].

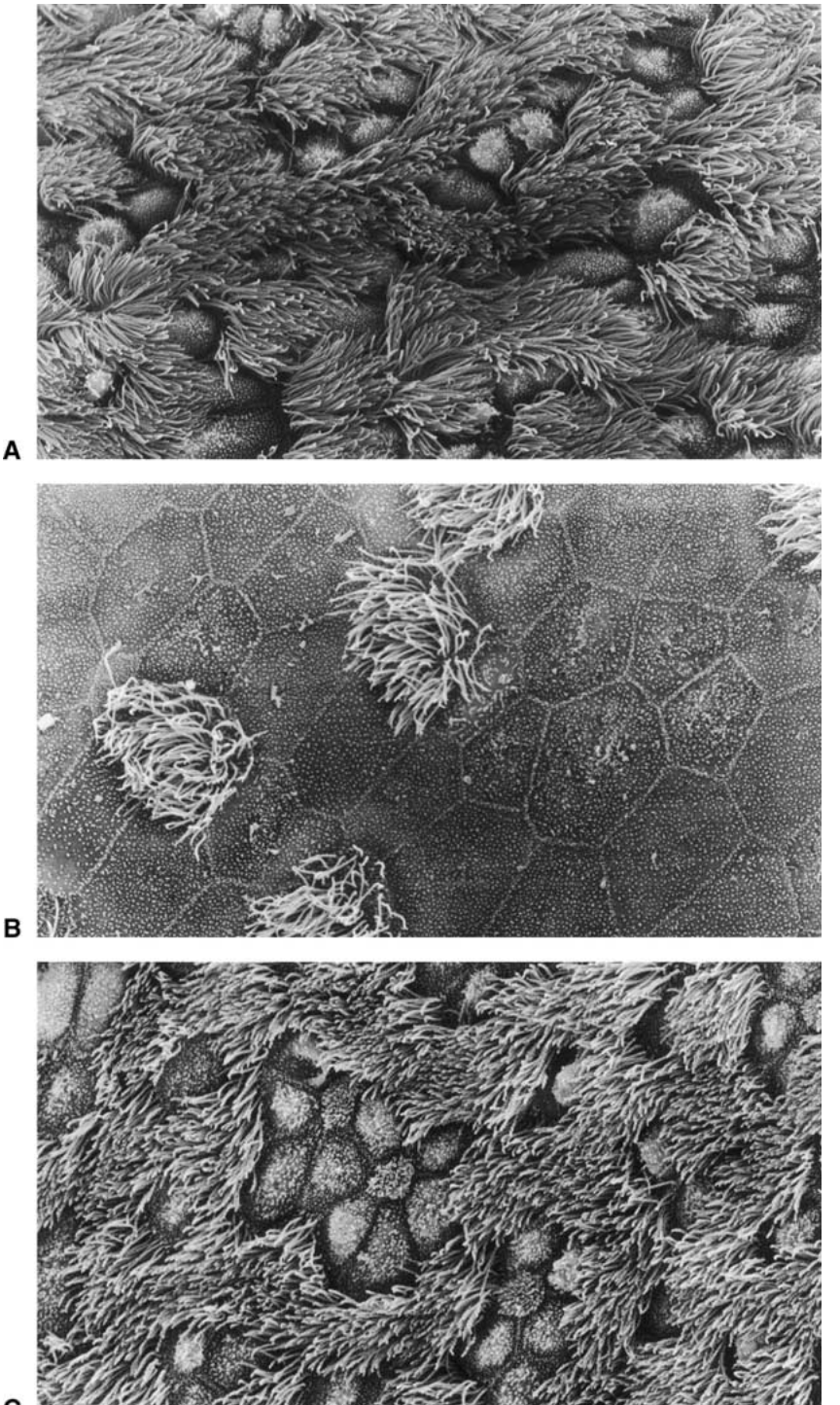

Fig. 2.

Morphometry

To evaluate the relative area of the tracheal surface that was occupied by cilia, random SEM micrographs of the samples were obtained with a magnification of ×1000. Quantitative analysis of ciliated and non-ciliated areas was obtained with the help of a transparent grid of 20 points, spaced 4 cm from each other, that was superimposed on the printed micrographs. The numerical values of the relative area of tracheal epithelium occupied by cilia for each rat was calculated using the following formula: total points of ciliated cells/total points of the grid inside the micrograph. The data are presented as the average area that cilia occupied in 100 μm^2 of tracheal epithelium.

Results

We have compared the relative area occupied by cilia on the rat tracheal epithelium between animals that were kept in silence (group A) or chronically submitted to two different protocols of exposure to LFHI noise. The two groups of noise-treated rats were the following: B - rats submitted to LFHI noise in utero and until reaching adulthood (a total of 2000 hours of exposure); C - rats submitted to 2000 hours of LFHI noise only after the animals reached adulthood.

SEM of Tracheal Epithelium

The luminal surface of the tracheal epithelium of the Wistar rats is dominated by the presence of cilia that occupy more than half of the full area of this portion of the respiratory tract. Non-ciliated regions of the tracheal epithelium show up by SEM either as a smooth surface or covered by short microvilli (Fig. 2A). We have observed that rats chronically submitted to LFHI noise in utero and up to adulthood presented a marked change in the ultrastructure of the luminal surface of the trachea that was expressed by decrease in the area of cilia seen on the epithelium (Fig. 2B), whereas loss of cilia was less evident in animals submitted to noise only in adulthood (Fig. 2C).

Quantitative Comparison of Ciliated Areas of Trachea

We have performed morphometric analysis of the tracheal epithelium using SEM micrographs of samples from the three different groups of Wistar rats. Our data are illustrated in Graph 1 (Fig. 3) where the three experimental groups are compared with regard to the average area of the tracheal epithelium occupied by cilia. Cilia of control rats occupied an average of 60 $\pm$ 18 μm^2 of a total area of 100 μm^2 of tracheal of epithelium. The ciliated surface of the tracheal epithelium was decreased in rats submitted to LFHI noise in utero and up to adulthood: in

Fig. 2. Scanning electron micrographs of the tracheal epithelium of Wistar rats not treated (controls, **A**), or submitted to LFHI noise in utero and up to adulthood (**B**), or submitted to LFHI noise only after reaching adulthood (**C**). A clear decrease in the area occupied by cilia is observed in Fig. B. 2000.

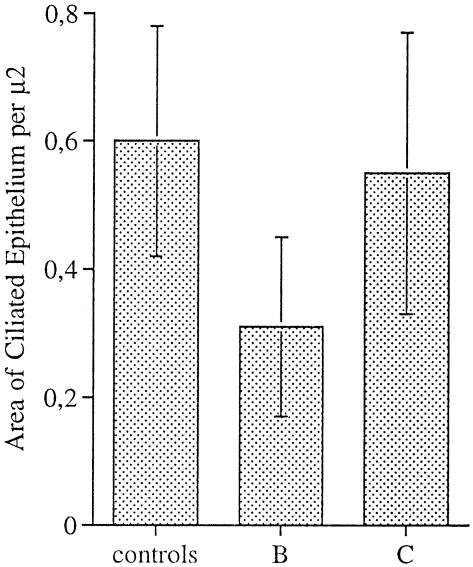

Fig. 3. Quantitative comparison of the area occupied by cilia out of 100 μm^2 of tracheal epithelia in rats submitted to LFHI noise in utero and up to adulthood (**B**) or only after reaching adulthood (**C**); controls are represented on the left of the graph. Data are depicted as average ± standard deviation. Group B is significantly different ($p < 0.05$) from controls.

these animals, the ciliated area of 100 μm^2 of trachea was just 31 ± 14 μm^2; the difference between the two groups was statistically significant ($p = 0.011$). In contrast, rats submitted to LFHI noise only after reaching adulthood did not present a statistically significant change in the area occupied by cilia on trachea (55 ± 22 μm^2 out of 100 μm^2 of epithelium), when compared with controls (60 ± 18 μm^2 out of 100 μm^2 of epithelium). These values of ciliated area of tracheas from rats submitted to LFHI noise in adulthood were not significantly higher than those of animals submitted to LFHI noise since they were generated and up to adulthood.

Discussion

Exposure of humans and laboratory animals to environmental LFHI noise causes pathological alterations in a number of organs and systems, as others and we have characterized before [2–4, 6–8,10, 17]. The respiratory system was shown to be a major target of noise-induced pathology, with clinical and histological changes observed in both men and rodents [5, 11, 13, 14, 16]. These alterations include (1) airflow limitation in VAD patients, a finding that was correlated with structural changes observed in CAT scans [16]; (2) moderate intersticial fibrosis of the lung detected in rats chronically submitted to LFHI noise [5]; (3) increased thickness of rat pleura with loss of microvilli by mesothelial cells [14]; and (4) ultrastructural abnormalities of cilia of the respiratory tract of rats submitted to LFHI noise [13].

Here, we have investigated whether noise exposure during the *in utero* and postnatal periods damages the respiratory tract and whether these putative changes are different from those induced by noise treatment in adult rats. Our qualitative observations by SEM and our quantitative morphometric analysis of

the tracheal epithelium indicate that cilia are particularly sensitive to LFHI noise if this environmental stress is present since the rats are born and until adulthood. Ciliated cells play a central role in the removal of particles and infectious agents that enter the respiratory channels [15, 18, 19]. Therefore, the herein-reported decrease in cilia is likely to cause significant malfunction in the sorting out of exogenous particles inhaled by individuals that are submitted to noise in utero and in childhood. Our data also indicate that the tracheal epithelium of adult rats is more resistant to LFHI-induced lesions than the deep lung or the pleura since we have reported before that marked cellular alterations are observed in these organs of the respiratory system after rats are exposed to LFHI noise [5, 13, 14].

That the tracheal epithelium is particularly susceptible to damage caused by LFHI noise during pregnancy and the postnatal period is a new concept to be added to the overall picture of VAD. However, the lack of measurable alterations in cilia when animals are exposed to noise only after reaching adulthood indicates that the vulnerability of the tracheal epithelium to LFHI noise may be restricted to the early stages of life. Our findings also suggest caution regarding exposure of pregnant women and children to LFHI noise, namely, in working areas such as in airplane and textile industries, where this type of environmental hazard occurs.

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