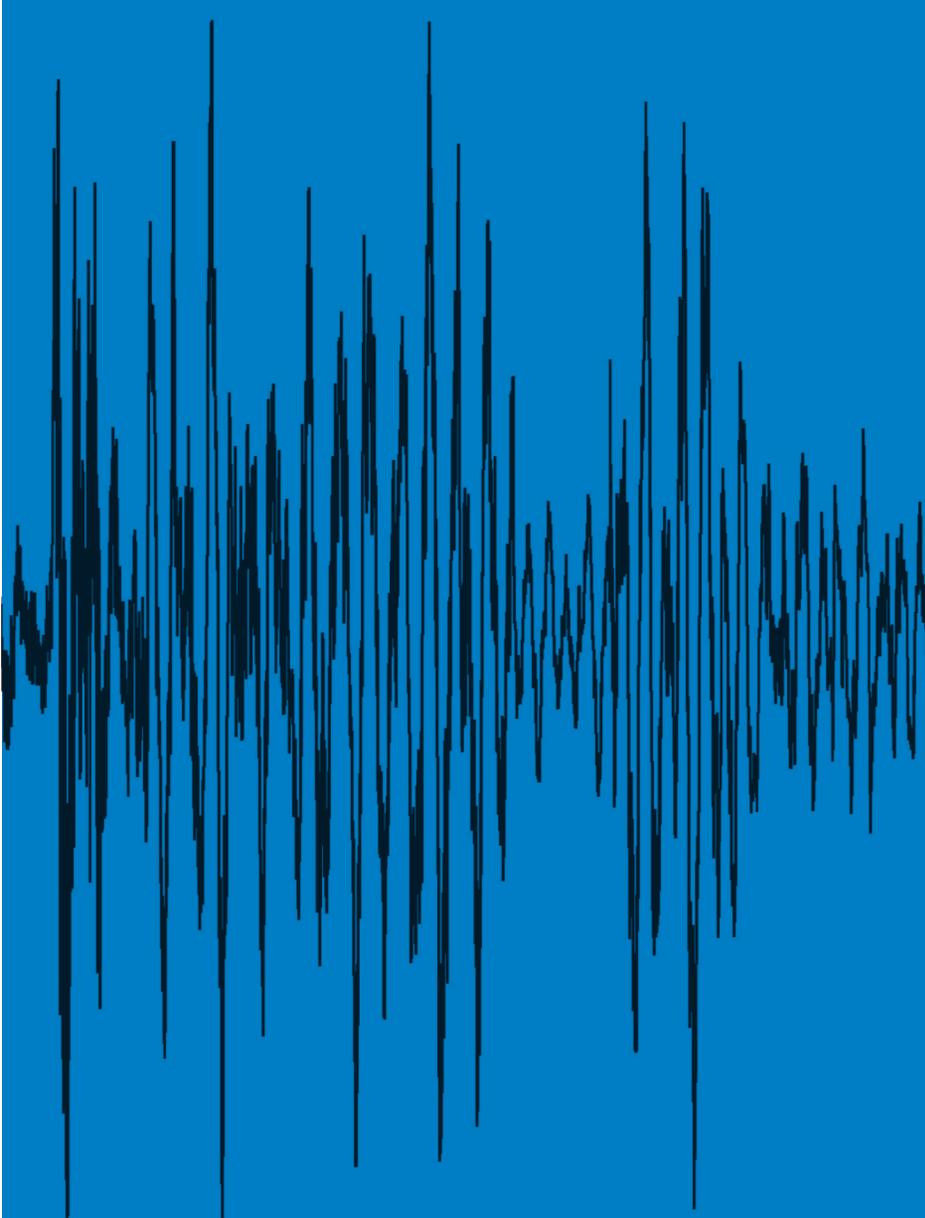


Indexed with  
MEDLINE, EMBASE & SCI

ISSN 1463-1741

Impact Factor® for 2011:  
1.254



# Noise & Health

A Bi-monthly Inter-disciplinary International Journal  
[www.noiseandhealth.org](http://www.noiseandhealth.org)

# Exposure to industrial wideband noise increases connective tissue in the rat liver

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## Abstract

Rats were daily exposed (eight hours/day) for a period of four weeks to the same high-intensity wideband noise that was recorded before in a large textile plant. Histologic observation of liver sections of the rats was used to perform quantitative comparison of hepatic connective tissue (dyed by Masson trichromic staining) between the noise-exposed and control animals. For that, we have photographed at random centrolobular areas of stained rat liver sections. We found that noise exposure resulted in significant enhancement in the area of collagen-rich connective tissue present in the centrolobular domain of the rat liver. Our data strengthen previous evidence showing that fibrotic transformation is a systemic effect of chronic exposure of rodents and humans to industrial wideband noise.

*Keywords:* Connective tissue, industrial noise, light microscopy, liver, wideband noise

## Introduction

Working-place noise has been identified as a significant cause of work-related health problems of our time; the harmful effects of work-related noise depend on the physical features of the noise, and on the extension and frequency of human exposure to it.<sup>[1]</sup> In addition to lesions to the hearing function,<sup>[2,3]</sup> noise may be deleterious to internal organs due to vibration that is strong enough to be transmitted to human body tissues.<sup>[4-6]</sup> Exposure to chronic noise may also affect the immune system,<sup>[7,8]</sup> and be magnified by other environmental co-factors.<sup>[9,10]</sup> As part of working-place noise, high-intensity wideband noise is often found in large industrial outfits with heavy machinery,<sup>[11]</sup> such as modern textile plants. This type of noise may contribute, as we have reported before, to health problems among textile workers.<sup>[12-14]</sup> In this study we have recorded the noise present in a large textile mill of our geographical region (northern Portugal), and we have reproduced the exact same noise in a noise-insulated animal room where rats were kept for a period of four weeks. Noise was activated according to a schedule of daily working time (eight hours). We report here that the

liver of these noise-exposed animals presented a significant enhancement in connective tissue when compared with data from controls rats kept in silent rooms.

## Methods

### Animals and experimental groups

We have used ten adult (two-month old) male Wistar rats that were purchased from a Spanish commercial breeder (Charles River Laboratories España, S. A., Spain). The rats were first kept for one week in the general animal house of our institute and had unrestricted access to food (commercial chow) and water. Half of the animals were then transferred to a noise-insulated special animal room equipped with noise-generating equipment where they were kept for a period of one month. Standard house conditions were used throughout the experiment and they involved keeping two rats in a plastic cage (42 × 27 × 16 cm) with a steel lid. Five rats were exposed to the same noise that was pre-recorded in a textile plant. Rat noise-exposure was performed for four weeks according to a working time schedule (eight hours/day; five days/week with weekends in silence). The other half of the rats was kept in silence in a separate animal room; They were used as age-matched controls of the noise treatment.

### Noise exposure

The wideband noise of a cotton-mill room from a large textile factory of Northern Portugal, was recorded and reproduced in a sound-insulated animal room. The spectrum of frequencies

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	DOI: 10.4103/1463-1741.102959
	PubMed ID: ***

and intensities of this wideband noise is documented in Figure 1.

### Light microscopy

All rats were sacrificed by a lethal intravenous injection of sodium-pentobarbital (40 mg/kg) and their vascular system perfused with a saline followed by paraformaldehyde fixation. The right lateral lobe of the liver was excised and routinely processed for light microscopy analysis. Five  $\mu\text{m}$  paraffin-embedded slices of liver samples were obtained and dyed by Masson Trichromic staining (for connective tissue dyeing) and counter stained with Haematoxylin.

### Quantification of connective tissue

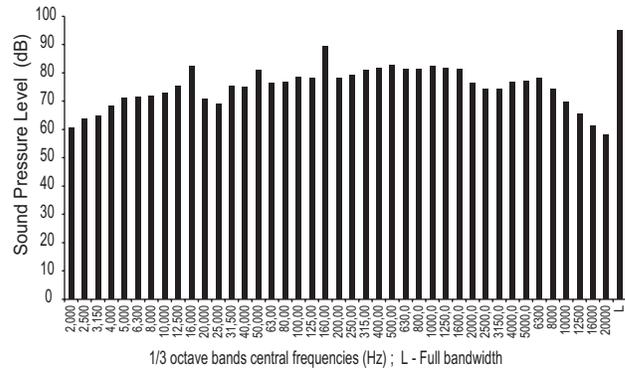
We have determined the average width of the connective tissue layer of the wall of centrolobular veins of the liver paraffin slices in both noise-exposed and non-exposed rats. Using a  $40\times$  magnification, random three to five centrolobular veins per rat were photographed and with the help of the Leica Q Win V3 software, measurements on the width of the connective tissue layer were made in three points per vein (chosen at random by superimposition of a transparent grid).

### Statistical analysis

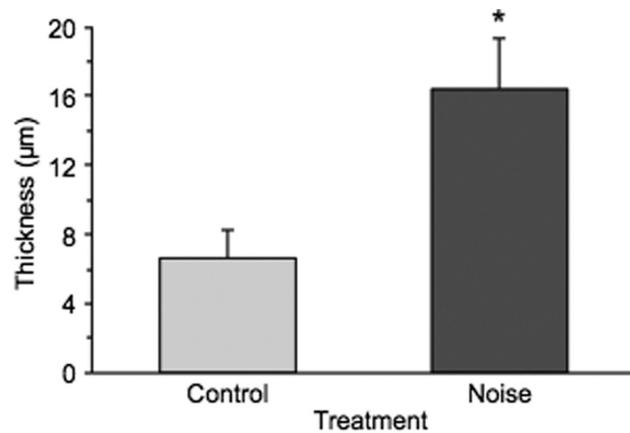
All values are reported as mean + standard error (SE). Differences in the width of the connective tissue layer of the wall of centrolobular veins of the liver were analyzed through the Mann-Whitney  $U$  test. The significance level was set at 0.05. The statistical analysis was carried out with IBM SPSS Statistics version 20.

### Results

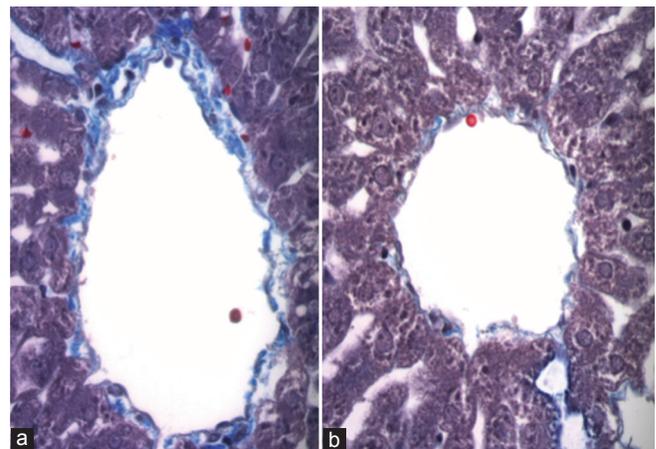
Paraffin tissue sections were studied by light microscopy to quantify the area occupied by connective tissue in the centrolobular area of liver samples of rats chronically exposed to wideband industrial noise, and to compare the values obtained in these animals with those measured in samples from age-matched controls kept in silence. Quantitative comparison of numerical data from the two groups of rats revealed that exposure to noise was associated with a significant increase (Mann-Whitney  $U$  test,  $U = 2.00$ ,  $P = 0.016$ ) in the area occupied by connective tissue on the wall of the centrolobular vein of the liver tissue [Figures 2 and 3]. Qualitative scrutiny of the overall topography of connective tissue in the noise-exposed rats indicated that the connective tissue was located in the same histologic domains where connective tissue is seen in normal liver, i.e. in addition to the centrolobular region, in the portal regions and in the narrow interlobular spaces. There was no evidence of disruption of lobule architecture due to abnormally located collagen fibers.



**Figure 1:** Spectrum of sound pressure level (dB) and intensities (Hz) of the textile-type noise that was recorded in a cotton-mill-room of a large plant of northern Portugal; the same wideband noise was reproduced in the animal room where the Wistar rats were kept



**Figure 2:** Quantitative comparison of the thickness of connective tissue layer of centrolobular areas of the liver between control rats and rats exposed to noise (means $\pm$ se). There is a significant difference between values from the two groups of animals ( $P = 0.016$ )



**Figure 3:** Light micrograph of a centrolobular vein of the liver from a noise-exposed rat (a) and an age-matched control rat (b). A thicker layer of connective tissue on the wall of the centrolobular vein can be seen in the liver of the noise-exposed rat (a) when compared with their age-matched control (b). Masson Trichromic staining  $400\times$ .

## Discussion

Our results document that there is enhancement in connective tissue of the centrolobular areas of the liver of rats if they are chronically submitted to industrial wideband noise. Rat noise exposure was done for a period of just 4 weeks indicating that a relatively short period of time is enough to activate liver fibroblasts into increasing their production of connective tissue matrix and fibers. Of the two major histologic domains where connective tissue is seen in the liver lobule, centrolobular area and portal regions, we have chosen to focus our attention on the former one because of the greater morphological homogeneity of the centrolobular area of the liver lobule in comparison with peripheral areas, such as portal regions.<sup>[15]</sup> Because the tissue accumulation of connective tissue in the liver of noise-exposed animals did not alter the general arrangement of liver lobules, it may be concluded that it is not likely that this reported noise-induced alteration of the liver microanatomy will evolve into disruption of liver architecture leading to cirrhosis.<sup>[16]</sup> The present study adds the liver to a number of other organs (both in humans and rodents) that have been found to enhance their content in collagen fibers after being chronically exposed to noise.<sup>[5,17,18]</sup>

The noise used to treat the rats in this study is similar to the one that we have recorded in a modern large textile mill of northern Portugal. The weekly schedule that we have submitted the rats to noise was similar to the weekly working hours of manufactory workers in Europe. It is thus plausible to conclude that our results may be relevant to address potential liver alterations in workers of large factories with intense noise due to heavy machinery.

## Acknowledgment

The authors are very grateful to Mr. António Costa e Silva and Dr. Madalena Costa for technical assistance. The authors also acknowledge Dr. Pinto de Almeida from Flor do Campo textile mill who allowed us to use the textile facilities in order to tape the cotton-mill noise. This work was funded by grants from FCT, Portugal.

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**How to cite this article:** Oliveira MR, Freitas D, Carvalho AP, Guimarães L, Pinto A, Águas AP. Exposure to industrial wideband noise increases connective tissue in the rat liver. *Noise Health* 2012;14:227-9.

**Source of Support:** FCT, Portugal, **Conflict of Interest:** None declared.