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ARTIGO ORIGINAL/ORIGINAL ARTICLE

O parênquima pulmonar do rato Wistar exposto a ruído de baixa frequência

The lung parenchyma in low frequency noise exposed Wistar rats

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RESUMO

Em estudos prévios do aparelho respiratório realizados em ratos expostos a ruído de baixa frequência (RBF), observaram-se lesões irreversíveis do epitélio respiratório. Um dos aspectos mais notáveis destas lesões diz respeito

ABSTRACT

Previous studies of low frequency noise (LFN) (≤ 500 Hz, including infrasound) exposed Wistar rat trachea and lung show that LFN induces irreversible lesions in the rodent respiratory system. Most notable was the behavior of the tracheal

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às imagens das microvilosidades das células em escova que apresentam alterações importantes na distribuição e na morfologia. Este estudo é dirigido às populações celulares dos alvéolos pulmonares de ratos expostos a RBF em comparação com ratos mantidos em silêncio. Dez ratos Wistar foram expostos continuadamente a 2160 horas de RBF e sacrificados após uma semana em silêncio. Dez ratos de controlo, do mesmo grupo etário, foram mantidos em condições idênticas mas em silêncio. Todos os animais foram tratados de acordo com a norma 86/609/CE. Fragmentos do parênquima pulmonar foram recolhidos e processados para microscopia óptica e electrónica de varrimento. Nos ratos expostos, as paredes alveolares apresentam-se grosseiramente espessadas e a estrutura distorcida ou mesmo apagada. Os macrófagos são extremamente frequentes nos ratos expostos, embora também se observem em menor número nos ratos de controlo. As paredes dos vasos estão espessadas e os pneumócitos de tipo I são raros. Em contraste, os de tipo II tornam-se na população celular alveolar mais frequente e importante. Nestas células, as microvilosidades distribuem-se irregularmente. Ainda nos pneumócitos tipo II, observa-se com grande frequência "buracos negros" na superfície. Observam-se ainda 2 tipos de células em escova: as intersticiais e as externas. Nos ratos expostos, as microvilosidades destas células apresentam-se fundidas. O RBF exerce uma acção nociva sobre o parênquima pulmonar.

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Palavars-chave: ruído de baixa frequência, pneumócitos tipo I e II, célula em escova, microvilosidade, fibrose, alvéolo.

brush cell (BC) where fused microvilli were frequently captured in scanning electron microscopy. This study focuses on the alvoelar BC in LFN-exposed and control rat populations. Ten Wistar rats were exposed to 2160 hours of continuous LFN, then kept 1 week in silence before sacrifice. Another 10, age-matched rats were kept in continous silence. All were fed standard rat food, and had unrestrained access to water (treated in accordance with 86/609/CE). Lung parenchyma fragments were excised and processed for scanning electron microscopy. In the non-exposed, alveolar walls were thin, and wall structure was well defined. In the LFN-exposed, walls were thickened and wall structure was almost effaced. Macrophages were found in the non-exposed specimens, but were not so frequent in the LFN-exposed. Both vein and artery walls were thickened. In the parenchyma of the exposed rats, an increase in type II pneumocytes was observed, and their microvilli were dramatically altered in terms of cell-surface distribution, sprouting direction, and amount. Unidentified black holes eruptin from the cell surface were observed. In the alveoli, 2 different types of BC's were observed: external and interstitial. In the exposed rats, microvilli of both types of BC were fused. These results confirm that LFN exposure can impinge on the entire respiratory system of living organisms.

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Key-words: Low frequency noise, Type I and II pneumocytes, brush cell, microvilli, fibrosis, alveoli.

INTRODUCTION

The use of animal models to investigate the effects of low frequency noise (LFN) (5500 Hz, including infrasound) on the respiratory system has been ongoing since 1992. The motivation for these studies has been a LFN-induced pathology designated as vibroacoustic disease (VAD)[1-3].

In VAD, a systemic, extra-aural pathology caused by long-term exposure to LFN[1-3], early signs include repeated oropharynx infections and bronchitis (in smokers and non-smokers alike)[2]. Although these may be aggravated by the alterations in the immune system, there is evidence to suggest that other mechanisms may be at play, especially given the intense and irreversible cellular changes observed in the trachea of LFN exposed rodents, and briefly described above.

The involvement of the respiratory system in VAD and in LFN-exposed workers (smokers and non-smokers alike) has already been demonstrated[4-7], and with the data collected to date from LFN-exposed animal models, some of

the human pathology has already been partially explained[5,6,8-10].

Previous studies of the tracheae of LFN-exposed Wistar rats showed that the respiratory brush cell (BC) had an extraordinary response to the presence of LFN: its microvilli fuse, forming a central indentation which, with time, continues deepening until the cell eventually dies[11].

The goal of this paper is to describe the morphological changes observed in the parenchyma and alveoli of LFN-exposed Wistar rats, and in particular, the BC.

METHODS

Noise exposure

A sound signal was generated by a digital noise generator, amplified and frequency filtered. Fig. 1 shows 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 chamber. The resultant acoustic energy was predominantly ≤500 Hz, with dBA levels at 85 and dBLin levels at 108. (Fig. 1). Sound pressure levels (in dBA and dBLin) were measured with a modular precision sound level meter (Bruel & Kjaer, 2231, Den-

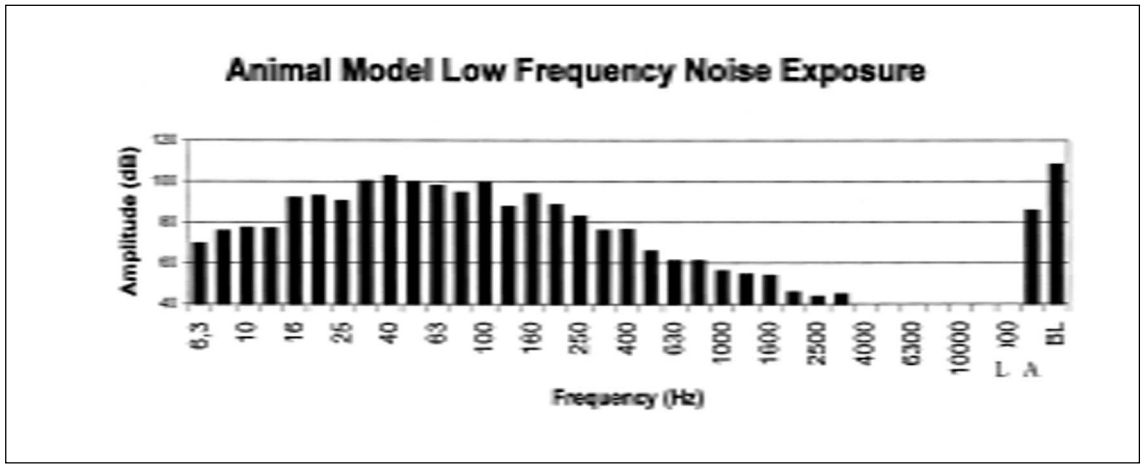

Fig. 1 — Frequency distribution of the acoustic environment of the exposed rat population. Acoustic energy is predominantly within the lower frequency range (≤500Hz). Average levels: 108 dB_{Lin} (dBL), 85 dBA.

mark). Frequency spectra were obtained using a real-time frequency analyzer (Hewlett Packard, 3569 A, USA) in 1/3 octave frequency bands (from 6.3 Hz to 20000 Hz). Microphone calibration was achieved with a 250 Hz pistonphone (Bruel & Kjaer, 4228, Denmark) to a sound pressure level of 124 dB re: 20 μPa.

Animals

Ten Wistar rats were continuously exposed to LFN for a total of 2160 hours, after which they spent one week in silence before sacrifice (to attenuate the effects of edema). Ten age-matched rats living in continuous silence were used as controls. Rodents were obtained from a local breeder (Portuguese Institute of Oncology, Lisbon, Portugal), had unrestrained access to water, and were treated in accordance with the European Commission on Animal Protection for Experimental and Scientific Purposes (86/609/CE).

Lung fragments were excised and prepared for light microscopy, and scanning (SEM) electron microscopy.

Microscopy

The animals were sacrificed by a lethal intravenous injection of sodium-pentobarbital (40mg • kg^{-1} BW) and the trachea was divided in two along the saggital line. The specimens for light microscopy were formalin-fixed, paraffin-embedded, and stained with hematoxylineosin, and fucsin-rhesorcin.

The specimens for electron microscopy were placed in a solution of 3% gluteraldehyde in 0.1 M phosphate buffer, pH 7.2 and then washed with several changes of 5% sucrose in 0.1 M phosphate buffer, pH 7.2, for ultrastructural studies.

Specimens for scanning electron microscopy (SEM) 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 15 kV.

RESULTS

Parenchyma

In the non-exposed, alveolar walls were thin, and wall structure was well defined (Fig. 2). However in the LFN-exposed, walls were thickened and wall structure was almost effaced (Fig. 3). Numerous macrophages were found in the non-exposed specimens (Fig. 4), but were not so frequent in the LFN-exposed. Both vein and artery walls were thickened. Artery intima were thickened and both internal and external elastic laminae exhibit disruptions. This feature was first observed in the autopsy of a VAD patient deceased in 1987, in large and small arteries and veins[12], and has since been observed in LFN-exposed Wistar rats[13].

The amount of type II pneumocytes increased in the exposed rats, while type I pneumocytes almost vanished. In the non-exposed, type II pneumocyte microvilli covered the entire surface of the cell open to the airway, without gaps, and all sprouted in the same general direction (Fig. 5). In LFN-exposed rodents, pneumocyte type II microvilli appeared decreased in number, interspersed with gaps, and with various sprouting directions (Fig. 6). On the surface of type II pneumocytes, unidentified black holes erupting to the hypophase were visible in both non-exposed and exposed albeit less frequently in the latter (Figs. 5, 6). This feature went initially unnoticed because of the extraordinary increase in the number of type II pneumocytes in exposed rodents. The frequency of black holes per type II pneumocyte is decreased in exposed. But given the increased number of type II pneumocytes, the number of black holes per alveollus is not visibly altered.

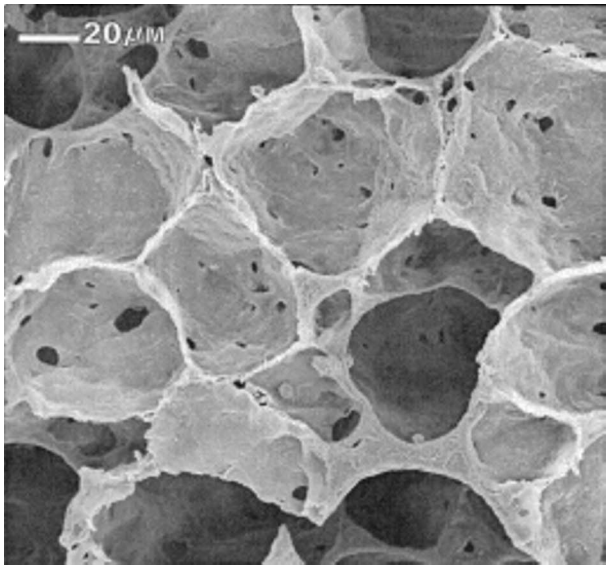

Fig. 2 — Lung Parenchyma Structure – control rat. Walls are thin, and details of wall structute are visible.

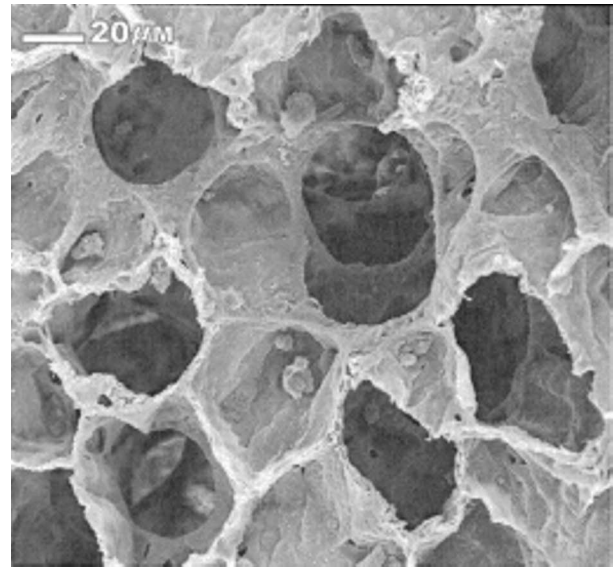

Fig. 3 — Lung Parenchyma Structure – exposed rat. Walls are thickned and details of wall structute are not as obvious as in controls.

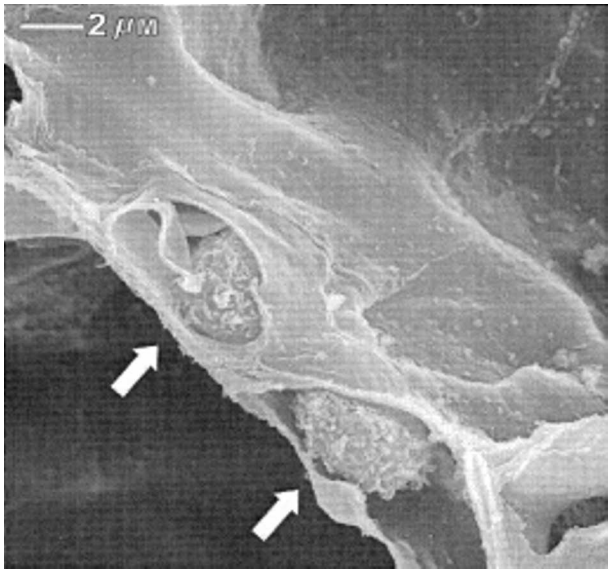

Fig. 4 — Lung Parenchyma Structure – control rat.Two macrophages are partially visible.

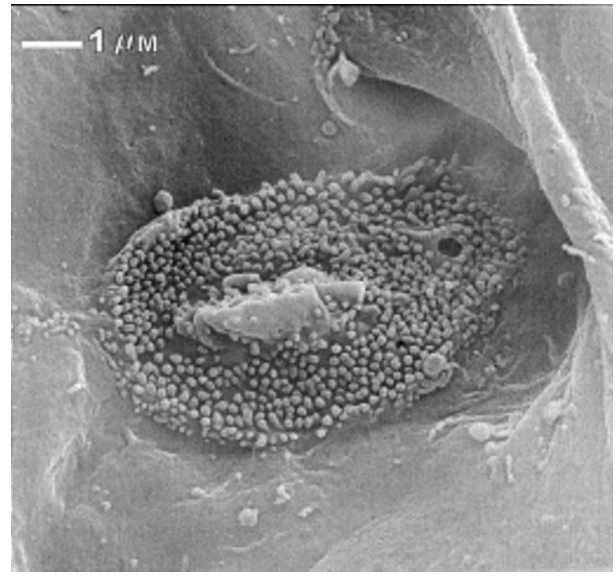

Fig. 5 — Type II Pneumocyte – control rat. Microvilli are evenely distributed over the surface of the cell and their sprouting directions are similar. The "black hole" is not an artifact as was observed in numerous micrographs.

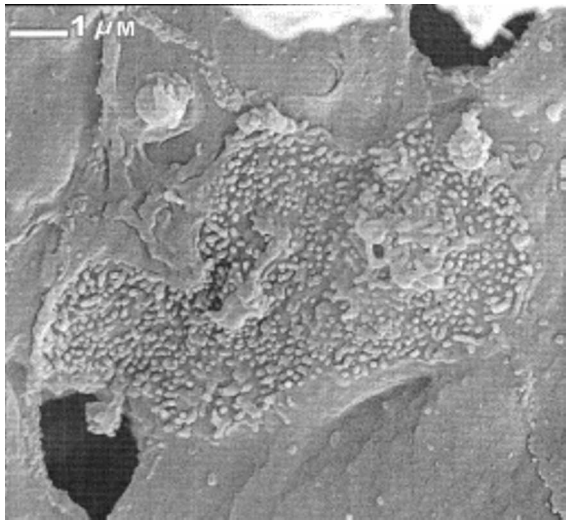

Fig. 6 — Type II Pneumocyte – exposed rat. Microvilli are disorganized. They are no longer evenely distributed over the surface of the cell, and sprouting directions are not uniform. The "black hole" are still very evident.

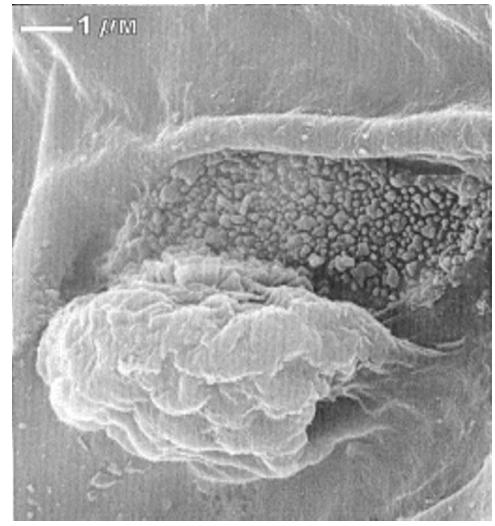

Fig. 7 — External BC – control rat. BC is resting upon a type II pneumocyte. BC microvilli are evenly distributed and can be individually identifiable.

The alveolar brush cell

Two distinct types of BCs can be found in the alveoli: the external BC which has its body external to the alveolar wall (Fig. 7), and the interstitial BC which has its body embedded within the alveolar wall (Fig. 9). Although still featuring an apical surface covered by microvilli, the body of the external BC, protruding from the alveolar wall, is connected by powerful external anchorage structures. The characteristic tuft of microvilli is also seen on the apical surface of the interstitial BC. In non-exposed, the amount of external BCs is approximately the same as that of interstitial BCs. In relation to the pleura, BCs are more numerous in the distal alveoli than in the proximal alveoli, as previously observed 14 in the non-exposed rodents, BC microvilli are frequently seen resting upon type II pneumocyte cells (Fig. 7). This is also seen in the LFN-exposed but additional external BCs with no apparent relationship to pneumocytes are distinguishable as well (Fig. 8). In exposed, groups of 3 or 4 external and interstitial BCs bunched together can be observed. No such gatherings are found in the non-exposed. In many micrographs, the wall of the external BC body seems more convoluted than in non-exposed.

In the LFN-exposed specimens both external and interstitial BC microvilli have fused together and seem to spread outward forming a central indentation (Figs. 8, 10). This is similar to what was observed in the tracheal and bronchial BCs[11].

DISCUSSION

Past studies investigating LFN-induced lesions on rat respiratory epitelia used an occupational schedule to present the acoustics stimulus, *i.e.*, the animals were exposed to LFN for 8 hours/day, 5 days/week and spent weekends in silence.

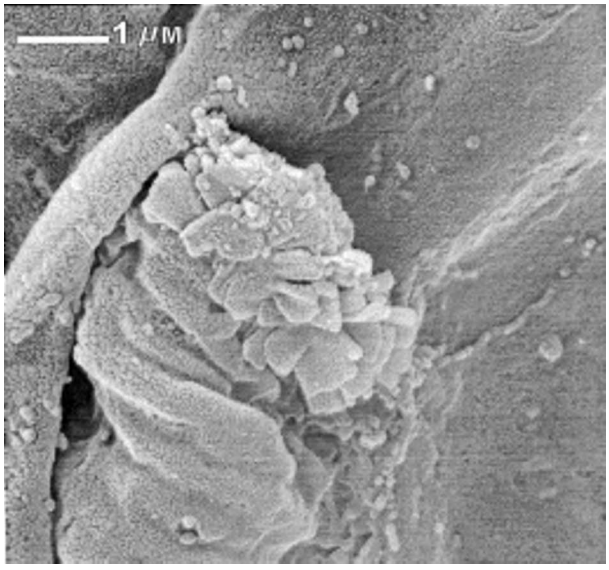

Fig. 8 — External BC – control rat. BC microvilli are fused. The outer of the BC appears disorganized and under extreme stress.

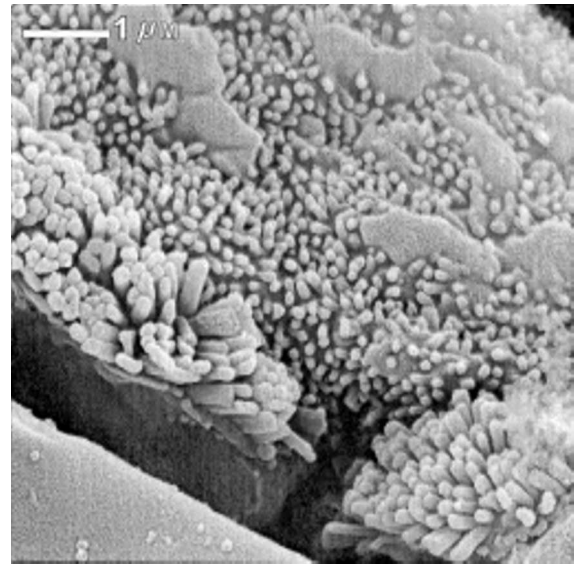

Fig. 9 — Interstitial BC – control rat. Two BC are resting upon a pneumocyte. BC microvilli are individually identifiable and are evenely distributed over the apical surface of the cells.

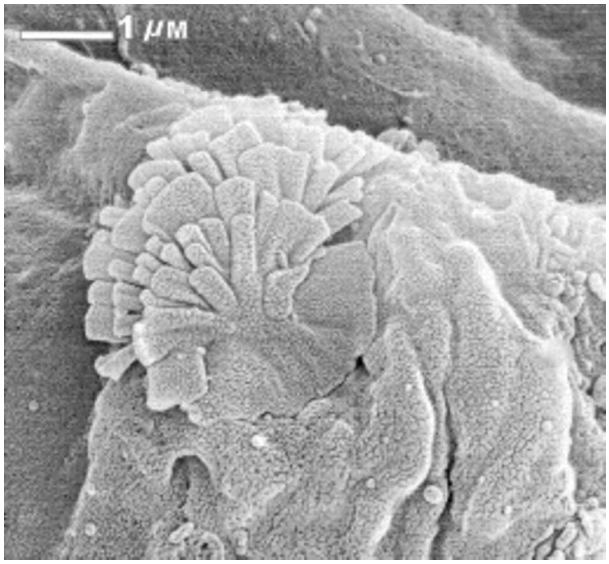

Fig. 10 — Interstitial BC – exposed rat. BC microvilli are no longer evenely distributed and microvilli appear fused. The body-like appearance of the BC is not the abnormal structure of the alveolar wall.

In this study, continuous exposure was chosen because it simulates other work environments, such as onboard ships, on oil rigs, or in space, where no external damping exists and all life support systems are sources of LFN. On the other hand, urban and suburban LFN can also be significant sources of exposure. For example, if the residential unit is located near high volume roads or highways, near subway or bus stations, the exposure to LFN may be constant while in the home, albeit at a lower amplitude.

Thickened artery intima and disrupted internal and external elastic laminae was first observed in the autopsy of a VAD patient deceased in 1987, in large and small arteries and veins [12], and has since been observed in LFN-exposed Wistar rats [13]. The structural changes with increased thickening corresponde to the fibrosis already observed in previous LFN-exposed animal studies[8,9,11] and human[5,12] studies. Similarly, effaced structures as well as cellular de-differentiation

have also been observed in previous studies[11]. The decrease in macrophages was expected given the acceleration of the cellular life-cycles, and the extraordinary amount of cellular death, already observed in both the human lung[15,16] and pericardium[17,18] of VAD patients. This feature requires further inquiry.

Decreased amounts of type I pneumocytes is a known and expected situation since other pulmonary stress situations elicit this response. However, here there is a simultaneous dramatic increase in type II pneumocytes and BCs.

Although the function of the respiratory BC is still unknown, some studies suggest that it functions as a neuropeptide modulator[19,20]. The black holes cannot be considered artifacts since they were very frequent, and were reported in other literature[11].

In conclusion, the evidence presented herein attests the deleterious effect of LFN on the parenchyma of rats, and the urgent need to monitor workers who must remain in LFN-rich environments for extended periods of time.

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REFERENCES

1. CASTELO BRANCO NAA. The clinical stages of vibroacoustic disease. Aviat Space Environ Med 1999; 70 (3, Suppl): A32-9.
2. CASTELO BRANCO NAA, RODRIGUEZ LOPEZ E, ALVES-PEREIRA M, JONES DR. Vibroacoustic disease: some forensic aspects. Aviat Space Environ Med 1999; 70 (3, Suppl): A145-151.
3. CASTELO BRANCO NAA, RODRIGUEZ LOPEZ E. The vibroacoustic disease — An emerging pathology. Aviat Space Environ Med 1999; 70 (3, Suppl): A1-6.
4. CASTELO BRANCO NAA. The respiratory system as a target of low frequency noise. Reports on human and animal models. Proc 8th Intern Cong Sound & Vibration, Hong Kong, P. R. China, July 2001: 1501-1508.
5. REIS FERREIRA JM, COUTO AR, JALLES-TAVARES N, CASTELO BRANCO MSN, CASTELO BRANCO NAA. Airflow limitations in patients with vibroacoustic disease. Aviat Space Environ Med 1999; 70 (3, Suppl): A63-69.
6. CASTELO BRANCO NAA, MARTINHO PIMENTA AJ, REIS FERREIRA J, ALVES-PEREIRA M. Monitoring vibroacoustic disease. Proc. SSGRR2003w, January 2003, L'Aquila, Italy: No. 102: 5 pages.
7. ALVES-PEREIRA M. Extra-aural noise-induced pathology. A review and commentary. Aviat Space Environ Med 1999; 70 (March, Suppl): A7-21.
8. GRANDE N, ÁGUAS AP, SOUSA PEREIRA A, MONTEIRO E, CASTELO BRANCO NAA. Morphological changes in the rat lung parenchyma exposed to low frequency noise. Aviat Space Environ Med 1999; 70 (3, Suppl): A70-77.
9. SOUSA PEREIRA A, ÁGUAS A, GRANDE NR, CASTELO BRANCO NAA. The effect of low frequency noise on rat tracheal epithelium. Aviat Space Environ Med 1999; 70 (3, Suppl): A86-90.
10. OLIVEIRA MJR, SOUSA PEREIRA A, ÁGUAS AP, MONTEIRO E, GRANDE NR, CASTELO BRANCO NAA. Effects of low frequency noise upon the reaction of pleural milky spots to mycobacterial infection. Aviat Space Environ Med 1999; 70 (March, Suppl): A137-140.
11. CASTELO BRANCO NAA, ALVES-PEREIRA M, MARTINS DOS SANTOS J, MONTEIRO E. SEM and TEM study of rat respiratory epithelia exposed to low frequency noise. In: Science and Technology Education in Microscopy: An Overview, A. Mendez-Vilas (Ed.), Formatex: Badajoz, Spain, 2002, vol. II: 505-533.
12. CASTELO BRANCO NAA. A unique case of vibroacoustic disease. A tribute to an extraordinary patient. Aviat Space Environ Med 1999; 70-3: A27-31
13. MARTINS DOS SANTONS J, GRANDE NR, CASTELO BRANCO NAA, ZAGALO C, OLIVEIRA P. Vascular lesions and vibroacoustic disease. Eur J Anat 2002; 6-1: 17-20.
14. CHANG LY, MERCER RR, CRAPO JD. Differential distribution of brush cells in the rat lung. Anat Rec 1986; 216: 49-54.
15. REIS FERREIRA J, MONTEIRO E, MARTINS AP, CASTELO BRANCO NAA. Aspectos morfológicos da traqueia em doentes com a doença vibroacústica. VVVV Congresso de Penumologia 2002; Stª Mª da Feira.

16. CASTELO BRANCO NAA, MONTEIRO E, REIS FERREIRA J. Aspectos Morfológicos do Pulmão em Doentes com a Doença Vibroacústica. XVIII Congresso de Penumologia 2002, Stª Mª da Feira
17. CASTELO BRANCO NAA, MONTEIRO E, ALVES--PEREIRA M, ÁGUAS AP, SOUSA PEREIRA A, GRANDE NR. Morphological changes in the pericardia of military helicopter pilots. Proc. Microscopy Barcelona 2001, 4-7 Sep 2001: 318-319.
18. CASTELO BRANCO NAA, ÁGUAS A, SOUSA PEREIRA A, MONTEIRO E, FRAGATA JIG, GRANDE NR. The pericardium in noise-exposed individuals. Internoise 2001, The Hague, Holland, August 2001: 1003-1006.
19. ZAGALO C, ÁGUAS AP, SOUSA PEREIRA A, MONTEIRO E, GRANDE NR, CASTELO BRANCO NAA. Bronchial reactivity in VAD patients and morphological changes in rodent brush cells. Aviation Space Environmental Medicine 2001; 72(3): 253.
20. ZAGALO C, CASTELO BRANCO NAA, MONTEIRO E, ALVES-PEREIRA M, ÁGUAS AP, PEREIRA, AS, GRANDE NR. Low fequency noise exposed rat trachea: a neuroendocrine function For the respiratory brush cell? Proc. Microscopy Barcelona 2001, 4-7 Sep 2001: 320-321.