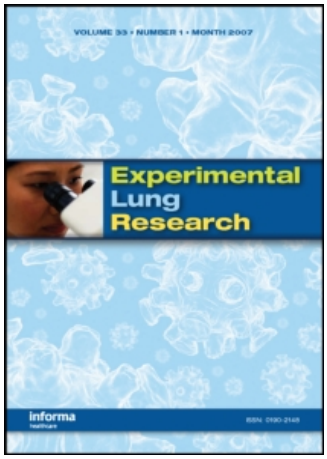

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REDUCTION OF RAT PLEURAL MICROVILLI CAUSED BY NOISE POLLUTION

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REDUCTION OF RAT PLEURAL MICROVILLI CAUSED BY NOISE POLLUTION

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□ *Scanning electron microscopy (SEM) was used to investigate whether chronic exposure to noise modifies pleural morphology. Rats were submitted to 8-h/day schedule of noise that is similar to the working hours at cotton-mill rooms. Morphometry of the area occupied by microvilli on the pleural surface showed a decrease in microvilli after 3 months of rat exposure to noise. The reduction of microvilli was 10% after 3 months of noise exposure (reaching 20% after 7 months of noise treatment) and is consistent with pleural effusions found in some of the patients working in noise-polluted environments.*

Keywords *mesothelial cells, microvilli, noise, pleura, scanning electron microscopy, vibration*

Mesothelial cells make up the lining of the pleural cavity, as well as of other coelmic cavities of the body. They do not significantly differ in morphology or in cytochemical features in the distinct cavities where they are located. Two features dominate the cytology of mesothelial cells: high density of microvilli and of pinocytic vesicles [1]. Both characteristics are aimed at

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keeping a constant amount of fluid in the pleural space. Pinocytic vesicles mediate transcellular fluid transport, and microvilli increase the luminal surface of the mesothelium, for absorption of pleural fluid [2].

Occupational exposure to noise and vibration may damage biological structures and cause a constellation of pathologies that make up the so-called vibroacoustic disease [3, 4]. Pulmonary and respiratory tract disorders are often found among vibroacoustic disease patients [5]. A previous investigation of ours has shown that chronic exposure of rats to jet-industry noise changes the histology of the pleura and also decreases the capacity of the animals to sort out particles injected in the pleural space [6].

Noise pollution is a common environmental feature of working facilities of the modern textile industry, namely in the so-called cotton-mill rooms of the plants. Industrial noise pollution, according to Portuguese and EC law (European Directive 86/188/CEE), occurs when noise is above 85 db. To investigate whether chronic exposure to cotton-mill noise modifies the fine structure of the luminal surface of pleural mesothelial cells, rats were chronically exposed to this type of environmental pollution.

MATERIALS AND METHODS

Animals and Experimental Groups

We used 45 adult male Wistar rats that were purchased from a Spanish breeder (Charles River Laboratories España, S. A., Spain). All animals 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). Standard house conditions were used and they involved keeping 2 rats in a plastic cage ($42 \times 27 \times 16$ cm) with a steel lid.

Thirty-five rats were divided into 7 experimental groups of 5 animals each and submitted to different periods of noise exposure, ranging from 1 to 7 months, according to an occupationally simulated time schedule (8 hours/day, 5 days/week with weekends in silence). The several groups of noise-exposed rats were sacrificed monthly (from 1 up to 7 months).

The remaining 10 Wistar rats were used in 2 control groups (5 animals each) and sacrificed either at the beginning or at end of the study, i.e., with the same age of rats submitted to 1 or 7 months of noise treatment.

Scanning Electron Microscopy (SEM)

The rats were sacrificed by a lethal intravenous injection of sodium pentobarbital (40 mg/kg) and the middle lobe of the right lung excised and processed for SEM. The lung samples were fixed *in toto* in a solution of 3% glutaraldehyde in 0.1 M phosphate buffer, pH 7.2, washed in several

changes of 5% sucrose in 0.1 M phosphate buffer, pH 7.2, dehydrated, critical point–dried, and coated with gold–palladium [7, 8]. Observations of the samples by SEM (JEOL JSM-6301F, Japan) were performed at an accelerating voltage of 10 kV.

Quantification of Microvilli Density

To evaluate the relative area occupied by microvilli on the rat visceral pleura, random SEM micrographs of the samples were obtained at a magnification of 2000. Ten micrographs were made of each sample in a near identical angle with respect to the detector; a total area of 0.03 mm^2 of the visceral mesothelial lining was used for quantitative analysis of each sample. The relative area of the pleura that was covered with microvilli was determined with the help of a transparent grid of 20 points, spaced 4 cm from each other, that was superimposed on the printed micrographs [8]. The numerical values of the relative area of the pleura that showed microvilli were calculated using the following formula: total points of microvilli-covered zones/total points of the grid inside the micrograph. The data are presented as the average proportion of area.

Statistical Analysis

All values are reported as mean $\pm$ standard error. Differences in the proportion of area covered with microvilli were compared using least-squares analyses of variance. Arcsine transformation of the data ($\text{angle} = \arcsin\sqrt{\textit{proportion}}$) was used because of non-normality. Statistical significance was accepted for $P < 0.05$. Statistical procedures were carried out on LSMLMW [9].

Noise Exposure

The environmental noise of a cotton-mill room from a large textile factory of Northern Portugal was used as the paradigm of the occupational noise studied in this investigation.

We have recorded and reproduced the noise present in the cotton-mill room of this factory. This was achieved with an electroacoustic set-up that used a PC-based system, with a DT2823 data acquisition and a SB Live 5.1 cards, 1 B&K 4165 microphone with preamplifier, one 2-channel power amplifier, and 16 monitor-type and 1 subwoofer loudspeakers in biamplification. The software was designed using the LabVIEW system. Sound-signal processing was done offline, applying LabVIEW and Matlab systems. Our apparatus was capable of recording and reproducing the specified noise sounds while monitoring the saturation level in the amplitude dynamic range. A 99.7% dynamic range was preserved for all signals. Signal-acquisition and

processing methodologies were designed in order to carefully measure and preserve the sound characteristics. Total signals duration was 1 hour. Frequency and amplitude characterization of signals was done for all samples. Reproduction of sounds, with spectrum very near the original one, at the original levels of approximately 90.8 dBA (92 dB measured with the sound meter B&K 2260) was achieved by equalization and distribution of sound output in the room. The spectrum of frequencies and intensities of the noise used in this study is documented in Figure 1.

The recorded noise was then reproduced in a noise-insulated animal room, where the rats were to be exposed to it. The sound characterization and room equalization was done by means of a 35-filter bank composed by 3 low-frequency octave-band band-pass filters and $32\frac{1}{3}$-octave pass-band filters for the upper bands. All filters have 50 dB selectivity. The average sound pressure level in the room, as well as the dispersion of values among cages, were carefully controlled. The final sound pressure values that were obtained, measured with a quality calibrated sound meter, were within a 3 dB tolerance relative to the original values, and the dispersion of values among cages was also inside a tolerance of 3 dB relative to the referenced average. The detailed spatial organization of the room where the rats were exposed to noise is illustrated in Figure 2.

RESULTS

SEM micrographs offered high-resolution views of the surface morphology of mesothelial cells of the visceral pleura of the rat; these images confirmed that microvilli were dominant on the luminal surface of the rat mesothelium (illustrated in Figure 3). It was, thus, possible to determine in each sample whether microvilli were present or absent from the surface of mesothelial cells of the rat pleura under observation. In control rats, we

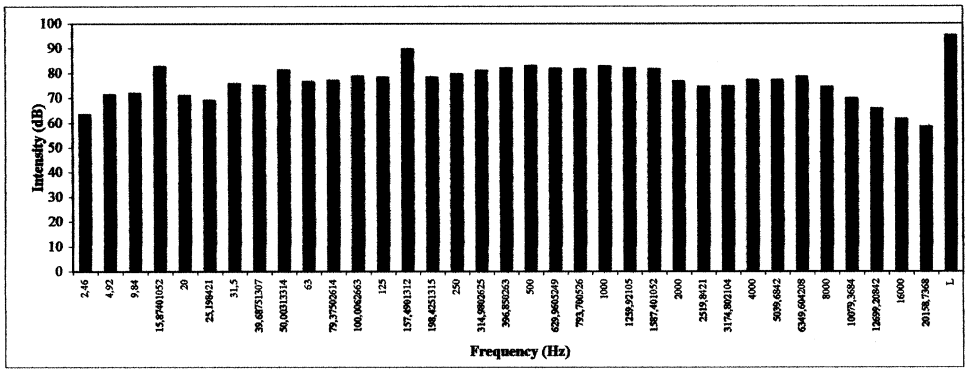

FIGURE 1 Spectrum of frequencies and intensities of the textile-type noise that was recorded in a cotton-mill room and reproduced in the animal houseroom where rats were kept. L = total sound pressure level.

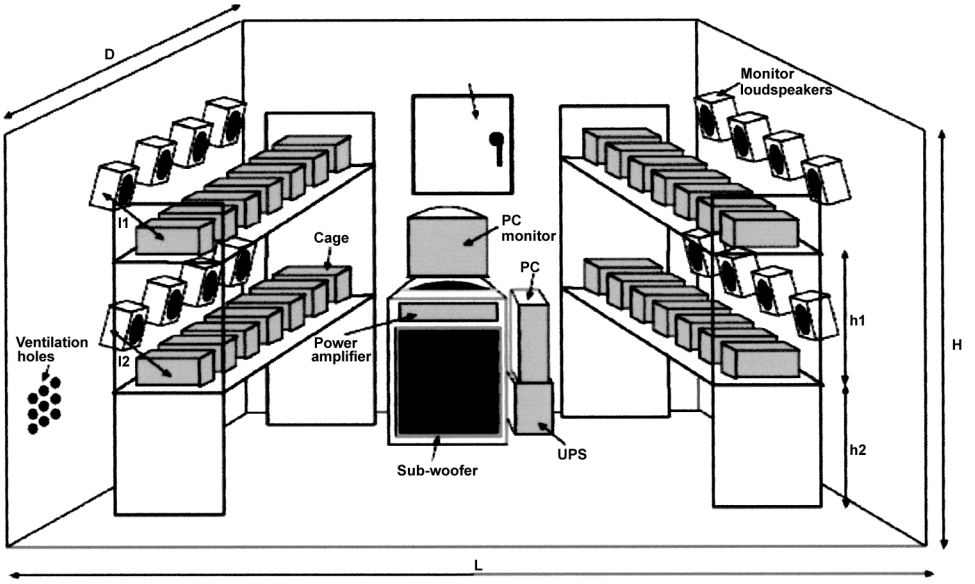

FIGURE 2 Spatial organization of the animal room where Wistar rats were exposed to noise recorded at a cotton-mill room of a textile plant. The dimensions of the room were the following: L = 3.02 m, D = 3.08 m, and H = 2.90 m.

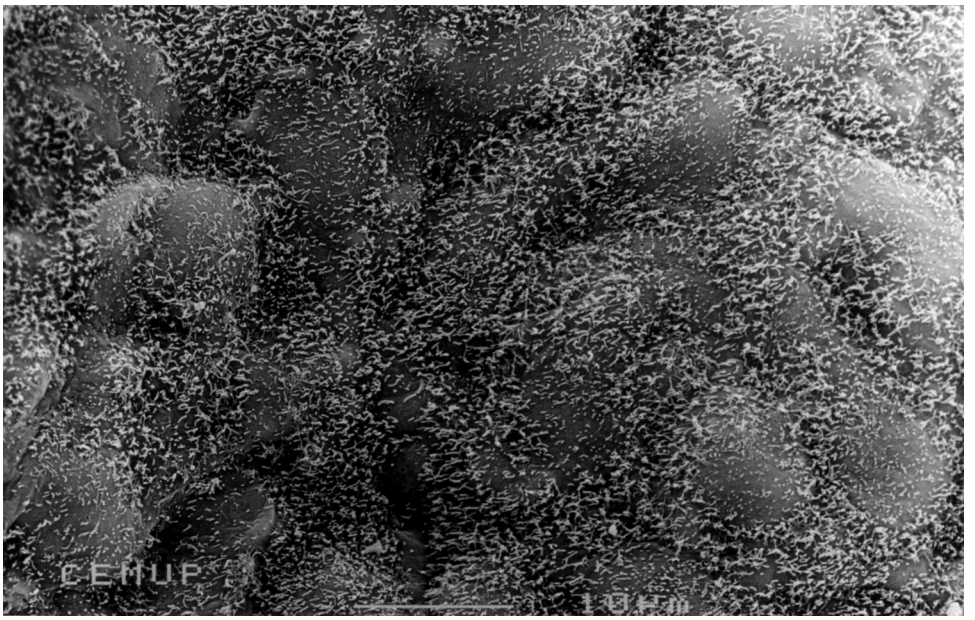

FIGURE 3 SEM micrograph of mesothelial cells of a control rat showing high density of microvilli covering the visceral pleura. × 2000.

have found that around 80% of the pleural surface was coated by microvilli; this proportion of microvilli-coated mesothelial cells is in accordance with a previous morphometric analysis of the rat pleura [10].

A cursory SEM screening of our samples suggested that the mesothelium of noise-treated rats was more frequently devoid of microvilli than the mesothelial surface of control rats (compare Figures 3 and 4). The classical ''combed'' arrangement in parallel rows of microvilli on the mesothelial surface was also altered in the samples from noise-treated rats. Here, the finding of patches of microvilli indicated that there was some degree of aggregation of these finger-like projections of the cell surface, as a result of noise exposure of the animals.

In order to quantify the apparent noise-associated loss of microvilli, we have taken random SEM micrographs of the visceral pleura of rats submitted or not to cotton-mill noise. We found that rats exposed to noise for up to 2 months did not present any quantitative change in the area of the pleura that was coated by microvilli. In contrast, from the 3rd month on after, noise exposure of the animals was associated with a significant decrease in the area of pleura that was coated by microvilli (Figure 5). This relative loss of microvilli was a moderate one because it was expressed, after 7 months of noise treatment, by a decrease of only around 20% in the area of the visceral pleura that was coated by the microvilli (Figure 6).

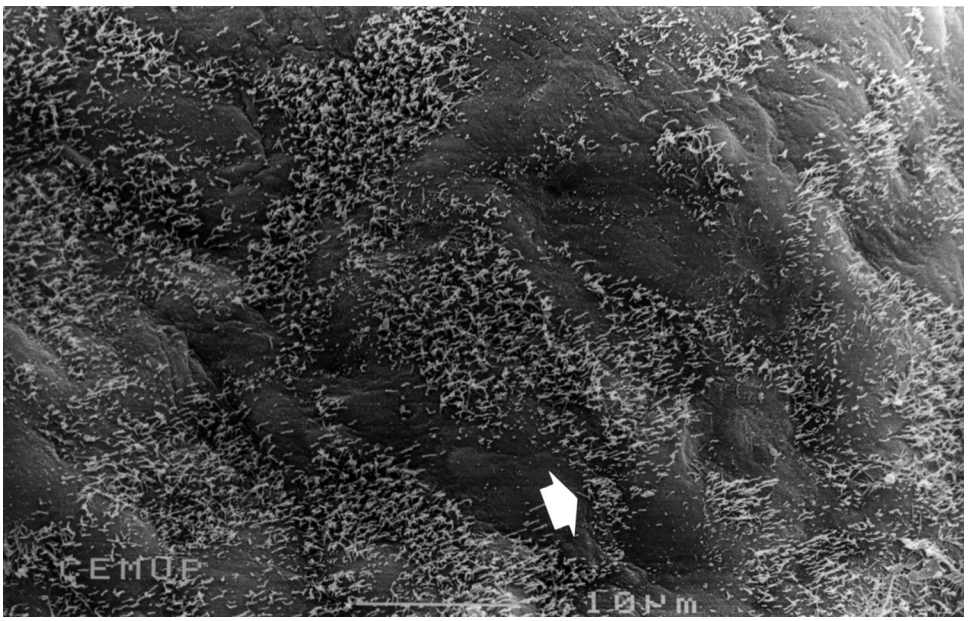

FIGURE 4 SEM micrograph of pleural surface of a rat exposed to textile-type noise for 7 months. It shows a decrease in the density of microvilli that cover the visceral pleura when compared with Figure 3. This micrograph also shows patches of microvilli (arrow). × 2000.

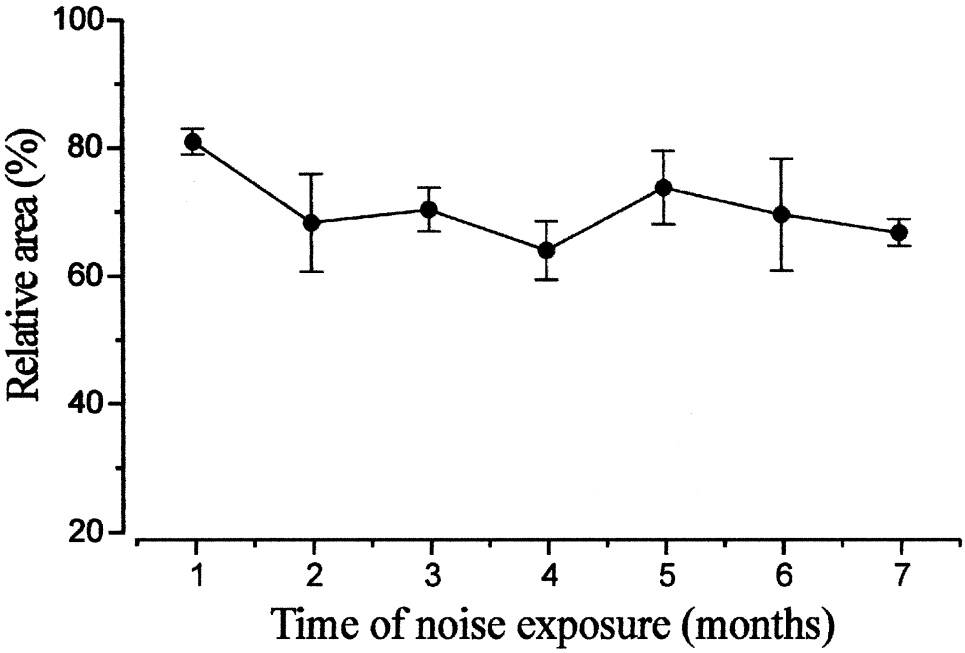

FIGURE 5 Kinetics of the relative area occupied by microvilli on the visceral pleura of treated rats subjected to textile-type noise for 1 up to 7 months. The microvilli density decreases after the 3rd month of exposure of rats to noise.

DISCUSSION

We report here the first evidence that chronic exposure of adult rats to cotton-mill noise causes 10% to 20% decreases in the relative area occupied by microvilli on the visceral pleura of the animals. This conclusion is derived from SEM quantitative data: a significant reduction in the area of the pleura covered by microvilli was found after the rats were submitted to noise for at least 3 months.

Previous publications have documented that chronic exposure of rodents or humans to noise of low frequency and high intensity leads to systemic disorders, namely to alterations of the epithelia and connective tissue of the respiratory system [4–6, 8, 11–13]. In rats, we have previously reported loss of cilia from the tracheal epithelium, enhancement in lung connective tissue, and decrease in the capacity of particle clearance from the pleural space [6, 8, 11–13]. In humans, chronic noise exposure has been associated with increased frequency of respiratory infections, pleural effusion, and also air flow limitation [5].

Microvilli are considered to be a fragile cell-surface differentiation of the mesothelium: they often undergo damage during pleural pathology and are rapidly lost when mesothelial cells are kept in culture [14–16].

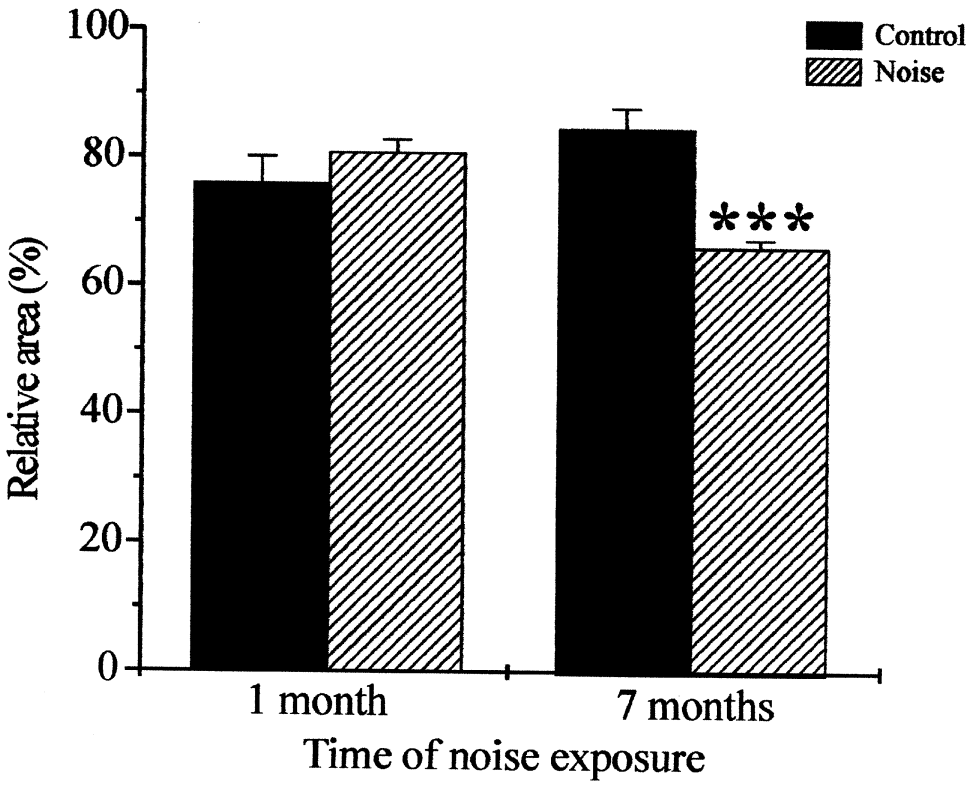

FIGURE 6 Comparison of the relative area occupied by microvilli on the visceral pleura, after 1 and 7 months of noise exposure of rats and their controls. The statistical analysis indicated that the relative area occupied by microvilli is significantly decreased (***) when the rats are exposed for 7 months to textile-type noise ($F(1,16) = 15.474$, $P = .001$).

The functional significance of the herein-reported loss of microvilli has to be found in the physiology of these cell-surface differentiations. Two main functions have been ascribed to pleural microvilli: increase in the area of the cell membrane that will be help in fluid absorption, and reduction of mechanical resistance between the 2 pleural leaflets during respiratory movements [17]. Conceivably, decrease of the mesothelial surface due to loss of microvilli may, therefore, result in a smaller capacity for fluid removal from the pleural space and, consequently, may facilitate the formation of a pleural effusion.

A possible explanation for the loss of microvilli by the mesothelium may be found in putative physical effects of noise-related vibration of tissues. In fact, being thin elongations of the cell membrane, microvilli may be electively prone to damage caused by vibration of the serosal surface from which they are sticking out. This is in accordance with clinicopathological studies of thoracic serosa that have revealed that loss of microvilli, followed by

increased serosal thickness, is often detected in patients suffering from prolonged exposure to low-frequency noise [18, 19]. Pleural effusion has also been observed in these patients [5]. Because only the rats exposed for longer periods of time to noise showed significant damage to mesothelial microvilli, it is pertinent to consider whether this cellular alteration is due either to aging of the pleura, making it more susceptible to noise-induced damage, or to the cumulative effects of noise exposure.

The mesothelium is made up of terminal cells that have a relatively long life span (33 days in the rat) and cannot be easily renewed [20]. Thus, lesions of mesothelial cells are not likely to be rapidly repaired, and it is conceivable that loss of microvilli may become a permanent feature of the noise-damaged pleura [1]. It is, therefore, pertinent to suggest that echographic evaluation of serosal membranes, namely of their thickness, may be a helpful clinical follow-up of workers that are chronically submitted to noisy environments.

Two previous investigations have revealed that another cell membrane differentiation, cilia of the tracheal epithelium, is harmed by chronic exposure of rats to noise [8, 21]. Together with the herein decrease in microvilli on the pleura, these 2 cellular lesions may indicate that long differentiations of the plasma membrane may be particularly vulnerable to damage caused by noise of cotton-mill rooms.

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