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Chapter

Acoustics and Biological Structures

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Abstract

Within the context of noise-induced health effects, the impact of airborne acoustical phenomena on biological tissues, particularly within the lower frequency ranges, is very poorly understood. Although the human body is a viscoelastic-composite material, it is generally modeled as Hooke elastic. This implies that acoustical coupling is considered to be nonexistent at acoustical frequencies outside of the human auditory threshold. Researching the acoustical properties of mammalian tissue raises many problems. When tissue samples are investigated as to their pure mechanical properties, stimuli are not usually in the form of airborne pressure waves. Moreover, since the response of biological tissue is dependent on frequency, amplitude, and time profile, precision laboratory equipment and relevant physiological endpoints are mandatory requirements that are oftentimes difficult to achieve. Drawing upon the viscoelastic nature of biological tissue and the tensegrity model of cellular architecture, this chapter will visit what is known to date on the biological response to a variety of different acoustic stimuli at very low frequencies.

Keywords: infrasound, low frequency noise, health, cellular biology, tissue morphology

1. Introduction

Airborne pressure waves are ubiquitous in all human environments and have played vital roles in the survival, evolution, and development of the human species. Under certain conditions, airborne pressure waves can be perceived as "sound" by the human auditory system. Under other conditions, they may be perceived as a whole-body or partial-body vibration. Some airborne pressure waves are not consciously perceived at all. As human societies developed and became more technological, airborne pressure waves emanating from human-made devices became ubiquitous and "noise" became a more serious issue. By the late nineteenth century, noise and health studies began to flourish. In the early twentieth century, the telephone and growing industrialization led to more in-depth studies of the human hearing function. In 2011, a WHO document on the burden of diseases reflected the seriousness of the ongoing "noise problem" [1].

The only airborne pressure waves considered of consequence for human health were those that could be *heard*, i.e., "what you can't hear can't hurt you" (**Figure 1**). This notion justified the development of acoustic measuring devices and methodologies that concentrated solely on the audible portion of the acoustical spectrum.

Within the audible segment (20–20,000 Hz), human auditory acuity is not evenly distributed, and is more sensitive within the 800–7000 Hz range than it is to airborne acoustic events occurring below 500 Hz or above 15,000 Hz. Thus, early on, scientists understood that in order to protect human hearing function and speech intelligibility, the entire audible segment need not be considered, but rather, only the frequencies at which the acuity was highest: 800–7000 Hz range. The development of the A-frequency weighting and the resulting deciBel-A (dBA) metric allowed acousticians and health professionals to assess acoustical environments simulating this variability of human auditory acuity.

Figure 2 shows the frequency response curve for the dBA metric, clearly following the human auditory response to airborne acoustic pressure waves.

While the dBA metric proved to be key for the protection of hearing and speech intelligibility, it was insufficient for the assessment of airborne pressure waves occurring outside of the 800–7000 Hz range. **Figure 3** emphasizes the 800–7000 Hz range within the dBA metric, and **Figure 4** shows its application at 10 Hz. The dBA metric is, therefore, unsuited for evaluating airborne pressure waves occurring at frequencies below 800 Hz. Health effects that may be developing due to exposures

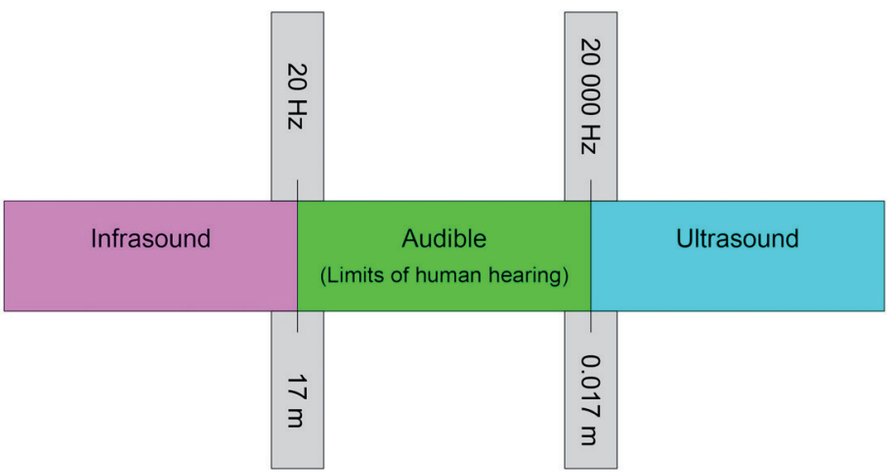

Figure 1.
Acoustical spectrum showing the classical three segments (infrasound, audible, and ultrasound) with the frequency and wavelength indicated at the cutoff of each segment.

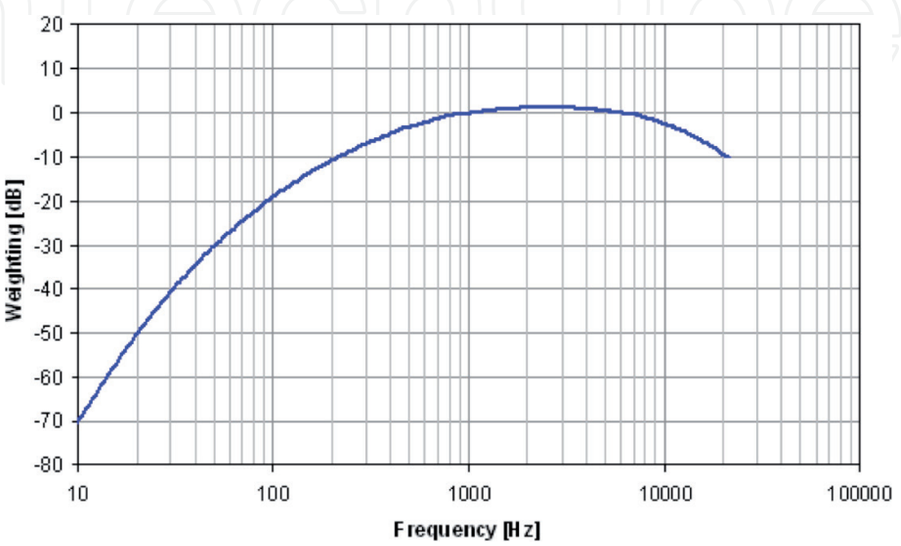

Figure 2.
Frequency response curve for the deciBel-A metric (dBA) commonly used in noise-related legislation [2].

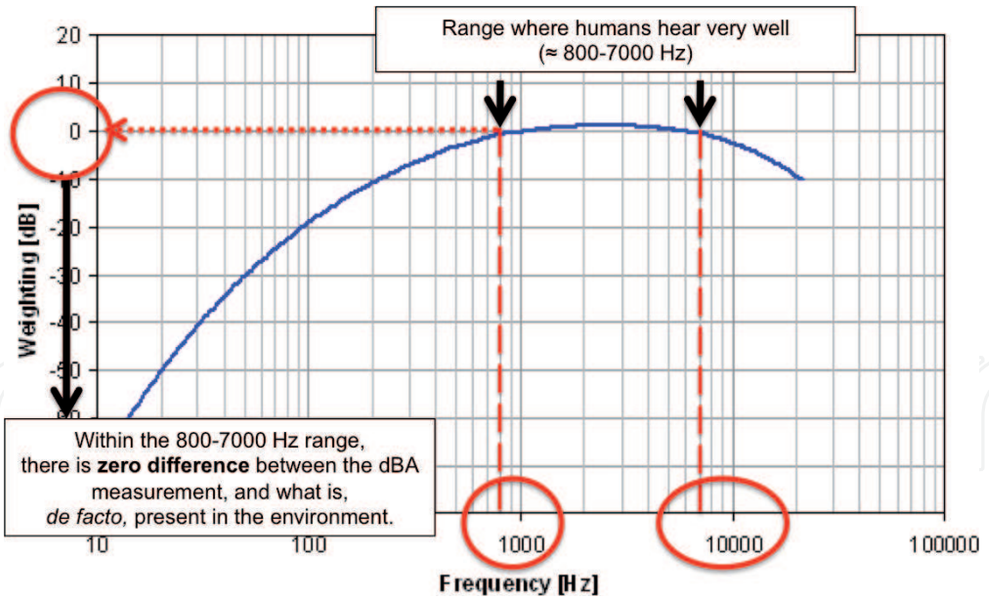

Figure 3.
Frequency response curve for the dBA metric applied to the range of highest human auditory acuity. Within this frequency range, the dBA measurement will accurately reflect the airborne acoustical energy present in the environment.

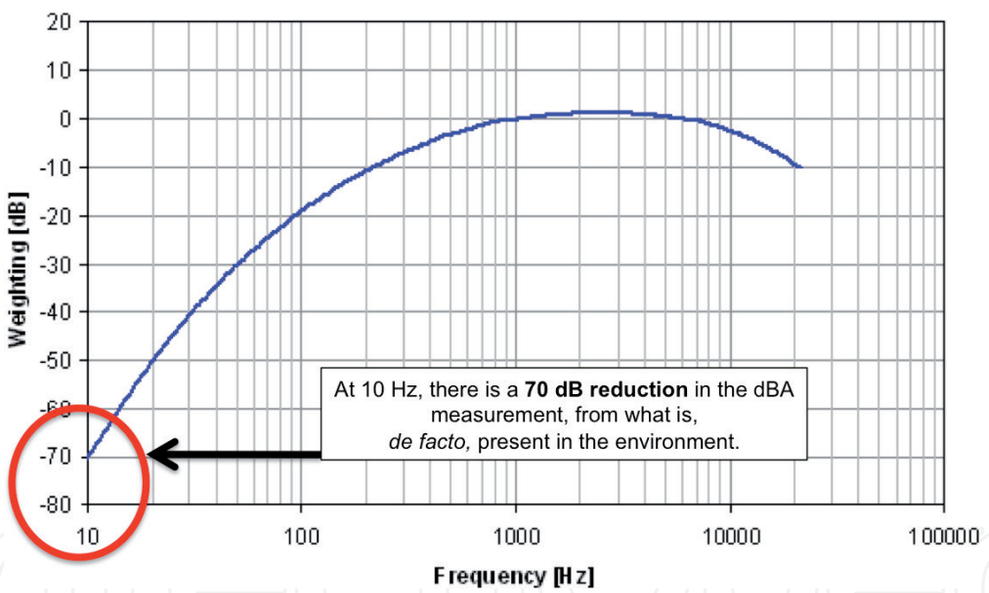

Figure 4.
Frequency response curve for the dBA metric applied to infrasonic frequency ranges, showing a 70 dB difference when evaluated at 10 Hz. Within these lower frequency ranges, the dBA metric will significantly underestimate the airborne acoustical energy present in the environment.

at these lower frequencies cannot be properly studied if the dBA metric is being used to characterize acoustical environments.

There is a shortage of studies that properly evaluate the biological response to infrasonic (≤20 Hz) or lower frequency (≤200 Hz) airborne pressure waves. Three important reasons for this have been provided above: the rudimentary segmentation of the entire acoustical spectrum into merely three "blocks" (compare to segmentation of the electromagnetic spectrum), the unsuitability of the dBA metric to quantify airborne acoustical pressure waves at these lower frequencies, and the ingrained notion that "what you can't hear can't hurt you." These major hindrances have been crystallized into mainstream science [3] and have served to significantly impede scientific inquiry and human health protection.

The goal of this chapter is to consolidate what is known on the biological response to airborne pressure waves occurring within the infrasonic and lower frequency ranges. A biomedical engineering approach is taken, whereby biological organisms are viewed as structures of composite materials, with significant viscoelastic components and organized in accordance with the principles of tensegrity architectures. When airborne pressure waves impact these types of structures, the biological response will depend on the type of biomaterial under study, it will exhibit anisotropic properties, and it will vary nonlinearly with exposure time. Depending on the physical properties of the airborne pressure waves (including time profiles) and on the biostructure under study, mechanical perturbations are relayed into cells and tissues through a variety of different pathways that, to date, still remain unclear.

2. Biomaterials and human anatomy

2.1 Viscoelasticity

Viscoelasticity is an attribute given to bodies that exhibit both viscous and elastic behaviors beyond the classical Hooke's elastic model [4]. Viscoelastic materials have three distinct properties not contemplated by Hookean models: creep, stress relaxation, and hysteresis. Most biological materials have viscoelastic behaviors.

In a Hookean (or purely elastic) material, total deformation depends on total load, and no further deformation occurs even if load is maintained. In viscoelastic materials, however, when sufficient stress is applied and maintained, they may continue to deform, even though stress load remains unaltered. This property is called *creep*.

In a purely elastic material, the strain within the material is constant throughout the application of the load; it does not vary with time, but only with the amount of applied stress. In viscoelastic materials, when stress is applied and maintained, strain can decrease with time. This property is called *stress relaxation*.

Consider repetitive or cyclical loads on materials. In purely elastic materials, periodic loads will not alter the stress-strain curve. The pathway taken by the material to deform is exactly the same pathway it takes to return to its original, equilibrium position. In viscoelastic materials, however, the return to equilibrium may be different than the pathway used to get to the point of deformation (The word pathway is here loosely used, and is meant to encompass all spatial, temporal and energetic components of these types of movements.) This property is called *hysteresis*.

2.2 Tensegrity structures

Many structures in the natural world are organized in accordance with the principles of tensegrity architecture—elements providing discontinuous compression are held together through elements of continuous tension [5]. **Figure 5** shows several examples of tensegrity structures.

Depending on the properties of the airborne pressure waves and biomaterial under study, the propagation of mechanical perturbations throughout these types of structures can reach long distances, without loss of structural integrity.

2.3 Cellular and tissue mechanotransduction

Cells and tissues are organized in accordance with the principles of tensegrity architecture [8, 9]. This means that in addition to biochemical signaling,

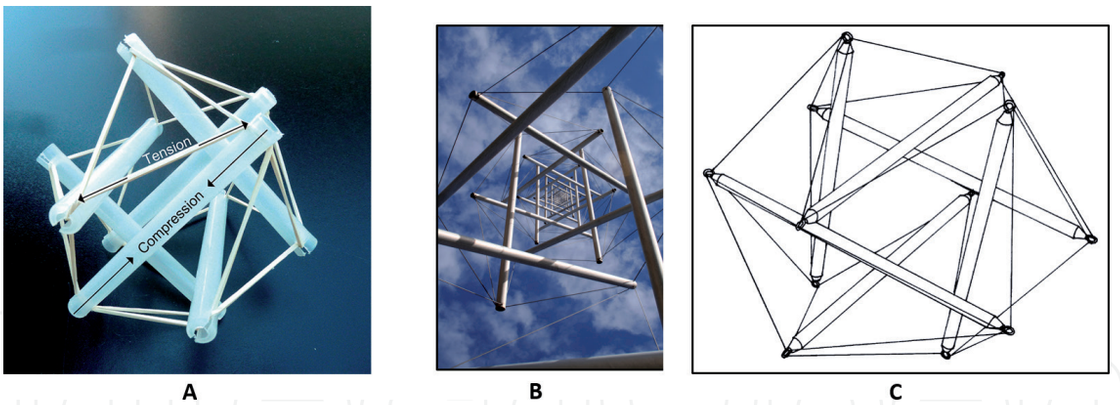

Figure 5.
Tensegrity structures. A. Model showing elements of continuous tension and discontinuous compression. B. Needle tower, by Kenneth Snelson, in the Hirshhorn sculpture garden (USA) [6]. C. Icosahedron, first designed by Buckminster Fuller in 1949 [7].

cells also communicate with their surroundings through mechanical signals. Mechanosensitive receptors exist on cell surfaces, and mechanosensitive junctions interconnect cells, thus forming tissues. Depending on the physical properties of the airborne pressure waves and biomaterials under study, external airborne mechanical perturbations can elicit a mechanical response, which, in a larger, macroscopic view, can lead to clinically pathological situations.

2.4 The fasciae

The fascia is a sheet of connective tissue that uninterruptedly extends from head to toe, suspended from the skeleton, and that provides the integrated supporting framework for maintaining anatomical and structural form [10, 11]. That external mechanical perturbations elicit responses at large distances away from the point of entry is a well-known concept among scientists and health professionals who study fasciae. When presented with external airborne pressure waves, fasciae can respond by changing their structural properties: *from a mechanical point of view, the fasciae are organized in chains to defend the body against restrictions. When a restriction goes beyond a specific threshold, the fasciae respond by modifying their viscoelasticity, changing the collagenic fibers, and transforming healthy fascial chains into lesioned chains* [10]. One of the fascia's key roles is that of shock absorption.

Connective tissue structures are ubiquitous forming all external surfaces of vessels, nerves, organs, and muscles, and at the cellular level, the extra-cellular matrix that surrounds and communicates with each individual cell. In addition to maintaining structural integrity, the fasciae are the first line of defense against external perturbations, playing important physiological roles in mobilizing the immune system.

3. Laboratorial studies, field studies, and biological outcomes

Studying the effects of infrasonic or lower-frequency airborne pressure waves on biological structures is a very complex undertaking, whether it be on cell cultures, on animal models, or on human populations. Laboratorial studies, occupational field studies, and residential field studies all have their own strengths and weaknesses. When the latter go unrecognized, however, experimental design flaws can ensue. In this section, the attributes of these different experimental setups are discussed, and their weaknesses and strengths are explored. Together

with the preceding section, this serves as a preamble to Section 4, where the results of experimental studies are described in detail.

3.1 Laboratorial studies

Laboratories where infrasonic and lower frequency airborne pressure waves can be applied in a controlled manner are in short supply worldwide, and those that do exist are mostly associated with military installations. Laboratories emitting airborne pressure waves with infrasonic and lower frequency components cannot be randomly placed within residential environments; issues with neighbor disturbance and public health would curtail its use. Moreover, the equipment used to generate the airborne pressure waves is, typically, very large and very expensive, and few sectors of society (other than military or space exploration industries) would have the need for an extensive use of these types of installations.

In these laboratory settings, continuous or pulsed-trains of single-tone airborne pressure waves can be applied, as well as, broadband exposures that can be accurately characterized. The fact that exposure times and acoustic parameters can be precisely controlled is one of the strengths of laboratorial studies, allowing for continuous time exposures, or occupationally simulated exposure schedules. Immediate (hours or days) versus long-term (weeks or months) effects can also be explored.

There are numerous types of biological outcomes that can be studied under laboratorial conditions. Light-, electron- and atomic-force microscopy can be used to study cellular and tissue structural properties, as well as their chemical composition and content of bio-reactive elements. Polymerase chain reaction (PCR) techniques can provide information on messenger RNA (mRNA) expression, allowing for the identification of key pathways. With pharmacological intervention or gene knockout specimens, specific signaling molecules and pathways involved in the elicited responses can be pinpointed. Additionally, control populations for comparison are fairly easy to achieve—they are simply not subjected to the laboratorial exposures.

3.2 Occupational field laboratories

Occupational environments are exceptional field laboratories, as both short-term (several months) and long-term (years) effects can be investigated in more realistic acoustic environments. Typically, different workstations have different acoustical features that can greatly depend on different machinery regimens. For occupational field laboratories, acoustical characterizations of the workplace(s) must be comprehensively undertaken and time exposures to each type of environment should be scored.

Exposure times at work must be differentiated from exposure times away from work, i.e., when the work shift ends, workers leave the field laboratory, but additional exposures to infrasonic or lower frequency airborne pressure waves may be incurred (e.g., recreational, transportation). These must be documented. Significant confounding factors may be introduced unless each subject's residential area is scrutinized and prior-exposure histories probed for fetal, childhood, and adolescent exposures.

Possible biological outcomes within occupational field studies are more limited when compared to laboratory exposures. Noninvasive testing can be imprecise, and the minimally invasive testing (such as a blood chemistry analysis, X-ray, or MRI) may also not be sufficiently precise to yield relevant data. It is also the case that scientific knowledge on relevant biological outcomes that can be noninvasively evaluated in exposed humans is still absent or, at best, very incomplete.

Survivorship bias is a well-known confounding factor in human population studies. In occupational environments, workers with more time on-the-job are those who have survived throughout the years of professional activity, while workers with less time in professional activity may exhibit more severe biological outcomes. This phenomenon is often misinterpreted leading to inconclusive or erroneous conclusions.

Control populations for exposures to infrasonic and lower frequency airborne pressure waves have been a very difficult proposition, given the ubiquitous nature of this stressor. One of the solutions to this profound problem is the scoring of subjects into different groups as per their exposure. Within this context, control groups are composed of individuals who have the least amount of cumulative (prior and present) exposure, and not of individuals with zero exposure.

Different professions can provide different field laboratories, both in terms of acoustic environment and time exposure schedules. For example, long-haul truck drivers are typically exposed for more than 8 hours daily and, oftentimes, sleep in the truck while it is idling, or while refrigeration systems are continuously operating. Workers onboard ships, submarines, offshore oilrigs, aircraft, and spacecraft (for example) can be exposed to significant amounts of infrasonic and lower frequency airborne pressure waves for weeks or months at a time. The wealth of information waiting to be gleaned from these types of field laboratories is breathtaking.

3.3 Residential field laboratories

Field laboratories in urban, suburban, and rural residential settings are generally designed to investigate environmental health effects due to human-made infrasonic and lower frequency airborne pressure waves. Typically, these sources are associated with industrial complexes or infrastructure that, in turn, are usually linked with important economic interests. In general, the amount and type of infrasonic and lower frequency airborne pressure waves contaminating a home will depend on the machine operation and/or the use of the infrastructure. For example, in most urban and suburban areas, airports must close down between the hours of midnight and 5 am. Some factories do not have night shifts and therefore also have daily shutdown periods. Large refrigeration units, hydroelectric dams, and large volume highways, however, must be kept running 24/7 and can also be viewed as continuous sources of infrasonic and lower frequency airborne pressure waves. Wind turbines are the latest addition to these type of sources although they are almost exclusively within rural areas.

Comprehensive characterization of the acoustic environments in the different residential areas must be undertaken (e.g., master bedroom, children's bedrooms, living-lounge areas), since room-resonance phenomena can significantly modify the acoustic environment that is originally being induced and driven by external, incoming airborne pressure waves. Additionally, wind can also influence the spectrum, intensity and type of infrasonic and lower frequency airborne pressure waves that exist within a room. This differentiation is readily achieved with proper acoustic evaluations.

Residential exposure times are much more difficult to control, as they can differ from room to room and on an hourly basis. Moreover, subjects may also be sleeping within the "contaminated" environments, which can severely aggravate biological outcomes. If exposure is concomitantly occurring during sleep and waking hours (e.g., homemakers, workers from home, farmers), then biological outcomes may be further aggravated. Leaving the home can be equated with a biological recovery period (i.e., nonexposure period).

Short-, medium- and long-term effects can be studied in residential settings when the implementation of a new infrastructure or industrial complex is known to be coming to the area. Biological outcomes should strive to be either noninvasive or minimally invasive, and prior-exposure histories are fundamental for achieving useful statistical data.

4. Past relevant studies

Numerous studies conducted over the decades have shed light on the biological response to infrasonic and lower frequency airborne pressure waves and associated symptomatic complaints. Due to space limitations, this discussion will only deal with some of the vascular and collagenous abnormalities, cardiomyocyte changes, and the hippocampus responses, as induced by different types of exposures. For reasons explained in the section "Introduction," all studies using the dBA metric have been eliminated from consideration (with one exception in an occupational setting). Selected studies mostly focus on the cellular and tissue changes observed in laboratory, occupational, and residential settings, using light and electron microscopy. The sequence in which the studies are presented does not follow the classical anatomical order.

4.1 Vascular changes

In the mid-1960s, within a military setting, the immediate exposure to 10–60 Hz, at 118–140 dB, for 2 minutes, induced disturbances of the visual field as reported by all five human subjects [12]. In 1985, laboratorial animal studies exposed rats to tonal 8 Hz at 100–140 dB, 3 hours daily, for 5, 10, 15, or 25 days, and examined the blood and lymph networks of the palpebral (eyelid) and bulbar (eye globe) conjunctiva. *Day 5*: narrowing of all parts of the conjunctiva blood network was observed, with decreased blood capillary lumens. Capillaries, precapillaries, and arterioles were twisted, and blood component agglomerations were identified in venous vessels. *Day 10*: conjunctiva capillaries were twisted and large vessel diameters were decreased. *Day 15:* blood and lymph vessel tonus had changed, and stagnation was present. *Day 25:* failure of tissue homeostasis was aggravated. Capillary penetration was increased, as seen through tissue enlargement, and significant agglutination was observed in the large vessels [13].

In a similar study, animals were exposed to 8 Hz at 100 dB, or to 16 Hz at 100 dB, 3 hours daily, for 1 month. Clinical and morphological evaluations were conducted at days 3, 7, 15, 30, and also post-exposure at days 30, 60, and 90. *Day 3:* clinical changes were not observed, but morphological changes were present: edema in the upper and middle areas of the eyelid derma and heterogeneous blood filling of vessels with extra-vascular erythrocytes were also observed. Fine focal hemorrhages were identified under the corneous layer of the eyelid. Sclera exhibited edema, and blood vessels were filled heterogeneously with stasis and extra-vascular intraconjunctive hemorrhages. In the 8-Hz group, moderate edema was present near the optical nerve, and the 16-Hz group exhibited perineural hemorrhages in the optical nerve. *Day 7:* in both groups, conjunctiva blood vessels had expanded and arteries in the oculus fundus were narrower and twisted. Eyelid edema of the derma was identified in both groups. The most pronounced vascular changes were found in the eyelid conjunctiva: stasis, edema, and pericapillary hemorrhages. Sclera capillaries were overfilled with blood and extra-vascular hemorrhages were observed. *Day 15:* in both groups, conjunctiva vessels were narrower and twisted, and ocular globe conjunctiva exhibited nonvascularized

areas. Vascular changes as seen previously were more expressed: edema, paresis state in capillaries (erythrocyte stasis), and extra-vascular erythrocytes. The iris exhibited narrower vessels. *Day 30:* narrowed and twisted vessels were clinically detected, with ocular fundus arteries and veins significantly narrowed and twisted, more pronounced in the 16-Hz group. In the eyelid conjunctiva, derma exhibited the same vascular changes seen before: edema and erythrocyte stasis. Sclera arteries and veins were larger, overfilled with blood, and with the presence of extra-vascular focal and diffuse hemorrhages with conjunctiva involvement. At all time points, the 16-Hz group disclosed more destruction than the 8-Hz group. *Day 60 (30 days post-exposure)*: clinical evaluations revealed less twisted and narrow arteries and veins, but morphological recovery was slower. In the 8-Hz group, moderate regeneration was observed in the eyelid conjunctiva epithelium. In the 16-Hz group, predominant retinal damage persisted. *Day 90:* no clinical changes were observed in either group [14].

Within an occupational setting (reinforced concrete factory), vessel changes in the palpebral and bulbar conjunctiva, and in the retina, were investigated among 214 workers (age range: 20–58 years), with 1–30 years of employment. Workers were divided into two groups:

- *Control group* (n = 54): not occupationally exposed to significant levels of infrasonic and lower frequency airborne pressure waves.

- *Exposed group* (n = 160): tonal 8 and 16 Hz at 96–100 dB, simultaneously with non-tonal 20–500 Hz at 91–93 dBA.

The exposed group was divided into subgroups as per years of professional activity. **Table 1** describes each subgroup and the vessel abnormalities found. No such abnormalities were found in the control population [14].

Within a different occupational setting (aircraft industry), ocular changes were studied in 23 male workers (average age: 42, range: 32–58 years). Lesions

Occupational exposure time	**1–2 yrs**	**3–10 yrs**	**11–20 yrs**	**20–30 yrs**
Number of workers	21	84	36	19
Palpebral and bulbar arteries (%)				
Enlarged	0	82	8	0
Narrow	0	17	91	100
Twisted	0	80	100	100
Retinal arteries (%)				
Enlarged	0	0	0	0
Narrow	0	91	100	100
Twisted	0	90	100	100
Retinal veins (%)				
Enlarged	0	87	11	0
Narrow	0	13	88	100
Twisted	0	75	97	100

Table 1.
Percentage of abnormal vessel changes seen in the palpebral and bulbar conjunctiva and retina among occupationally exposed workers [14].

were observed in the blood-retinal barrier in 19 workers (lesion types: 13 inactive, 2 active, 4 mixed). Choroidal circulation was altered in 14 workers (late perfusion with chronic features). Changes in retinal circulation were observed in four workers (type: 1 occlusive, 1 exudative, 2 mixed). Three workers presented with optic neuropathy (1 papillitis, 2 optic atrophy), and one exhibited sensorial retinal macular detachment [15]. The immediate effects of tonal exposures with 8 Hz at 130 dB, 2 hours daily, for 1, 7, 14 and 21 days, also revealed a breakdown of the blood-retinal barrier in the rat eye [16].

These studies strongly suggest that under the impact of infrasonic and lower frequency airborne pressure waves, a vascular response is mounted by ocular structures and could be related to decreased visual acuity in workers. Data in **Table 1** seem to indicate that, as exposure time progressed, vessels that were initially enlarged ceased to exist, apparently being replaced with narrower and twisted vessels. Enlarged vessels usually suggest the need for an increased blood supply. However, given the sustained mechanical insult, making the vessels narrower and twisting them throughout the structures may, in fact, reflect a more efficient blood delivery system.

This concept is further reinforced by the observation of narrow and twisted blood vessels in the gastric mucosa of rats, exposed to non-tonal, occupationally simulated (aircraft industry) acoustic environments characterized as 6.3–25 Hz at 70–90 dB and 40–500 Hz at 90–100 dB. Continuous exposure was applied, and evaluations occurred at 1, 3, 5, 9, and 13 weeks. In *3–5 weeks*, the gastric submucosal layer exhibited significantly increased thickness, when compared to non-exposed controls. This increased thickness was due to the proliferation of type IV collagen. Arterial walls disclosed significant intima and media thickening, ruptured internal elastic lamina, and thrombotic changes. In *9–13 weeks,* neoangiogenesis was observed, with the appearance of tortuous and twisted vessels. The authors concluded that, in the stomach, continuous exposure induced fibrosis that could be linked with neoangiogenesis, since collagen type IV is also an early marker of neoangiogenesis [17]. One of the earliest studies investigating the long-term effects of airborne pressure waves on gastric complaints was conducted in 1968, in a residential setting where changes in gastric function were associated with aircraft noise [18]. Within occupational settings, an increase in gastric complaints was documented among boiler-plant workers, 2 years after the implementation of mandatory hearing protection devices [19]. Among aircraft industry workers, gastrointestinal problems were among the earliest to appear after 1–4 years of professional activity [20].

Vascular changes were also identified in the liver structures of animals exposed to 2, 4, 8, or 16 Hz, at 90–140 dB, 3 hours daily, for 5–40 days. Exposures to 2 or 4 Hz induced less damage than exposures to 8 and 16 Hz. *Single, 3-hour exposures:* with 2 or 4 Hz and 90 dB, no changes were observed in the hepatic structures, while at 100–110 dB, liver parenchyma disclosed single fine hemorrhages. At 120 dB, increased arterial wall diameters were observed, as well as capillary lumen expansion, indicating the development of ischemia. At 130–140 dB, the number of hemorrhagic events increased, as did the number of affected hepatocytes. With 8 or 16 Hz exposures, damaged hepatocytes were present in the ischemic and non-ischemic areas. *Days 5–15:* more pronounced hepatocyte changes were seen. *Days 25–40*: a gradual death of changed hepatocytes was observed [21].

Hemorrhagic events in the lung were documented as early as 1969, within the Soviet and US space exploration studies, in dogs exposed to occupationally simulated (spaceflight) wide-band frequency range at 105–155 dB, for 1.5 or 2 hours. Hemorrhages up to 3 mm in diameter were observed beneath the pleura. As exposure time and decibel level increased, the number of hemorrhages increased but never

exceeded 3 mm in diameter. Microscopic analyses of the hemorrhagic sections disclosed ruptured capillaries and larger blood vessels [22]. In a laboratory setting, rats received tonal exposures to 2, 4, 8, or 16 Hz at 90–140 dB, 3 hours daily, for 40 days. Analysis time points were conducted after 3 hours, at 5, 10, 15, 24, and 40 days of exposure, as well as during post-exposure times. *Single, 3-hour exposures:* with 2 or 4 Hz at 90–110 dB, mosaic hemorrhages were observed under the pleura, covering the entire lung surface. With 8 Hz at 110 dB, more hemorrhagic expression was observed. With 8 or 16 Hz at 120–140 dB, larger hemorrhagic foci were disclosed. Within the alveolar capillary network and postcapillary venules, vessel diameters were increased with 2 or 4 Hz at 90–110 dB, leading to large hemorrhages and perivascular edema. Erythrocyte overflow in alveolar capillaries was observed with 8 or 16 Hz at 110 dB. With 8 or 16 Hz at 120 or 140 dB, lung tissue exhibited large hemorrhagic foci in the connective tissue septa of the bronchi-pulmonary segments. In all exposure types, capillary changes were followed by alveolar epithelium desquamation and basal membrane denudation. *Longer exposures:* with 8 Hz at 120 dB, acinuses became filled with erythrocytes, and interstitial hemorrhagic foci caused a strong deformation of the respiratory bronchioles. With 8 or 16 Hz at 140 dB, ruptured vascular walls were observed leading to decreased alveolar lumen [23].

The highly invasive bronchoscopic evaluation with biopsy was performed among a group of volunteer subjects, with occupational or residential exposures to infrasonic and lower frequency airborne pressure waves, as detailed in **Table 2**.

Bronchoscopic observations in all patients revealed small submucosal, vascular-like lesions ("pink" lesions), located distally in both tracheal and bronchial trees, and uniformly distributed bilaterally near the spurs. Biopsies were performed on the abnormal mucosa (pink lesions) and on the apparently normal mucosa (outside of the pink lesions). In the non-pink areas, some vessel wall thickening was visible. In the pink areas, the basal membrane disclosed abnormal neovascularization, with thickened blood vessel walls and scarce lumen. No gender differences were identified [24].

4.2 Collagen and connective tissue

Collagen, composed of triple-helix tropocollagen chains, is the most abundant protein in the human body, a key component of the fasciae, and is produced by fibroblast cells. It has long since been considered as the "steel" of the human body [25], but its energy storage capacity has been shown to be 10 orders higher than in spring steel [26]. Different types of collagen have different mechanical properties. Type IV collagen (increased in the exposed gastric mucosa [17]—see above), is organized into X-shaped structures and is commonly found in the basal membrane of arterial walls, hence its increased expression during angiogenesis.

In *day 5* of the eyelid-and-bulbar-conjunctiva animal studies (see above [13]), collagen fibers in the connective tissue were enlarged, as were some fibroblast nuclei; on *day 10*, adipose cells in the connective tissue had been redistributed and positioned in the vascular areas of the conjunctiva. In the second animal study described above [14], *day 3* included edema of the sclera causing separation of collagen filaments in the 16 Hz group, and by *day 7,* this was observed in the 8-Hz group as well; *day 15:* focal and disseminated disorganization of sclera collagen fibers was observed in both groups; *day 30:* homogenization and disorganization of collagen in the derma while, in the sclera, collagen fibers were persistently separated due to edema, with some undergoing dystrophic and necrotic changes. Slow regeneration was observed during the post-exposure periods.

In the lungs of dogs studied within the scope of space exploration (see above [22]), focal enlargement of the alveoli involved the stretching of connective tissue

Profession/type of exposure	Gender	Age	Smoking
Aircraft technician	Male	48	Mild
Aircraft technician	Male	52	No
Aircraft technician	Male	59	Mild
Combat pilot	Male	61	No
Helicopter pilot	Male	59	Moderate
Aircraft pilot	Male	54	No
Merchant marine	Male	37	No
Military helicopter nurse	Female	56	No
Flight attendant	Female	36	No
Flight attendant	Female	39	No
Flight attendant	Female	40	No
Homemaker	Female	54	Mild
Homemaker	Female	59	No

Table 2.
Description of subjects who received bronchoscopic evaluations with biopsy [24].

structures of alveoli walls. In the biopsy images of the bronchoscopic study (see above [24]), non-pink areas disclosed a thickened basement membrane with abnormal amounts of collagen, while the pink areas disclosed an even thicker membrane with very large amounts of collagen. The abnormal neovascularization was embedded within collagen bundles. Retraction of structures neighboring the collagen fibers was not observed. A marked reinforcement of the cytoskeleton and intercellular junctions was seen in the pink areas, as compared to non-pink areas. The five individuals that disclosed images of collagen fiber degeneration and disruption also tested positive for antinuclear antibodies.

Under an occupationally simulated acoustic environment, characterized as 20–200 Hz at 70–90 dB (aircraft industry), and occupationally simulated exposure schedules (8 hours daily, 5 days weekly, weekends in silence), focal interstitial fibrosis was found in the lung parenchyma of rats after a cumulative 4000-hour exposure. Additionally, thickened alveoli walls and dilated alveoli were observed [27]. Tracheal epithelium in similarly exposed rats disclosed significant subepithelial fibrosis [28, 29], and with longer occupationally simulated exposures, the subepithelial layer became composed of hyperplastic collagen bundles, some with a degenerative pattern. Cellular edema was also observed [28, 30].

Within an occupational setting (aircraft industry) and investigating long-term outcomes, high-resolution CT scans of the lungs and respiratory function tests were provided to 21 nonsmoker male workers, who were divided into two groups: with (n = 7, average age: 42) and without (n = 15, average age: 36) complaints of airflow limitations. There was a significant relationship between the presence of symptoms and images of lung fibrosis through the CT scan. No differences existed among the groups when comparing the percentage of predicted values of lung function [31].

Fasciae abnormalities have been most prominently studied in the pericardia of exposed workers, subsequent to autopsy findings in an aircraft industry worker that disclosed a grossly thickened pericardium [32]. Pericardial morphological changes were studied among 12 male workers: three aircraft technicians, four fixed-wing aircraft pilots, four helicopter pilots, and one long-haul truck driver. Pericardial samples were removed with informed consent of the patient and Ethics Committee

approval, at the beginning of cardiac surgery (prescribed for other reasons by the National Healthcare Service). In all cases, there were no visual adherences, or inflammatory aspects and pericardia were grossly thickened. The classical, three pericardial layers were identified: serosa, fibrosa, and epipericardium. However, in all cases, the fibrosa had split in two and, in between, a new layer of loose tissue was observed, consisting of vessels, nerves, arteries, and lymphatics surrounded by adipose tissue. Both fibrosa layers were composed almost entirely by wavy, interwoven collagen bundles, surrounded by numerous cytoplasmic extensions (whose mother cell was difficult to identify), and interspersed with some elastic fibers. The new, loose tissue layer sandwiched in between the split fibrosa contained blood and lymphatic vessels, adipose tissue, and nerves. Both the loose tissue layer and the fibrosa layers contained macrophages and vascular hyperplasia, also seen in lymphatic vessels [33–36]. Pericardial and cardiac valve thickening has also been confirmed through echocardiography studies in occupational settings (aircraft [37] and commercial-airline industries [38]), with thickness increasing with increasing exposure time. In residential settings, pericardial and valve thickening [39] and increased arterial stiffness [40] were observed in populations chronically exposed to military-training exercises [39], and transportation systems [40].

4.3 Heart cells and tissues

In 1983, electron microscopy techniques were used to study animal myocardia exposed to single and multiple infrasonic exposures of 4–16 Hz at 90–150 dB, 3 hours daily, for 45 days, and post-exposure time points were included. No changes were observed with single exposures at 4–6 Hz and at less than 100 dB, when compared to non-exposed controls. *Single exposure with 4–10 Hz at 120–125 dB:* induced decreased arterial diameter and capillary expansion, with resulting focal ischemia. Images of intracellular myocytolysis were frequently found. These processes were reversible. *Multiple exposures with 4–10 Hz at 120–125 dB for 5–25 days*: ventricle fibrillation and subsegmental contractures in ischemic foci were identified. Myofibril fragmentation was observed in the Z-line, sarcoplasmic reticulum structures were absent, cell nuclei were deformed, and chromatin was found accumulated under the nuclear membrane. *post-exposure*: intracellular regeneration was concomitant with damaged cells. In surviving cells, mitochondria were increased in number and size, and both myofilaments and sarcoplasmic reticulum elements were being created. Intracellular regeneration was slow and ended with the creation of Z-lines, after which myofibrils became normal and myocardiocytes completely recovered. *Single exposure with 10–15 Hz at 135–145 dB:* more pronounced myocardial damage, with partial death of myocardiocytes, resulting in myocardiocyte dystrophy. Damaged cells included chromatin condensation and redistribution to the nuclei membrane. Less damaged cells regenerated after 5–10 days post-exposure. *Multiple exposures with 10–15 Hz at 135–145 dB:* persistent myocardial ischemia related to vascular changes and accompanied by cardiocyte damage. After 15–25 days post-exposure, recovered cells began functioning normally despite the presence of abnormal structures within the cellular cytoplasm, namely, giant mitochondria [41].

Cardiac injury was studied in rat cardiomyocytes exposed to tonal 5 Hz at 130 dB, 2 hours daily, for 1, 7, or 14 days. *Days 1–7:* SERCA2 (sarcoplasmic reticulum Ca^{2+} ATPase 2, an enzyme with calcium-transporting properties and involved in the decomposition of ATP into ADP) was significantly increased, and swollen mitochondria were observed in the cardiomyocytes. *Day 7:* SERCA2 was significantly decreased and an increased number of swollen mitochondria were observed. *Day 14:* SERCA2 was significantly decreased and platelet aggregation was found in the intercellular substance. Intercellular calcium ion (Ca^{2+}) concentration significantly

increased with increasing exposure time [42]. With similar exposure protocols, another study repeated the SERCA2 and intercellular Ca^{2+} concentrations, but also included evaluations of the expression of whole cell L-type Ca^{2+} currents (WLCC) and the mRNA expression of a subunit of the L-type Ca^{2+} channel (LCC). SERCA2 and intercellular Ca^{2+} concentrations behaved as described immediately above, while the expression of WLCC and mRNA expression of LCC increased with increasing exposure time [43].

For three continuous months, rats were exposed to non-tonal, occupationally simulated (aircraft industry) acoustical environments characterized as 6.3–25 Hz at 70–90 dB and 40–500 Hz at 90–100 dB. Ventricular cardiac muscle and interstitial fibrosis were quantified and compared to non-exposed controls. Exposed rats disclosed a 97.5% increase in fibrosis in the left ventricle, an 81.5% increase in the interventricular septum, and an 83.7% increase in the right ventricle. No significant differences were found in the mean values of cardiac muscle in the left and right ventricles, when compared to non-exposed controls. However, the fibrosis-to-muscle ratio was significantly higher in the exposed rats, indicating significant ventricular myocardial fibrosis [44].

In another study, rats were exposed to a non-tonal, occupationally simulated (textile mill) environment rich in infrasonic and lower frequency components, under an occupationally simulated schedule (8 hours daily, 5 days weekly, weekends in silence), for 1, 3, 5, and 7 months. Ventricular coronary artery caliber, artery wall thickness, and size of arterial perivascular tissue were quantified in a total of 130 arteries (61 exposed and 69 controls). No changes were observed in arterial lumen caliber, and in arterial wall thickness, when compared to non-exposed controls. Perivascular tissue was more prominent in the exposed samples and seemed to exhibit fibrotic development. Lumen-to-wall ratio showed no differences, while wall-to-perivascular-tissue ratio showed a significant increase, as compared to non-exposed controls [45].

In animals exposed to 2–20 Hz peaking at 114 dB, for 28 continuous days, ventricular arteries were studied as to the dimensions of lumen, wall, and perivascular space. An additional group of animals received the same exposure but were treated with dexamethasone (a corticosteroid). Blind evaluation of 31 arteries disclosed increased perivascular spaces in the exposed groups, reflected in the significantly reduced wall-to-perivascular-space ratio, as compared to non-exposed controls. No changes were observed in the lumen-to-wall ratio. With dexamethasone treatment and exposure, no differences were observed in the wall-to-perivascular-space ratio, as compared to controls, suggesting an underlying inflammatory mechanism [46].

Gap junctions are a fundamental component of intercellular communication, allowing inorganic ions and small water-soluble molecules to pass directly from one cell's cytoplasm to another. Gap junctions are formed by protein complexes (connexons) each composed of six subunits made of the protein connexin. Cardiac connexin43 (Cx43) is a component of gap junctions, and its reduction in combination with increased collagen deposition and interstitial fibrosis has been associated with ventricular arrhythmias [47]. Within this context, rats were exposed to non-tonal, occupationally simulated (aircraft industry) acoustical environments characterized by 6.3–25 Hz at 70–90 dB and 40–500 Hz at 90–100 dB, for three continuous months. Immunohistochemical quantification of Cx43 was conducted on the left ventricle, interventricular septum, and right ventricle. Significantly decreased Cx43-to-muscle ratios were found in the exposed rats, as compared to non-exposed controls, suggesting the possibility of arrhythmogenic consequences [48].

4.4 The hippocampus

Prior studies have shown that the hippocampus is involved in learning and memory impairment, such as that seen in rodents after infrasound exposure [49]. The hippocampus—located between the cerebral hemispheres and the brainstem—was classically considered as part of the limbic system. The hippocampus proper is divided into four regions (CA1, CA2, CA3, and CA4), each with different input and output pathways. The Dentate Gyrus (DG) is an additional hippocampus structure and that contributes to the formation of new episodic memories, and spontaneous exploration of novel environments. In the central nervous system (CNS), neuroglia consists of the non-neuronal cells (oligodendrocytes, astrocytes, ependymal cells, and microglia) and is often referred to as the connective tissue of the brain. Glial cells surround neurons to hold them in place, supply them with oxygen and nutrients, insulate them from one another, destroy pathogens, and remove dead neurons.

Glial fibrillary acidic protein (GFAP) is an intermediate filament protein expressed by numerous cells within the CNS, and although its exact function remains unknown, it appears to be involved in maintaining the mechanical strength of astrocytes. The expression of GFAP was studied in the brains of mice exposed to 16 Hz at 130 dB, 2 hours daily, for 1, 7, 14 21, or 28 days. GFAP expression was increased in the hippocampus, cortex, and hypothalamus in a time-dependent manner [50].

Corticotrophin releasing hormone (CHR) is a peptide hormone involved in the stimulation of the pituitary synthesis of ACTH (adrenocorticotropic hormone) as part of the hypothalamic-pituitary-adrenal axis' response to stress. Corticotrophin releasing hormone-receptor 1 (CHR-R1) has wide expression in the CNS. It plays important roles in fear learning and consolidation in the amygdala, in stress-related modulation of memory function in the hippocampus, and in arousal regulation in the brainstem. Prior studies showed that infrasound exposures caused an upregulation of CRH and CRH-R1 in neurons of the hypothalamic paraventricular nucleus [51]. Recent studies have also shown that CRH is expressed in activated microglial cells [52]. Within this context, rats and *in vitro* cultured microglial cells were exposed to 16 Hz at 130 dB for 2 hours, after which changes in CHR-R1 were examined. *In vivo* exposure disclosed activation of microglial cells and an upregulation in the expression of CRH-R1 in the hypothalamic periventricular nucleus. *In vitro* exposure disclosed that, in the absence of neurons, microglial cells were activated and CRH-R1 expression was upregulated. These data suggest that both neurons in the hypothalamic periventricular nucleus and microglial cells are effector cells for infrasound-elicited responses [51].

The transient receptor potential cation channel, subfamily V, member 4 (TRPV4) protein acts as a calcium channel that is also mechanosensitive. It plays important roles in the systemic regulation of osmotic pressure by the brain, in skeletal growth and structural integrity, in airway and lung function, retinal and inner ear function, and in pain. Animals were exposed to 8 or 16 Hz at 90, 100 or 130 dB, 2 hours daily, for 14 days. Rat learning and memory abilities were most severely impaired with 16 Hz at 130 dB at days 7 and 14, with prominent loss of hippocampal CA1 neurons, as compared to non-exposed controls. Significant astrocyte and microglial activation was seen in the hippocampus after days 1 and 7, and before neuronal apoptosis became evident. *In vivo* pharmacological intervention causing the inhibition of glial activation protected against neuronal apoptosis. *In vitro*, exposed glial cells released proinflammatory cytokines, a key factor for neuronal apoptosis. In both *in vivo* and *in vitro*, expression levels of

TRPV4 were increased as compared to non-exposed controls. Pharmacological or knock-out intervention of TRPV4 in cultured glial cells decreased the levels of inflammatory cytokines and attenuated neuronal apoptosis. This study also demonstrated the involvement of calmodulin and protein kinase C signaling pathways in the response to infrasonic exposures. These data suggest that TRPV4 expressed by glial cells is potentially a key factor in infrasound-induced neuronal impairment [53].

Neonatal rat hippocampal astrocyte cultures were exposed to 16 Hz at 130 dB for 15, 30, 60, 90, 120, and 240 minutes. Extra-cellular glutamate levels increased with increasing exposure time, and at 90 min, there was a 100% increase over baseline. The astroglial expression of Cx43 (connexin43—see above) was increased, as compared to non-exposed controls, as was the synthesis of Cx43 mRNA. Through additional evaluations using pharmacological and knock-out interventions, the authors concluded that infrasonic exposures induced astrocytes to release glutamate, and that Cx43 gap junctions were required for the exposure-induced glutamate release [54].

The endocannabinoid system includes lipid-based retrograde neurotransmitters, expressed throughout the CNS, and involved in fertility, pregnancy, pre-and postnatal development, appetite, pain-sensation, mood, and memory. Animals were exposed to 16 Hz at 130 dB, 2 hours daily, for 14 days. Cannabinoid (CB) receptors 1 and 2 in the CA1 hippocampal region of the exposed rats were down-regulated in a time-dependent manner, as compared to non-exposed controls. Apoptotic cells in the CA1 only became obvious after day 5, and cell death coincided with the decreased expression of CB receptors. Through pharmacological intervention, activation of CB receptors significantly reduced the number of apoptotic cells, ameliorated the behavior performance of exposed rats, and reduced the infrasound-elevated levels of proinflammatory cytokines. These data suggest that CB receptors could potentially serve as promising targets for future treatments against infrasound-induced injury [55].

Fibroblasts synthesize extracellular matrix (glycosaminoglycans, reticular, and elastic fibers) and collagen, and, in addition to their structural role, fibroblasts are also important for mounting the immune response to tissue damage. Fibroblast growth factors (FGF) signal through fibroblast growth factor receptors (FGFR). The fibroblast growth factor 2/fibroblast growth factor receptor 1 (FGF2/FGFR1) signaling pathway was investigated in animals and in cultured astrocytes, exposed to 16 Hz at 150 dB, 2 hours daily, for 1, 3, or 7 days. In both experimental models, astrocyte activation increased with exposure time and astrocyte-expressed FGFR1 was downregulated as compared to non-exposed controls. Pharmacological intervention using FGF2 exerted an inhibitory effect on infrasound-induced astrocyte activation, inhibited the elevation of proinflammatory cytokines, upregulated the expression of FGFR1, and alleviated neuron loss in CA1 hippocampus region. Inhibition of the FGF2/FGFR1 pathway aggravated astrocyte-mediated inflammation after infrasonic exposure. The authors concluded that astrocyte-mediated inflammation was involved in infrasound-induced neuronal damage and that the FGF2/FGFR1 pathway played a key role [56].

In a laboratory setting, rats were exposed to tonal 8 Hz at 140 dB, 2 hours daily, for 3 days. A post-exposure, 1-week time point was also established. Significant damage of hippocampus morphology was observed in exposed rats, and recovery was seen after 1 week of post-exposure. Neuronal apoptosis was significantly increased after 24- and 48-hour exposures, as compared to non-exposed controls, and then decreased after 1 week post-exposure. Expression of heat shock protein 70 (HSP70) peaked at 24 hours and was decreased at 48 hours [57].

5. Conclusions

Exposure to infrasonic and lower frequency airborne pressure waves can cause cellular and tissue damage depending on frequency, dB-level, and exposure time, while the viscoelastic properties inherent to biological tissues impart a nonlinear response to this type of acoustic stressor. The complex mechanosensitive and biochemical cellular signaling pathways mediating this cellular damage have not yet been pinpointed, although fasciae structures and connective tissues (including the neuroglia) seem to be the most sensitive under longer term exposures. Immediate exposures appear to induce inflammatory processes that do not seem to be maintained with longer exposures.

Widespread vascular involvement (not limited to the biological structures addressed herein) was observed in palpebral and bulbar conjunctiva and retina, gastric mucosa, liver structures, lungs, pleura and tracheae, alveoli, pericardia, and coronary arteries. This vascular response may (unsuspectingly) be the underlying cause of many symptomatic complaints. Cognitive deficits oftentimes documented within residential field laboratories may not merely be due to sleep deprivation, but also to hippocampal neuronal damage. Fasciae morphogenesis speaks to the demand on the whole-body structural integrity elicited by this type of external mechanical insult, while collagenous growths and hemorrhagic events of a focal nature may reflect concomitant resonance phenomena.

Recovery periods are not linear, and 2-hour daily exposures imply a 22-hour nonexposure period. This presents a problem for continuous exposures, such as those encountered in some professional activities and most residential environments. The underlying objectives of most of the studies discussed herein are related to occupational exposures and do not consider continuous exposures at less than 90 dB, nor are pressure pulsed trains presented within the laboratorial acoustic environments. In residential environments, however, these attributes are often present. The simulation of residential exposures does not appear to have yet been integrated into laboratory settings and protocols.

The whole-body response also elicits the immune system, affects organs of the reproductive system, changes receptor cells in the vestibular semicanals and auditory cochlea, and induces genotoxic effects, including teratogenesis. This is a pioneering field of science, still in its infancy and urgently requiring scientists from multidisciplinary areas of study because, ultimately, the health of human populations and their offspring must be protected.

Conflict of interest

None.

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