Chronic Exposure of Rats to Cotton-Mill-Room Noise Changes the Cell Composition of the Tracheal Epithelium

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The work environment of cotton mill rooms of modern textile plants is characterized by noise pollution. We have taped and reproduced this noisy environment to study its effects on experimentally exposed rats. Because we have previously documented that chronic noise causes alterations in the respiratory epithelium, we have focused our investigation on the morphology of the tracheal lining. Wistar rats were exposed to the textile-type noise from 1 up to 7 months, with an average 40 hours weekly exposure of the animals. The rats were sacrificed monthly and the tracheas were studied by scanning electron microscopy (SEM) to quantify the areas of the airway lining that were covered by ciliated, serous or other cells of the epithelium. We found that noise exposure of the rats caused a significant loss of tracheal ciliated cells; an increased density of serous cells on the epithelium balanced this change. This modification of the rat trachea was already established after 1 month of noise treatment of the animals; it did not change significantly throughout the 7-month course of the herein investigation. Loss of ciliated cells was more intense in areas of the tracheal epithelium located between the regions of cartilage rings. We conclude that the ciliated cell is an elective target for damage caused on the respiratory epithelium by the workplace noise occurring in cotton mill rooms. This modification of the respiratory epithelium is likely to impair clearance of the airways since this function depends on the activity of ciliated cells. (J Occup Environ Med. *2002;44:1135–1142*)

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DOI: 10.1097/01.jom.0000044127.59147.c3

Noise has become a common feature of working environments of modern man.[1–4] Textile industries, in particular, use machinery that exposes its operators to high levels of noise pollution. We have shown before that in other working environment where noise pollution is prevalent, eg, jet engine repair rooms, systemic disorders may occur, particularly with regards to nervous and respiratory diseases;[5–8] we have reproduced before this pathology in experimental models using rodents.[9–12]

Here, we have investigated the effect of chronic exposure of rats to the type of noise that occurs in cotton mill rooms of a modern textile plant. This type of noise is distinct from that of jet engine repair rooms. Our research was aimed at changes of the respiratory epithelium of the trachea caused by noise exposure of the animals. To reach this goal, we have used high resolution "en face" views of the luminal surface of the rat trachea obtained by scanning electron microscopy (SEM). Random SEM micrographs of the samples were made to quantify the relative areas occupied by the different cell types that are characteristic of the rat trachea.

Our data document the elective susceptibility of ciliated cells to noise aggression. They also suggest that an adaptative response of the respiratory epithelium occurs. This response balances the loss of ciliated cells with an increase in the area of the tracheal surface that becomes covered by serous cells after the animals are exposed to noise. We propose that textile noise represents a potential health hazard that may damage the respiratory lining of operators working in cotton mill rooms.

Materials and Methods

Animals and Experimental Groups

We have used 45 adult male Wistar rats that were obtained from a local breeder (Gulbenkian Institute of Science, Oeiras, Portugal). 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 two rats in a plastic cage (42 × 27 × 16 cm) with a steel lid. No signs of infection or inflammation were seen in the histological slides of organs of the rats.

Thirty-five of the animals were divided in 7 experimental groups that were submitted to increasing lengths 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 as controls and sacrificed either at the beginning or at end of the study, ie, with the same age of rats submitted to 1 or 7 months of noise treatment.

SEM

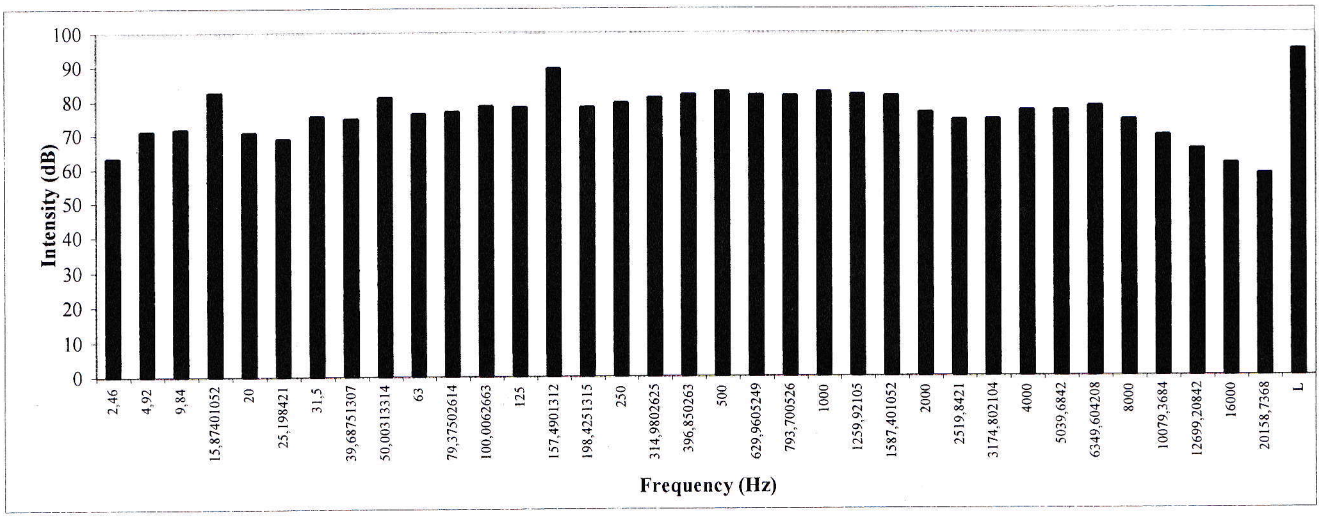
The rats were sacrificed by a lethal intravenous injection of sodium-pentobarbital (40 mg/kg) and the cervical trachea was excised and processed for SEM. Each trachea was divided in halves along its saggital line. The samples were then fixed in a solution of 3% glutaraldehyde in 0.1M phosphate buffer, 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.[13,14] Observations of the samples by SEM (JEOL JSM-35C, Japan) were performed at an accelerating voltage of 10 kV.

Quantification of Density of Epithelial Cells of Rat Trachea

Different densities of the major epithelial cells of the trachea are seen on the areas of the airway that are located over or in-between the cartilage rings, our quantitative analysis was carried out, for each sample, in separate for the two epithelial domains of the tracheal lining.

Ciliated and serous cells are the major cell types of the tracheal epithelium of the rat, together they cover more than 90% of the inner surface of the airway; this is in accordance with data from other workers.[15,16]

To evaluate the relative area of the tracheal surface that was occupied by ciliated cells, serous cells, brush cells and other unidentified cells, random SEM micrographs of the samples were obtained at a magnification of ×1000, as we have done before.[12] Twenty micrographs were made of each sample; a total area of 0.22 mm^2 of the epithelium surface of the trachea was used for quantitative analysis of each sample. The relative area occupied by each cell type, was determined with the help of a transparent grid of 320 points, spaced 1 cm from each other, that was superimposed on the printed micrographs. The numerical values of the relative area of ciliated and nonciliated cells of the tracheal epithelium were 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 proportion of area that ciliated, serous, brush, and other uniden-



Graph 1 (Fig. 5) Spectrum of frequencies and intensities of the textile-type noise that was recorded in a cotton-mill-room and reproduced in the animal house room where rats were kept.

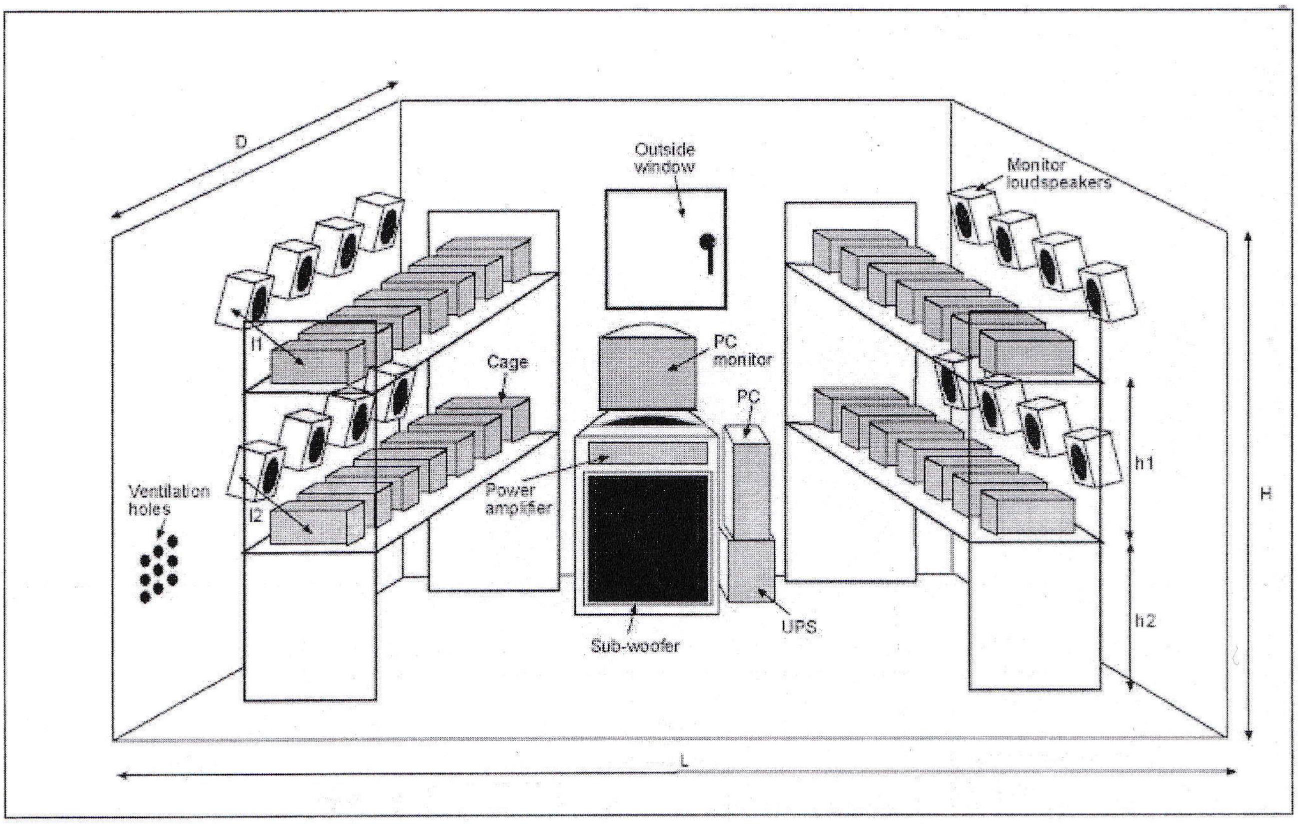


Fig. 1. Spatial organization of the animal room where the Wistar rats were exposed to the textile-type noise that was recorded at a cotton-mill-room of a plant. The dimensions of the room were the following: L −3.02 m, D −3.08 m, and H −2.90 m.

tified cells occupied on the whole tracheal epithelium.

Statistical Analysis

All values are reported as mean ± SE. Computing the partial correlation coefficient assessed the relationship between the area occupied by ciliated and serous cells, after controlling for age, duration of exposure, and localization over or in-between trachea rings. Because a strong correlation ($r = -0{,}95$, $P < 0.001$) between the relative areas occupied by ciliated and serous cells has been found, we only analyzed differences in the relative area occupied by ciliated cells located in regions in-between or over the cartilage rings of the trachea. Differences between the experimental groups in the proportion of area occupied by ciliated cells were compared using least-squares analyses of variance. Arcsine transformation of the data ($\text{angle} = \arcsin\sqrt{\text{proportion}}$) was used because of non-normality. Statistical significance was accepted for $P < 0.05$. Statistical procedures were carried out on LSMLMW.[17]

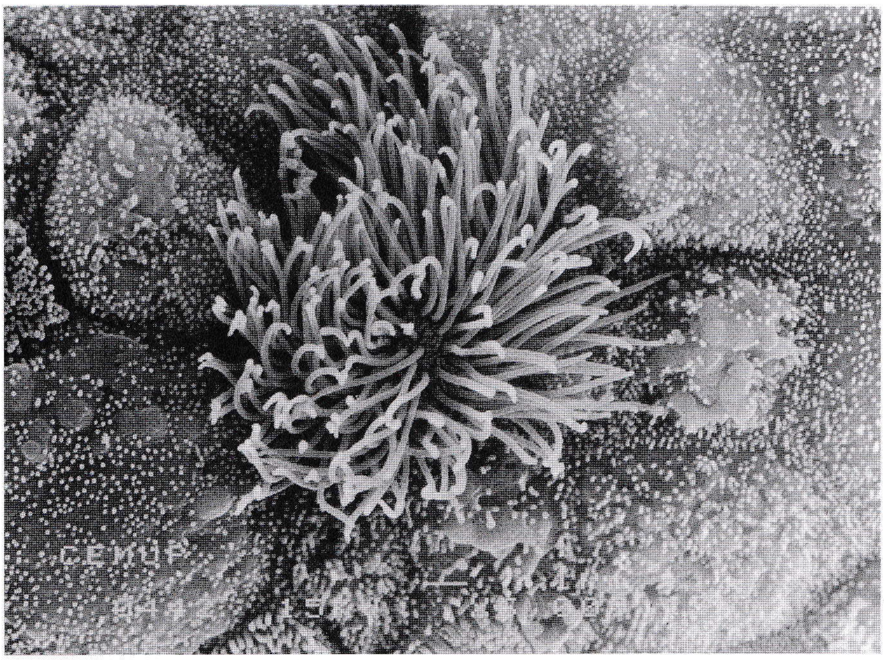


Fig. 2. High magnification SEM micrograph of a ciliated cell of the tracheal lumen of the rat surrounded by serous cells containing numerous secretory vesicles under their apical surfaces. ×6000.

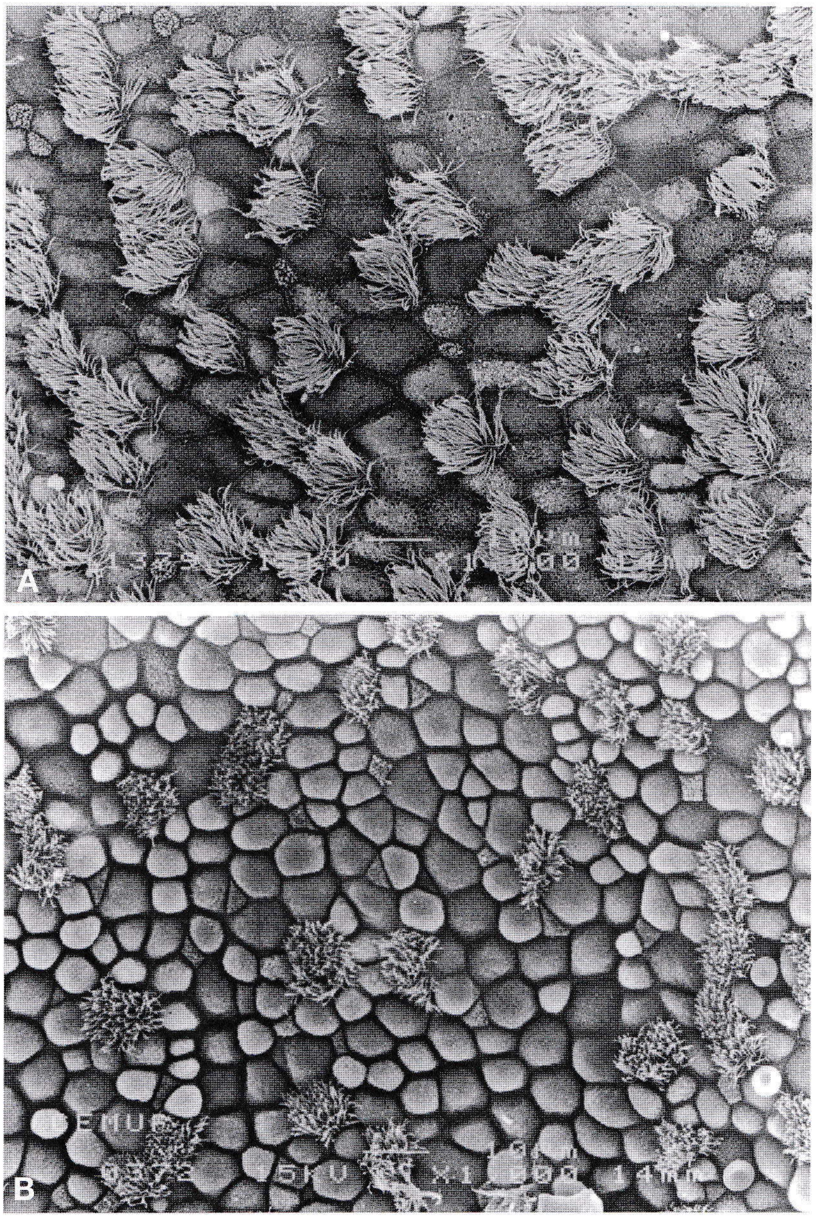


Fig. 3. SEM micrographs of cilia-poor area of the tracheal lumen of a control rat (A) and a 1-month noise exposed rat (B). Both micrographs show areas of the tracheal epithelium that are located at the midpoint in-between two adjacent cartilage rings. Serous cells are the most common cell type in both micrographs, followed by ciliated cells and a few brush cells. A decrease in the density of ciliated cells is observed in B in comparison with A. ×1000.

Noise Exposure

Textile industry is a major component of the economy of Northern Portugal. We have visited several cotton-mill rooms of textile plants in our region, and we have chosen one of these factories as the paradigm of environmental noise occurring in these type of plants.

Recording and reproduction of the noise present in the cotton mill room of this factory, was performed with an electro-acoustic set-up that used a PC based system, with a DT2823 data acquisition and a SB Live 5.1 cards, one B&K 4165 microphone with preamplifier, one 2-channel power amplifier, 16 monitor-type and 1 sub-woofer loudspeakers in bi-amplification. The software was designed using the LabVIEW system. Sound signals 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 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 at the original levels of approximately 92 dB (with spectrum very near the original one) 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 graph 1 (Figure 5).

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 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 was carefully controlled. The final sound pressure values that were obtained, measured with a quality calibrated soundmeter, 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 Fig. 1.

Results

We have used the SEM to examine the ultrastructure of the tracheal epithelium of Wistar rats after chronic exposure of the animals to noise recorded in a cotton-mill room. The cellular composition of the respiratory epithelium of the trachea was quantified and compared with that of

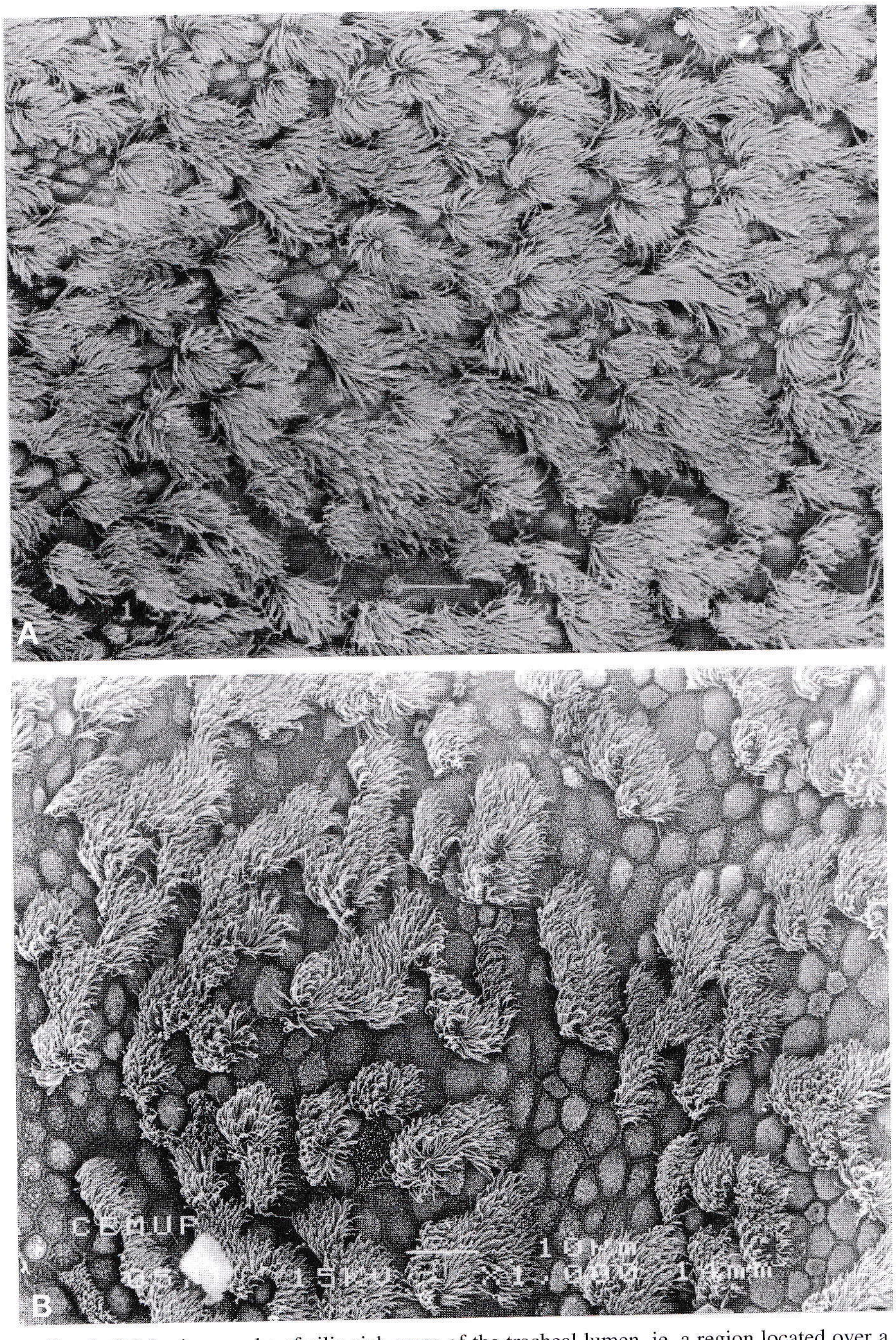


Fig. 4. SEM micrographs of cilia-rich areas of the tracheal lumen, ie, a region located over a cartilage ring. A - control rat; B – 7 months exposed rat. Ciliated cells occupy most of the epithelium surface in this region of the trachea but a decrease of the area occupied by these cells is observed in B in comparison with A. ×1000.

control animals. Figure 2 shows a SEM micrograph of a ciliated cell surrounded by a number of serous cells containing secretory vesicles under their cell surfaces. We found no significant differences between these two differently-aged control groups of rats with regards to the relative area occupied on the inner tracheal surface by ciliated, serous, and others cells of the epithelium.

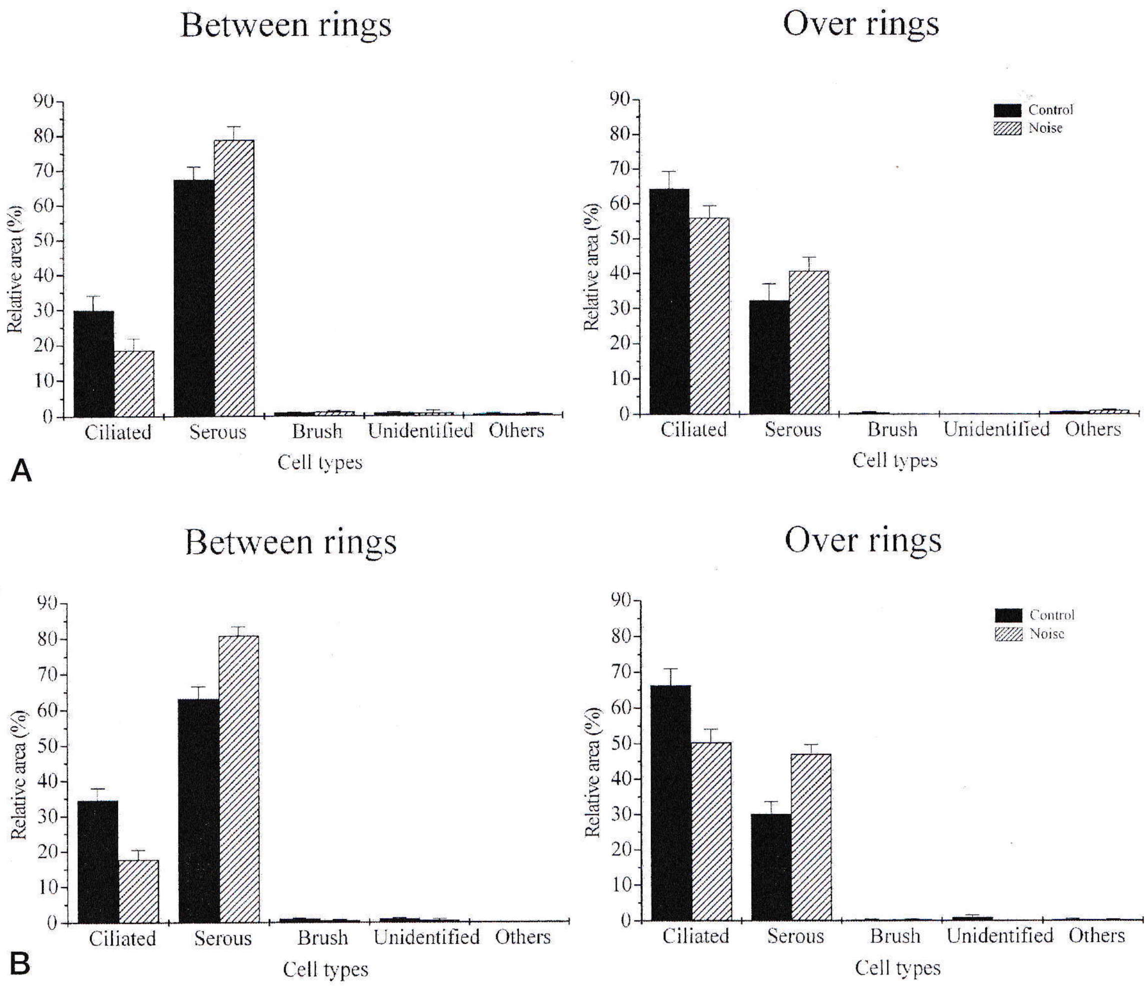
We have found that the exposure to the textile-type noise caused a significant decrease in the density of ciliated cells in both cilia-rich and cilia poor domains of the tracheal epithelium of the rats. This loss of ciliated cells was balanced by enhancement in the area occupied on the epithelium by serous cells. The phenomenon is illustrated in Figs. 3 and 4 showing the distribution of ciliated and serous cells on the tracheal lumen of control and noise-treated animals. The disorganized appearance of cilia of cells of noise-treated rats contrasted with the parallel distribution of cilia observed in samples of control trachea (Figs. 3 and 4). Quantitative comparison of these cell densities in the two domains of the trachea is depicted in graph 2 (Figure 6); here, the data compare control rats with those exposed to noise for 1 or 7 months. We also observed that the areas of the tracheal lining located in-between the cartilage rings (cilia-poor areas) showed a higher degree of loss of ciliated cells than the regions of the epithelium located over the rings.

The noise-induced alterations of the tracheal lining were observed all along the course of the exposure of the rats (from 1 to 7 months), but lacked any significant change throughout this 7-months period of treatment with regards to the degree of the cellular alterations that we have quantified. This finding indicated that after being established, (ie, after 1 month of noise exposure) the cellular change of the tracheal epithelium is kept at the same level, ie, with no quantitative evidence of either amelioration or aggravation of the proportion between ciliated and serous cells (graph 3, Figure 7).

Examination of the rats by veterinary doctors, along the 7 months of the experiment, revealed no clinical alterations in noise-exposed rats in comparison with control animals.

Discussion

The herein investigation demonstrates that the cellular composition of rat tracheal epithelium is altered if the animals are kept in an environment that reproduces the same noise found in a cotton mill room of a modern textile plant. We have submitted our rats to 40 hours/week schedule of noise that is similar to

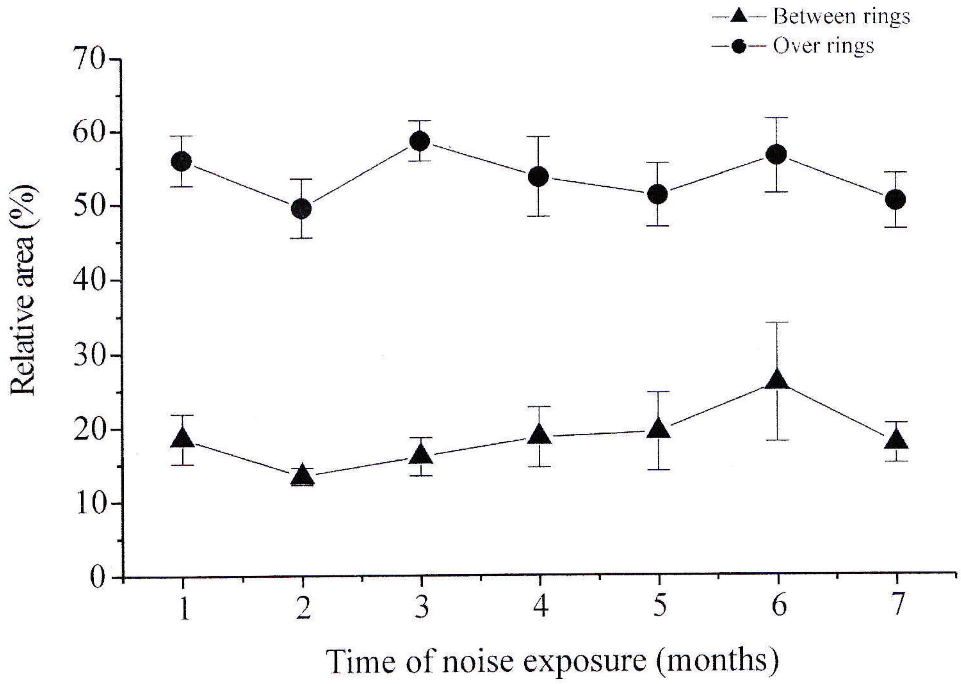


Graph 2 (Fig. 6) Comparison of the relative area occupied by ciliated and nonciliated cells in cilia-poor regions, and in cilia-rich regions between control and 1-month noise exposed rats (A) and between 7-months noise exposed rats and their controls (B). The statistical analysis indicate that the relative area of the tracheal epithelium occupied by ciliated cells is decreased after 1 and 7 months of exposure of rats to textile-type noise, both in the regions over and in-between the cartilage rings ($F(1,36) = 24.026$, $P < 0.001$, $R^2 = 0.83$), when compared with their controls.

the exposure time of textile workers that operate the machinery of the cotton mill room. Ciliated cells of the rat trachea were found to be particularly vulnerable to the noise aggression. In fact, the area of the trachea that was covered by ciliated cells was significantly reduced and the tracheal lining of noise-treated rats showed an increased area of serous cells. This epithelial change was installed early, ie, it was already seen at 1 month of noise treatment of the rats, but was not aggravated by continued exposure of the animals to noise for as long as 7 months. This is the first report documenting alterations of the cellularity of the respiratory epithelium caused by exposure of animals to workplace noise present in a modern cotton mill room.

It is well established that workers of cotton mill rooms may develop different health disorders.[18–21] Among these disorders are respiratory diseases because of chronic inhalation of cotton dusts.[18] Other pathogenic cofactors, such as cigarette smoking, have been identified before as having important contributions to respiratory diseases found among cotton mill workers.[19–21] Our investigation adds workplace noise as a putative contributor for the pathogenesis of respiratory disease among textile industry workers. In fact, the relative loss of ciliated cells that we have experimentally observed in the rat trachea, is likely to be associated with a decrease in the clearance capacity of the respiratory lining. Our previous studies on the effects of a different type of aggressive noise had already suggested that the ciliated cell is the main target for cellular damage induced by noise on the respiratory epithelium.[11,12]

We have recently observed that the inner lining of the rat trachea presents different densities of ciliated and serous cells in areas of the epithelium that are located either over or in-between the cartilage rings (data not published). Thus, we have separated here the two epithelial domains of the tracheal lining in our



Graph 3 (Fig. 7) Chronology of the relative area occupied by ciliated cells in regions located in-between (▲) and over (●) the cartilage rings of tracheas of treated rats submitted from 1 up to 7 months of textile-type noise. The decrease in ciliated cell density that is observed after 1 month is not significantly altered by a longer exposure of the animals to the noise.

quantitative analysis of the density of cell types counted in SEM micrographs of the samples. This discrimination allowed us to conclude that the noise-induced loss of ciliated cells was more intense in the region of the tracheal epithelium located in-between cartilage rings. It should be underlined that this area of the epithelium already has a lower density of ciliated cells than the over the ring regions of the airway.

In conclusion, the present investigation offers the first experimental evidence that textile-type noise alters the composition of the respiratory epithelium of rodents. It also points to the need to perform human studies to determine whether individuals chronically exposed to this type of workplace aggression will show evidence of respiratory impairment.

Acknowledgment

The authors are very grateful to Dr Daniela Silva and Prof. Carlos M. Sá (CIMUP, UP) for expert help with scanning electron microscopy. We thank Mr António Costa e Silva, Mrs Alexandrina Ribeiro, and Mr Emanuel Monteiro for technical assistance, Mr Duarte Monteiro for technical assistance and artwork, Mr Humberto Andrade Fonseca and Mr Hugo Silva Santos for the help in the setting up of animal room, for noise exposure, Dr Pinto de Almeida who allowed us to use the textile facilities to tape the cotton-mill noise and Mr José Aurélio Mexedo for photography. This work was funded by grants from IDICT, Portugal.

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