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FOCUSED ULTRASOUND STIMULATION OF THE PERIPHERAL NERVOUS SYSTEM: PHYSICAL BASIS AND PRACTICAL APPLICATIONS (REVIEW)

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Abstract

The subject of this review is the description and analysis of researches on application of focused ultrasound for stimulation of somatosensory, hearing and other neural structures of the peripheral nervous system. The main parameters of focused ultrasound, factors determining its biological effects, and methods of stimulation of peripheral nervous structures are discussed. Different stimulation effects and sensations are considered. A variety of practical applications of ultrasound stimulation of peripheral nervous structures is presented, including diagnostics of neurological and dermatological diseases, evaluation of the efficiency of anesthetic and analgesic drugs, principles of the development of an ultrasound tactile display, diagnostics of hearing disorders, possibilities of ultrasound hearing prosthetics, etc. The part of the review related with the methods and results (Sections 3-7) is based, first of all, on the data of the studies carried out in a number Russian Institutes from the beginning of 1970s and until last years. These results were published presumably in Russian journals and books and not well known to investigators in other countries. Some of the obtained results and offered approaches still have a current interest and practical significance. During the last decade, investigations of ultrasound stimulation of peripheral nervous structures began to develop actively in a number of countries. A review of a part of them is also presented.

Keywords and phrases: focused ultrasound, HIFU, peripheral nervous system, somatic nervous system, sensory information, receptors, sensations, stimulation, nerve fiber, radiation force, diagnostics of neurological diseases.

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1. Introduction

Searching for artificial stimuli that can activate neural structures noninvasively and locally and induce different sensations has always been an important task of physiology and medicine. Such stimuli would be extremely useful, for example, in diagnosis of diseases associated with changes in various sensations, as well as in physiological studies. Ideally, it is necessary to exclude any damage in a stimulated site or surrounding tissues, as well as to provide prolonged and repeated use of such artificial stimuli. It is also important to execute accurate measurements of various parameters of stimuli.

A wide-spread and classical means for stimulation of neural structures is an electric current. However, sometimes it is impossible to use this method for local stimulation of single receptors or other neural structures without affecting the neighboring ones. If it is necessary to activate a deep located structure, such the method could not be considered as a noninvasive one because the electrodes need to be in a direct contact with an activated structure.

The subject of this review is the description and analysis of researches on application of focused ultrasound for stimulation of somatosensory, hearing and other neural structures of the sensory-somatic nervous system. It is well-known that over the past several decades, focused ultrasound has become one of the most popular, safe and effective method for application in medicine amongst existing physical technologies. In fact, high-intensity focused ultrasound, or HIFU, is used widely for local ablation of diseased or damaged tissues [1-3].

The studies analyzed in this review are related mainly with research into application of focused ultrasound for activation of peripheral receptor structures. As shown below, the method of activating receptor structures with focused ultrasound entirely satisfies the requirements of contactless, noninvasive, local, and dosed effects. Functional effects arising from the use of ultrasound are extremely diverse. They range from tactile sensations and hearing to deep pain feeling and stimulation of acupuncture points. The review is focused on the stimulating (i.e., activating or irritating) effect of focused ultrasound on the receptor structures that can be repeated many times (sometimes, for years) without any risk of damaging the structures and surrounding tissues. Pulses (stimuli) of focused ultrasound in the frequency range from 0.5 up to 3.5MHz and with the duration ranging from parts of milliseconds to hundreds milliseconds were used in these studies. The advantages of the proposed method of stimulating of neural structures are the following:

● The method is noninvasive, since it doesn't require a destruction of tissues to access deep structures.

● The size of a stimulated region can be controlled and varied by changes in ultrasound frequencies and parameters of a transducer, which provides selectivity and locality of the effect on neural structures.

● It is possible to control precisely the parameters of an ultrasound stimulus, such as the intensity, duration, volume and area of action, and repetition frequency of stimuli.

● It is possible to affect not only the superficial structures (e.g., located in the skin), but also tissues located deeply in the body.

The use of amplitude-modulated ultrasound for inducing of hearing sensations has become a separate and important field of research useful for application in diagnostics of different hearing disorders as well as for prosthetics of hearing function of the deaf people. An important part of this review is investigations of the possibilities of practical use of these effects including their applications in clinical medicine for diagnostics of different neurological, dermatological and hearing diseases.

Investigations of neurostimulation and neuromodulation of the central nervous system, and first of all, the brain structures of humans and animals, are separate and actively developing field of studies, discussed in a number of excellent reviews [4-7], and do not consider in this article.

The part of the review related with the methods and results (Sections 3-7) is based, first of all, on the data of the studies carried out in a number Russian Institutes from the beginning of 1970s and until last years. These results were published presumably in Russian journals and books and not well known to investigators in other countries. Some of the obtained results and offered approaches still have a current interest and practical significance.

During the last decade, investigations of ultrasound stimulation of peripheral nervous structures began to develop actively in a number of countries and in many laboratories. A review of a part of them is also presented.

2. Main Parameters of Focused Ultrasound and its Physical Mechanisms

Effects of focusing of sound waves due to reflection from concave surfaces were known from immemorial times. Focusing of sound and ultrasound is similar to focusing light. In both cases effects of focusing can be implemented, for example, by means of collecting lenses, systems of mirrors or reflectors. However the most convenient, at least, for the purposes of medical ultrasound, appeared to be so called spherical focusing radiators [8] with a concave spherical surface. Another example of a device for focusing of ultrasound is the use of phased arrays allowing not only to steer electronically the focus over the space, but also to create several foci simultaneously [9].

The size of the focus is comparable with the wavelength of ultrasound. That means that if the size of a focal spot should be of the order of millimeter, it is necessary to use MHz-range ultrasound. Using of spherical focusing radiators allows focusing the ultrasonic energy near the center of curvature of the radiating surface. In this case, a wave front converging in the focus has a spherical form. Thus, the intensity on the surface of the converging front increases in inverse proportion to a decreasing a surface of the front, i.e., according to the law $1/r^2$, where r is the radial coordinate counted from the center of the focal region.

However because the geometric approach near the focus is inapplicable [8], the intensity in the center of the focal region does not achieve an infinity, and has a quite certain size.

Figure 1 [10] presents the main geometrical characteristics of a spherical focusing radiator: R is the radius of a radiator; F is the focal length; α_m is the angle of convergence; h is the depth; r_0 is the radius; and l is the length of the focal region.

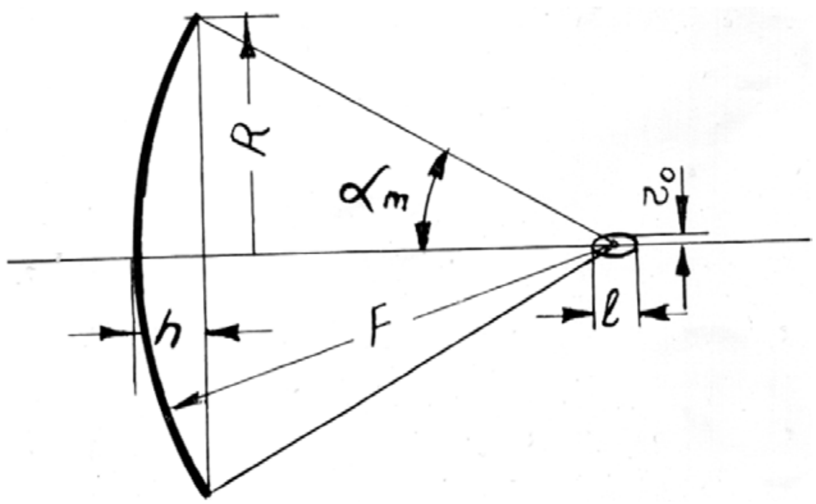


Figure 1. Main geometrical characteristics of a spherical focusing radiator [10].

Let's present the main relations for a single spherical focusing radiator [8]. Formulae are received in the assumption that the distribution of amplitude of the particle velocity on the surface of the radiator is continuous and uniform.

Radius of the focal region is

$$r_0 = 0.61 \frac{\lambda F}{R}, \tag{1}$$

where $\lambda = c/f$ is the wavelength of ultrasound with the frequency f and velocity of propagation of ultrasound in the medium c.

Length of the focal region is

$$l = \frac{2\lambda}{1 - \cos\alpha_m}. \tag{2}$$

Both of these parameters are determined by the zeros of the intensity nearest to the main focus. The maximal intensity in the center of the focal region with a not very large angle of convergence $\alpha_m (\alpha_m < 45^{\circ})$ can be expressed as a ratio of the source area and cross-sectional area of the focal region [8]:

$$I_F = 3.7 I_0 \frac{\pi R^2}{\pi r_0^2}, \tag{3}$$

where I_0 is the intensity at the surface of the radiator. The multiplier 3.7 shows that the intensity in the center of the focal region higher than the intensity averaged over its area, and also considers that through the focal spot passes only 84% of the focused energy, and 16% corresponds to the secondary maxima [8].

The pressure gain due to focusing is

$$K_p = 2\pi \frac{h}{\lambda}, \tag{4}$$

the oscillatory velocity gain is

$$K_v = K_p \cos^2 \frac{\alpha_m}{2}, \tag{5}$$

the intensity gain is

$$K_I = K_p^2 \cos^2 \frac{\alpha_m}{2}. \tag{6}$$

For example, for a radiator with the resonant frequency of 1MHz, with the radius and focal length equal to 42.5mm and 70mm, and the angle of convergence 36°, the diameter d and length l of the focal region are 3mm and 15mm, and the pressure and intensity gains are correspondingly ≈ 60 and 3225.

The distribution of the acoustical pressure amplitude in the acoustical field of a focused transducer is presented in Figure 2.

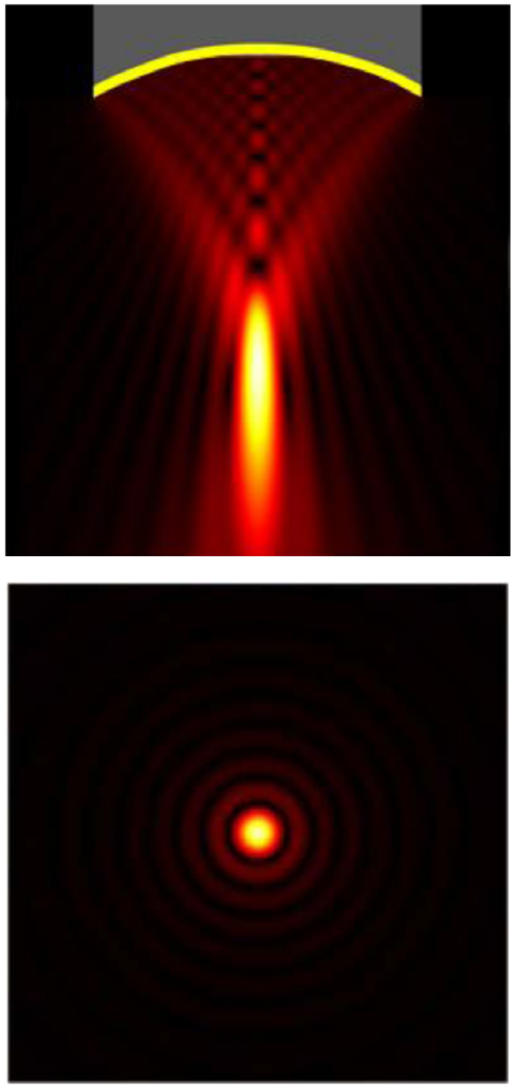


Figure 2. Distribution of the acoustical pressure amplitude in the acoustical field of a focused transducer along the axes (above) and in the focal plane (below). Courtesy of Prof. O. Sapozhnikov.

Investigations of acoustic fields of focusing transducers show that through the focal region within the main diffraction maximum at small angles α_m plane wave propagates. Therefore, for calculations of parameters of the sound field in the focal region, the plane wave approach is used [11]:

$$I = \frac{1}{2}\rho c\omega^2 A^2 = \frac{1}{2}\rho c V^2 = \frac{P^2}{2\rho c}, \tag{7}$$

where I is the intensity of ultrasound; A is the displacement amplitude; V is the velocity amplitude; P is the pressure amplitude; $\omega = 2\pi f$ is the angular frequency; and ρc is the characteristic acoustic impedance of a medium with the density ρ and velocity of ultrasound c.

When a plane ultrasonic wave propagates through a homogeneous medium, the intensity of ultrasound decreases with the distance exponentially [11]:

$$I = I_0 e^{-2\alpha x}, \tag{8}$$

where I_0 is the initial intensity; x is the distance from a source; and α is the amplitude attenuation coefficient of ultrasound in the medium. The attenuation coefficient, as well as the absorption coefficient, is expressed in nepers per unit length, i.e., as Np/cm or Np/m. The attenuation coefficient represents the sum of the absorption coefficient α_0 and the scattering coefficient α_s. The latter includes refraction and reflection. In practical applications of ultrasound, it is convenient to express attenuation in decibels. An attenuation coefficient expressed in dB/cm is 8.686 times larger than the same coefficient in Np/cm.

Let's consider the main physical factors that determine the ability of focused ultrasound in the megahertz frequency range to stimulate the peripheral nervous structures using single pulses (stimuli) with the duration of typically 1-10ms and less often to 100ms.

One of the most important factors determining numerous biological effects of focused ultrasound is heating of tissues due to absorption of acoustic energy in tissues and transformation into heat. The review of biological effects caused by thermal effects of ultrasound is in [12, 13].

If the plane wave is completely absorbed in the medium, the temperature T after time t will be [12, 13]

$$T = \frac{2\alpha_0 It}{\rho c_m} + T_0, \tag{9}$$

where T_0 is the temperature at $t = 0$, while ρ and c_m are the density and the specific heat per unit mass of the medium. For soft tissue $\rho = 1\text{g/ml}$ and $c_m = 4.2\text{J/g}$; for several tissues $\alpha_0 \approx 0.03\text{Np/cm}$ at 1MHz and the temperature rise in degrees Celsius is

$$T - T_0 = 0.14\ It.\ (I \text{ in W/cm}^2). \tag{10}$$

Equation (9) can be used only if the heat transfer by blood flow or other means of convection can be ignored, and also if the time t is short enough so that the temperature of a tissue does not decrease due to heat conduction (also called diffusion). For the short ultrasound pulses such an assumption is quite possible.

The second factor defining the biological action of ultrasound is its mechanical effects. Calculations using expression (7) show that, for example, at the frequency of 1MHz and the intensity of 1000W/cm^2 (which are realized rather simply with the use of modern focusing systems), the values of parameters of ultrasound are the following: the displacement amplitude is 0.6 microns, particle velocity is 3.6m/s, acceleration is $2.3 \cdot 10^9 \text{cm/s}^2$ (that exceeds the acceleration of terrestrial gravitation in $2 \cdot 10^6$ times), sound pressure is 55atm and pressure gradient along the distance of one-half wavelength $\Delta P = 1500\text{atm/cm}$. It is clear, that so intensive mechanical action on the medium can lead to various effects, including destroying in some cases. In particular, the gradients of the sound pressure existing in ultrasound field sometimes appear to be so great, that their action can be essential along the small distances, comparable with the sizes of some cellular structures (units and tens of microns).

It is worth comparing the mechanical action of ultrasound with the action of ionizing radiation, for example, X-rays. For increase of the intensity of the latter, it is required to increase a number of the charged particles, but the energy of each of them remains constant. At the use of ultrasound all parameters are inextricably linked to each other. Therefore, increase of the ultrasound intensity at the fixed frequency entails the increase in the values of all other parameters (displacement, particle velocity, sound pressure, acceleration, etc.).

Mechanical action of the focused ultrasound is increased with an occurrence in the biological medium ultrasonic cavitation, i.e., the formation and activity of gas or steam bubbles (cavities) in the medium irradiated by ultrasound. The physical nature of cavitation is considered in a number of reviews and books [13-15].

Cavitation is usually divided into two different types: inertial cavitation and non-inertial cavitation. Inertial cavitation is the process where a bubble in a liquid rapidly collapses, producing a shock wave. That type of cavitation formerly was called “transient”, “vapour”, “true”, “real” or “collapsing”. Non-inertial cavitation is the process where a bubble in a fluid is forced to oscillate due to ultrasound action. Such type of cavitation formerly was called “stable” cavitation.

Inertial collapse of a spherical gas bubble in a liquid is characterized by a relatively slow (i.e., a timescale on the order of half an acoustic cycle) growth of an initial bubble nucleus to many times its original size. This is followed by a rapid collapse, the initial stages of which are dominated by the inertia of a spherically converging liquid [14]. During the collapse, the gas temperature rises as it is compressed, and shocks can propagate within the gas.

In non-inertial cavitation, the intensity of the acoustic field is insufficient to cause a total bubble collapse. Bubbles in a liquid are forced to oscillate with only a small increase and decrease of the radius. This form of cavitation causes significantly less destructions than inertial cavitation. However, it is not only influences the inertial cavitation and its consequences, but also produces effects of its own. They are related, first of all, with secondary mechanical effects of ultrasound (acoustic streaming, radiation force).

The destructive effects of ultrasound cavitation can be substantially increased by the adding to a medium gas bubbles serving as cavitation nuclei. The same effects can be obtained with the use of contrast agents containing microscopic gas bodies, which provide echogenic interfaces when introduced into the sound beam. Studies with laboratory animals have shown that the probability of acoustic cavitation occurring in the body is increased when gas bubbles, in the form of contrast agents, are introduced into the body [16].

It is worthwhile to emphasize, that there is a fundamental difference between biological effects of heat and cavitation. Thermal changes in tissues occur not instantly, and a certain time is needed (usually not less than several seconds) to produce any effect. Destructions at inertial cavitation occur only after one or a few periods of ultrasonic oscillations. Therefore it is possible to characterize most adequately the thresholds of thermal destructive effects by the time-averaged power or intensity of ultrasound. The thresholds of cavitation can be more adequately estimated by the peak (in space and time) values of the sound pressure. Nevertheless, some researchers express the values of cavitation thresholds, measured by them, in terms of the intensity, instead of sound pressure. Some justification of such approach is that these values can be transferred to each other with a comprehensible accuracy.

Alongside with the mechanical effects caused by sign-variable oscillations in the medium, i.e., when the time-averaged force is equal to zero, there are also other effects for which such averaged forces differ from zero. Thus tissues or biological liquids are exposed by the unidirectional force, more often at some distance from a radiator, and also rotary forces. The physical nature of such effects is related with second order properties of an acoustic wave. Amongst them are the effect of radiation forces, and also occurrence of acoustic streaming. Biophysical consequences of these effects in ultrasonic fields were a subject of interest and research for a long time [13, 15, 17].

The radiation force acting on a certain target has already become, for a long time, a basic measured parameter for standard methods of measurements of the acoustic power. Radiation forces can be divided into two groups, according to the size of the targets (objects or structures) which they impact. The first group corresponds to objects, whose size is larger than the wavelength of ultrasound. They can be, for example, absorbing mediums with the sizes exceeding the dimensions of an ultrasonic beam, like rather large and homogeneous organs as the liver, brain, etc. Such targets can be also large interfaces of media with different acoustic properties (skin-air, soft tissues-bones, etc.).

The second group corresponds to the targets with the sizes smaller than the wavelength. They can be, for example, blood cells or cells in suspensions and also liquid inclusions in intercellular substance of a tissue. All the mentioned targets can be found in any practical situation and in any macroscopic volume of a tissue.

Let's consider the case of the large target. For a plane wave, the value of the radiation force at sound normal incidence can be found as [18]

$$F = D\frac{IS}{c}, \qquad (11)$$

where D is the coefficient depending on properties of a target or interface between two media. For an ideal absorber $D = 1$, and for an ideal reflector $D = 2$. If the ultrasonic beam falls on an ideal reflector under an angle θ, $D = 2\mathrm{Cos}^2\theta$, and if it passes through not reflecting interface of two media with various velocity of sound c_1 and c_2, $D = 1 - c_1/c_2$. For example, if $c_1 = 1450$m/s, and $c_2 = 1600$km/s (an interface between fat-muscle tissue), coefficient $D = 0.093$, i.e., less in 10 or 20 times than in the case of falling of sound on a completely absorbing or completely reflecting target. Thus if $c_1 < c_2$, as in the case considered above, the force is directed from a source and if $c_1 > c_2$ is directed to a source. At last, if the plane beam falls on rather thin absorbing layer with amplitude attenuation coefficient α, the coefficient $D = 2\alpha$ [17]. For example, if $\alpha = 0.01\text{mm}^{-1}$ at the thickness of a layer of 1mm, $D = 0.02$, i.e., it is two orders of magnitude less than for an ideal reflector. As absorption grows in tissues with the frequency, the force acting on tissues at the given intensity rises with the increase of frequency. From the presented estimations follows, that the radiation pressure appears most noticeably at interfaces of media with different acoustic properties (for example, soft tissues - bone, etc.). In the medium with a homogeneous attenuation, it is worthwhile to have in mind a gradient of the radiation force or radiation pressure in the direction of distribution of ultrasound [17]. This gradient is the main reason of the occurrence of acoustic streaming in the liquid medium.

In Table 1 [17], data describing values of the radiation force and radiation pressure typical for serially manufactured diagnostic equipment are presented. The parameters are the following: the pulse mode, the time-averaged intensity is 1W/cm^2, the peak intensity is 500W/cm^2, the beam area is 10mm^2, the frequency is 3MHz, and the attenuation coefficient is 0.015mm^{-1}.

Table 1. Estimated radiation forces and pressures in a typical diagnostic beam [17]

Totally absorbing surface	
Time-average force	65μN
Time-average radiation pressure	6.5Pa
Pulse-average force	32.5mN
Pulse-average radiation pressure	3.25kPa
Tissue-like attenuating medium	
Time-average force gradient	0.2μN mm^{-1}
Time-average radiation pressure gradient	0.02Pa mm^{-1}
Pulse-average force gradient	0.1mN mm^{-1}
Pulse-average radiation pressure gradient	10PaN mm^{-1}

Radiation pressure, undoubtedly, plays an essential role in occurrence of some physiological effects under the action of ultrasound. One of such effects is the occurrence of hearing sensations at the action of amplitude-modulated focused ultrasound on an ear labyrinth of humans and animals (see in Section 5).

3. Methods of Ultrasound Stimulation of Peripheral Nervous System

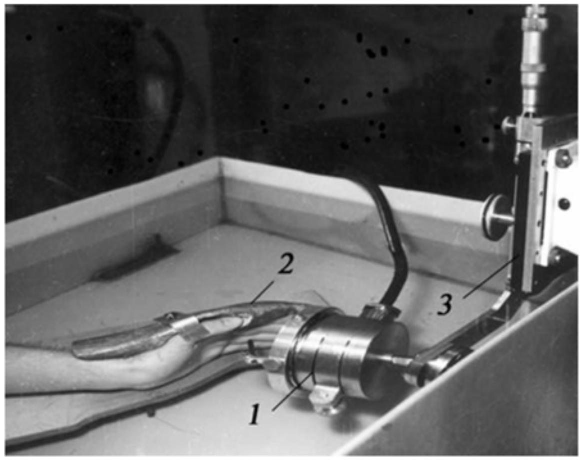
The possibility of activating sensory receptor structures in humans using short (with duration on the order of fractions or units of milliseconds) pulses of focused ultrasound was first shown in the middle of 1970s [19-22]. From the beginning, the subject of investigation was the somatic nervous system, i.e., part of the peripheral nervous system, which is the entire nervous system except the brain and spinal cord. It was found that focused ultrasound pulses directed on the skin can cause all the sensations that people routinely receive through the skin, such as tactile, thermal (warmth and cold), tickling, and itching, as well as various types of pain, including deep-seated pain in muscles, periosteum, etc. In the other words, focused ultrasound can be an artificial stimulus for practically all somatic senses whose receptors are associated with the skin, muscles, joints, etc.

An experimental set-up used for the research on the human's hand and forearm is shown in Figure 3 [21, 22]. Ultrasonic frequency varied widely; however, in most experiments, the focusing transducers with the frequencies of 0.48, 0.88, 1.95, and 2.67MHz were used [19-22, 10]. A focusing transducer and the hand and forearm of a subject were placed in the test bath with settled or distilled water, and the hand and forearm were fixed in a special casting (Figure 3). A removable pointer of the focus mounted on an ultrasonic transducer allowed the researchers to control the position of the center of the focal region. In most cases, the diameter of the radiating element of the focusing transducer was 85mm, the radius of curvature was 70mm, and the convergence angle of the transducer was 36°. As is well known, the form of the focal region is a spheroid or an ellipsoid of revolution. The calculated dimensions of the focal region of some focused transducers used in experiments are presented in Table 2 [20].

Table 2. The sizes of focal regions of the focused transducers used in experiments

f (MHz)	d (mm)	l (mm)	S (mm^2)	V (mm^3)
0.48	6.4	34	32	725
0.88	3.4	18	9.1	110
1.95	1.5	8	1.8	9.6
2.67	1.1	6	1.0	4.0

Here f is the operating frequency of the focused transducer; d is the diameter; l is the length; S and V are the area and the volume of the focal region within the main diffraction maximum. Although the maximum sizes determined by points where the ultrasound intensity diminished to zero is relatively large, the region where the intensity approximates its peak value would have dimensions considerably less than those shown in Table 2. Thus, using the focused ultrasound transducers of relatively high resonance ultrasound frequency, one can stimulate sensory receptor structures within relatively small volumes.



(a)

(b)

Figure 3. (a) The focusing radiator (1), casting (2) for fixation of the forearm in a water bath, a part of a coordinate system (3) is also seen [21, 22], (b) Experiment.

A schematic drawing of the set-up for the study of the stimulation effects of focused ultrasound on the sensory receptors of the hand and forearm is presented in Figure 4 [10, 23]. The focused transducer was mounted in 3D coordinate system (positioner) such that a threedimensional location of the transducer could be controlled with an accuracy of 0.1mm. The hand and forearm of a human were constrained in a specially made casting and submerged in a bath with water of controlled temperature [21, 22]. The transducer focal distance (usually 70 mm) permitted to stimulate not only the skin and superficial neural structures, but also subcutaneous or deep-seated sites of the hand and forearm.

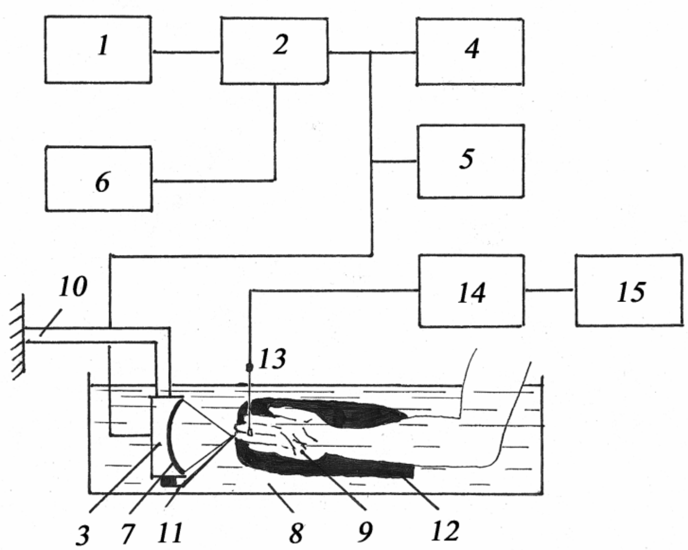


Figure 4. A schematic drawing of the set-up for the study of the stimulation effects of focused ultrasound on the sensory receptor structures of the hand and forearm. (1) ultrasound generator; (2) modulator and power amplifier; (3) housing of the focused transducer; (4) voltmeter; (5) oscilloscope; (6) generator of rectangular electrical pulses; (7) concave piezoceramic plate of the focused transducer; (8) bath with water of controlled temperature; (9) hand and forearm under investigation; (10) 3D coordinate system (positioner); (11) removable pointer of the center of the focal region; (12) casting to keep a hand and forearm in physiologically convenient position; (13) miniature hydrophone for measurements of cavitation; (14) selective voltmeter-amplifier tuned to the frequency of one half the ultrasound frequency; and (15) oscilloscope [10, 23].

Sensory receptor structures usually were stimulated by single pulses of specified durations (from 100μs to 100ms) with an intensity in the focal region from a few W/cm^2 to thousands of W/cm^2 (for the shortest pulses) [20-22]. In some experiments, especially when studying pain, series of pulses with different repetition frequencies were used. Stimuli were applied, after a warning, at irregular intervals, the intensity increasing from stimulus to stimulus until a sensation was reported by the volunteer. He/she was asked to describe its character. On the bases of the testee's reports after the action of ultrasonic stimuli, the absence or presence of sensations and descriptions of subjective characteristics were recorded and the thresholds of sensations were measured. The thresholds of different sensations were usually measured in the units of the intensity of ultrasound required to induce sensations, and, for persons with normal sensitivity, the thresholds were well reproducible. The variation of measured values did not exceed the data scattering in standard psychophysical experiments. From the values of the threshold intensity, other parameters, such as the displacement amplitude, oscillating velocity, sound pressure amplitude, temperature rise and radiation force could be calculated by means of expressions for a plane wave (Equation (7)) given above.

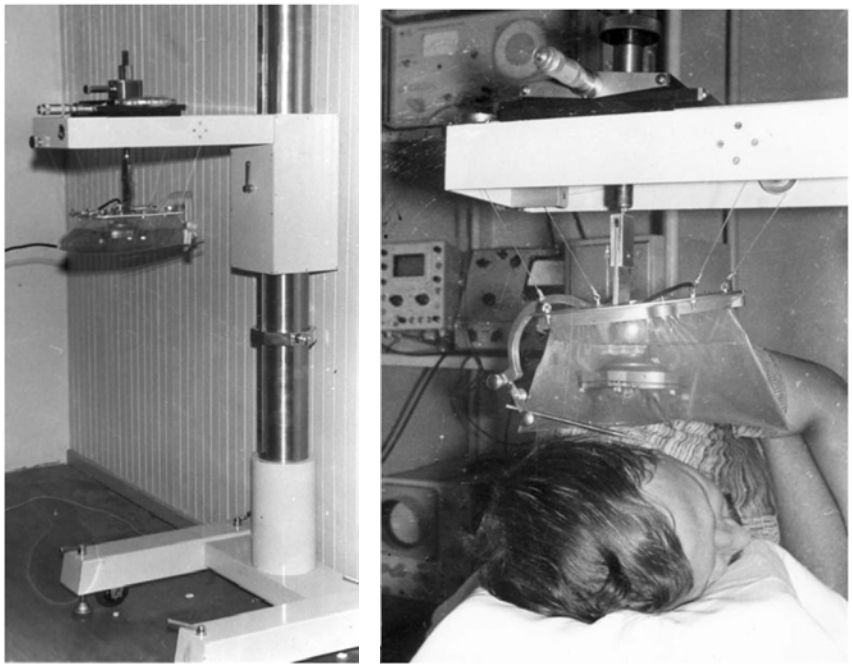
In a special series of experiments, the appearance of cavitation in soft tissues was checked with registration of subharmonic components of the acoustic noise which appeared with ultrasonically induced cavitation in the medium. The set-up included a miniature hydrophone (see Figure 4) placed in water 2-3cm from the center of the focal region; a selective amplifier tuned to a half ultrasonic frequency, and an oscilloscope. The appearance of cavitation resulted in a signal on the screen of the oscilloscope, its amplitude depending on the degree of development of cavitation. The intensity of ultrasound at which the signal amplitude exceeded by 40dB the noise level was taken as a threshold to induce cavitation [22].

Other methodological peculiarities of the research into the stimulation effect of focused ultrasound on somatic neural structures have been described in a number of papers [9, 20, 22-26] and books [10, 21, 27, 28].

During this research, a method of ultrasonic input of hearing information to humans was also proposed [10, 24, 27, 29]. The essence of the offered method is that the ear labyrinth is affected by amplitude-modulated focused ultrasound. The carrier frequency is significantly higher than the upper threshold of human hearing frequencies (for example, within 0.5-5MHz), and modulating frequencies correspond to the transmitted acoustic information. Since the ear labyrinth is affected by amplitude-modulated ultrasound, the human is subjected to the actions of oscillations with frequencies of f and $f \pm F$, where f and F, respectively, are the carrier frequency and the frequency of modulation, i.e., oscillations in the megahertz frequency range which are inaudible to humans. It was shown that with the propagation of amplitude-modulated ultrasonic oscillations in a medium, demodulation of ultrasonic signal occurs corresponding to auditory information transmitted.

Since the amplitude of the low-frequency signal increases with an increase in ultrasonic intensity, the sound pressure of the informative low-frequency signal is maximal in the focal region of the transducer. In the case of significant defocusing of an ultrasonic beam, it is maximal in the place of the maximum intensity of ultrasound. Thus, using the proposed method, auditory information can be inputted into the human ear labyrinth, avoiding the usual natural way of the sound waves to the ear labyrinth.

A testing of the proposed method of input auditory information into the ear labyrinth was performed in experiments in volunteers with normal hearing [24, 29]. A 3D coordinate system used for studying hearing sensations induced by amplitude-modulated focused ultrasound is shown in Figure 5(a) [27]. The error of determination of the coordinates for each of three mutually perpendicular directions was no more than 0.1mm. Part of the experimental setup is shown in Figure 5(b) [30]. A testee and a focusing transducer placed in a sound transparent bag are shown in the photo. The patient lying on the back on a bench, stayed in a horizontal position during the experiments.



(a) (b)

Figure 5. (a) A 3D coordinate system used for studying hearing sensations induced by amplitude-modulated focused ultrasound [27]; (b) Experiment on stimulation of human's auditory system [30].

A bag with a focusing transducer was filled with distilled water and tightly contacted with the head of a subject in such a way that there was no air gap. A focusing transducer was equipped with a removable focus pointer, the tip of which was coincided with the center of the focal region. The tip of a focus pointer was aligned with a point on the surface of the subject (i.e., skin), conventionally accepted as a coordinate origin. Then a focus pointer was removed, and the transducer was moved inside the bag in three mutually perpendicular directions so that the focal region of the transducer coincided with an intended site of irradiation.

The frequency of focused ultrasound was ranged from 0.67 to 3.7MHz. Ultrasonic generator provided the operations in a pulsed mode, as well as in the modes of amplitude-modulated oscillations and pulse-amplitude modulation.

In the case of a pulsed mode, the duration of ultrasound stimulus was adjustable from 0.1 to 1ms at a repetition frequency from 5 to 1000Hz. In the mode of amplitude-modulated oscillations, the modulation frequency ranged from 20 to 20000Hz, and the modulation index ranged from 0 to 1. In addition, the signals from the microphone, tape recorder, radio, etc. were used as modulating signals.

In the case of affecting the ear labyrinth with focused ultrasound in a continuous mode of irradiation without any modulation in amplitude, all subjects did not perceive any auditory sensations when increasing the intensity of ultrasound in the focal region up to $120 \mathrm{W/cm}^2$ (the intensity was not increased more for safety reasons). In the pulse mode, the subjects heard slightly distorted tones, the pitch of which corresponded to a pulse repetition frequency. For a control comparison, the pure tones supplied by the sound generator the headphone were used. The threshold auditory sensations were observed in subjects at an intensity of ultrasound in the focal region of the transducer of about $0.1 \mathrm{W/cm}^2$ and at a frequency of 0.67MHz. The presented here and below threshold intensities of ultrasound at the focal region, corresponding to the occurrence of auditory sensations in humans, are given without taking into account the attenuation of ultrasonic energy in bones and soft biological tissues on the path of propagation of the converging ultrasonic beam to the ear labyrinth. Thus, the real values of the threshold intensities in the tissues are significantly less than the mentioned.

With the use of focused ultrasound amplitude-modulated by sinusoidal oscillations with frequencies ranging from 50 to 15000Hz, the subjects experienced auditory sensations of tones, the pitch of which corresponded to the frequency of modulating signals. The volume of the audible tones increased with the modulation index. By varying the frequency of the sinusoidal modulating signal at a fixed modulation index and recording the intensities of ultrasound corresponding to the appearance of threshold auditory sensations, one could draw frequency-threshold curves analogical to audiometric examination. The frequency-threshold curves for a person with normal hearing are shown in Figure 6 [10]. The vertical axis represents the thresholds of ultrasound intensity averaged over the period of modulating oscillations. The horizontal axis represents the frequency of modulation. The parameter is the modulation index. The carrier frequency was approximately 1MHz. It is evident that the character of the curves, especially at modulation frequencies of 1-2kHz, is similar to the curves of the perception thresholds of sound signals by humans [31]. It is also evident from the graph that, for each fixed modulation frequency, auditory sensations appear when the product of the coefficient of modulation m on the intensity exceeds a certain constant value.

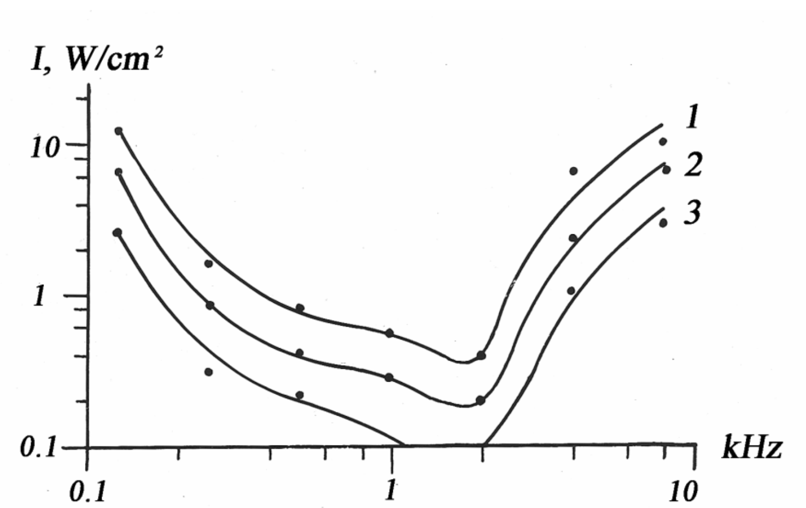


Figure 6. Typical dependences of threshold intensities of ultrasound in the focal region corresponding to the appearance of auditory sensations from the modulation frequency and modulation index m; (1) $m = 0.25$; (2) $m = 0.5$; (3) $m = 1$. The carrier frequency is 1MHz [10].

In essence, this method has no limitations in the frequency of modulating (audio) signals. When using focused ultrasound modulated in amplitude by oscillations of complicated form, for example, signals from a microphone or tape recorder, the subjects heard the corresponding transmitted auditory information.

4. Some Stimulating Effects and Sensations Induced by Focused Ultrasound

Directing ultrasound beam to the certain points on a skin of a palm surface or in the deep tissue of a forearm it was possible to induce practically all the sensations arising in given area of a body in natural conditions. In dependence on the location of the focal region, intensity and duration of a stimulus, temperature of water in the bath, the sensations of touch, pressure, warmth, cold, pain, tickling and itching were induced. At gradual increasing of the stimulus intensity, the sensations described by persons as «hardly feeling touches», «touching by a small soft brush» were elicit from the beginning. At the further increase of the intensity, these sensations changed to «a light push», «break of a falling drop», etc. These special sensations were precisely differed from the others: temperature, pain, tickling and itching.

When the center of the focal region was moved into the tissues under the skin the thresholds of sensations were increased. The tactile sensations disappeared when the focal region was entirely in the soft subcutaneous tissues. However, if the focal region was projected onto the skin of the opposite surface of the hand, for example, through the soft tissues between the thumb and the forefinger, the tactile sensations appeared again but with higher thresholds.

Stimulation of the same spots could result either in cold or in warmth sensations in dependence on the temperature in the bath where the forearm of a person was. Stimulation of some spots on the palm and, much more frequently, on the forearm resulted in sensation of no more than one or two modalities, e.g., tactile or tactile and temperature or only pain sensations, etc. In the deep tissues it was possible to induce only the pain. With the highest ultrasound frequency (2.67MHz), the pain sensation in the skin appeared less frequently than it did at the lower frequencies. This effect may be due to a smaller focal region or to the fact that the maximum stimulus associated with this frequency was not enough to elicit pain sensation.

Table 3 gives some representation of the order of threshold intensities (W/cm^2) corresponding to the origin of different sensations using focused ultrasound with the stimulus duration of 1ms and different ultrasound frequencies [20, 21]. These data were obtained from the lowest intensities observed at the same points in two persons. A number of spots investigated in every location varied from 5 to 21, and a number of measurements in every spot was varied from 4 to 42 (see details in [20, 21]). It is reasonable to assume that the lowest thresholds involve some conditions which are more optimal for stimulus action upon the receptor structures, e.g., due to a more precise coincidence of the focal region center with a sensitive structure. Because of this, the comparison of the lowest thresholds is probably more informative than the conventional procedure of calculating the mean and confidence intervals.

Table 3. The threshold values of the ultrasound intensities in W/cm^2 corresponding to the induction of different sensations with the use of stimulus duration of 1ms and different ultrasound frequencies [20, 21]

Sensation	Localization	Frequency (MHz)			
		0.48	0.88	1.95	2.67
Tactile	Finger	8	15	80	120
	Palm	16	80	250	350
Warm	Finger	55	90	1420	3200
	Palm	130	820	2940	4500
Cold	Finger	–	–	–	–
	Palm	130	820	2000	3000
Pain	Finger	55	140	2860	–
	Palm	290	350	–	3000

From the results of experiments with the study of temperature sensations induced by focused ultrasound follows that sensations of warmth and cold could be induced with the use of very short durations of stimuli, e.g., equal to 1ms. Simple calculations fulfilled with the use of formulae for evaluation of the temperature rise due to absorption of ultrasound in tissues (see, e.g., Equation (9)) showed that even for relatively high intensities of ultrasound the temperature rise ΔT will not exceed 0.5-1^{o}C, which is too small to induce a real temperature effect on the tissue. That means that in these cases the effective factor responsible for the origin of temperature sensation is the mechanical one rather than the thermal action of ultrasound. For this particular situation, thermoreception can be considered as a type of mechanorecerption. On the other hand, for relatively long ultrasound pulses the temperature rise in tissues ΔT can achieve 10-20^{o}C during the pulse and therefore should be taken into account for evaluation of main effective factors inducing temperature sensations. Very approximately, it is possible to consider that for ultrasound stimuli shorter than or equal to 10ms the mechanical factor is prevailing, but for the durations of longer than 50ms the thermal action can change the temperature relationships in the region of temperature-sensitive structures.

To investigate thermal stimulation without participation of mechanical effects, it was offered to apply pulses of a trapezoidal form [32]. For example, pulse with the duration of 400ms could consist of the elevating and falling off components with the duration 100ms each and a plateau of 200ms. Such the stimulus acting on the skin of human fingers did not elicit tactile sensations, but induced the warmth sensation changing by the burn pain. The threshold sensation of warmth arose at the intensities of about 70-120W/cm^2 (frequency of 2.6MHz), which corresponded to the temperature rise of 0.9-1.4^{o}C.

With further increasing of the intensity of stimuli and action on some spots, a sensation of pain is appeared [10, 25]. It occurred more often with stimuli of 10-100ms duration than with shorter stimuli (1ms). Depending on which spot in the skin was stimulated, the pain sensation could either be preceded by other sensations or not; in several points this sensation could not appear even with the maximum intensity of stimulus injuring the skin.

Unlike the other sensations, the pain was appeared not only when the center of the focal region is located in the skin but also when it was shifted into the tissues under the skin, i.e., into muscles, bones and joints. While studying the pain sensations produced by directing the focal region into different tissues, it was found possible to compare them, according to their associated subjective coloring and the degree of "unpleasantness". The pain sensations from deep tissues were characterized by the subjects as "more unpleasant", "much more unpleasant", or "the most unpleasant" as compared with those from the skin layer.

In addition to tactile, temperature and pain sensations, focused ultrasound was capable to evoke the feeling of itching and tickling [10, 20-22]. Thresholds for tickling sensation were close to (but always lower than) those for warmth sensation. The same relationship holds for thresholds for itching sensations. The feelings of warmth or cold were replaced sometimes by the tickling sensation, and the pain sensation in the skin was often followed by the itching sensation.

Alongside with somatic sensation some other sensations and stimulating effects were studied. One of the most convenient objects for the study of stimulation action of focused ultrasound are various receptor structures, i.e., specialized structures of the organism most sensitive to the action of mechanical, thermal, physical and chemical factors [21]. The essential interest in this relation has electroreceptor formations of fishes, for example, ampullae of Lorenzini. Being typical electroreceptors, they represent channels in length up to 10-15cm located under a skin of a fish, e.g., sharks and skates. Ampullae of Lorenzini possess high sensitivity to the gradient of the electric potential in sea water and they react to temperature and mechanical effects. The effects of focused ultrasound have been investigated on the structures of electroreceptor system of Black Sea skates [33]. In brief, it was shown that focused ultrasound evoked excitation (onset of impulse activity) when it was directed to the pore of electroreceptors and inhibited the activity when it affected to the ampulla itself [33].

It was of interest to study the possibility to use pulses of focused ultrasound to stimulate central nervous structures of invertebrates. Such investigations have been carried out and described in [34]. The edible snails without shells were used as an object of investigation. This object has the following advantages: Accessibility for microscopic observation of the stimulated areas, the ease of an electrode introduction, simplicity of matching the focal region to irritated areas, and the possibility of good acoustic contact between the focusing transducer and the object because the propagation of the ultrasound energy is implemented through water. Bioelectric responses from central and receptor neural structures of the snail evoked by ultrasonic stimuli were registered. At gradual increase of the intensity of single stimuli of focused ultrasound, there was elicited an evoked impulse activity characterized by essential increase of the repetition frequency of bioelectric pulses in comparison with the background activity. The threshold intensity of ultrasound in the center of the focal region, at which the activity of receptor cells of statocyst was induced, varied in different experiments from 90 up to $270 \mathrm{W/cm}^2$ with the duration of 1ms. If the intensity of stimuli exceeded $1000 \mathrm{W/cm}^2$, the evoked impulse activity of cells of ganglion was appeared. With the subsequent increase of the ultrasound intensity, the activity increased and did not show the tendency to reduction up to the maximal value of the intensity equal to $2000 \mathrm{W/cm}^2$. Thus, a principle possibility to induce a stimulation of some central nervous structures of invertebrates has been demonstrated.

There is a curious question: whether ultrasound has a taste? If to direct the focal region of the focused transducer to a tip of a tongue, a testee feels a peculiar and quite unpleasant taste. It reminds a taste which can be perceived after connecting electrodes of a low-voltage battery to a tip of a tongue [10, 23].

5. Mechanisms of Some Ultrasound Induced Sensations

Obviously, understanding the mechanisms of activating action of focused ultrasound on the structures responsible for skin and tissue sensitivity, as well as for the emergence of hearing and other sensations, is necessary for the efficient and safe use of ultrasound as a means of inputting sensory information to humans. Therefore, studying these mechanisms has always evoked a special interest [23, 30, 35].

Let's discuss the mechanisms responsible for the induction of tactile and thermal (warmth and cold) sensations. The presence of sensations of cold produced by focused ultrasound stimulation allowed to assume at once that the nature of sensations resulting from ultrasonic stimulation of sensory receptor structures is not always related to the action of adequate stimuli (for example, the action of mechanical stimulus on the mechanoreceptors or using a heat stimulus to evoke the sensations of warmth), because ultrasound itself obviously does not carry cold. The data on taste sensations elicited by focused ultrasound (see above) and reactions in the nerve fibers of skates obtained using focused ultrasound [33] also lead to the conclusion that activation of neural structures is caused by the actions of one or several stimuli, which are not adequate for many sensations. Attempts to identify the factors responsible for the activation of structures related to skin sensitivity are presented in a number of studies [10, 20-23, 30, 35]. Their goal was to determine which parameters of the ultrasound changed minimally when the threshold of tactile and thermal sensations appeared in the same sensitive points on the skin with the use of different ultrasound frequencies.

The frequencies were ranged from 0.48 to 2.67MHz. The changes in some parameters of focused ultrasound (intensity, sound pressure, temperature rise, displacement amplitude, and acoustic power) for thermal and tactile sensations caused in the human fingers when the frequencies were varied in the above-mentioned range has been studied [23]. It was shown that there were considerable changes (sometimes within several orders of magnitude) in some parameters of focused ultrasound. For example, the amplitude of the sound pressure varied approximately by a factor of 6, the temperature rise increased 100-fold, and the intensity increased by more than 30 times. The last value indicates that the radiation pressure should be excluded from consideration, since its magnitude is proportional to the intensity of ultrasound and will change significantly with changes in the ultrasonic frequency.

Formally, the amplitude of displacement (an alternating factor) was the most independent on the frequency, although it seemed more logical if the most independent factor would be not an alternating but unidirectional mechanical effect of ultrasound related to demodulation of high–frequency ultrasonic oscillations [10, 20-23, 30, 35]. The radiation force, which is proportional to the acoustic power and in the linear approximation does not depend on frequency, could be such parameter. However, the existence of a direct correlation between the thresholds of tactile and thermal sensations and the magnitude of the acoustic power causes a question, why the threshold of the radiation force does not depend on the area of its impact. Indeed, the focal spot area (32mm^2 for the frequency of 0.48MHz and 1mm^2 for the frequency of 2.67MHz) varied in our experiments by more than 30 times. This, however, did not have a significant effect on the threshold values of the radiation force.

A well-known mechanism explaining how shear waves with relatively high displacement amplitudes appear under the effect of the radiation force was involved to explain this fact. In the study of Sarvazyan et al. [36], it was shown that amplitude-modulated ultrasound with a carrier frequency of 3MHz, modulation frequency of 1kHz, the speed of shear waves in tissues of 3m/s, and the intensity on the axis of ultrasonic beam of 10W/cm^2, produced a displacement of about 30-40μm in the tissue. An expression for the maximum displacement in the medium $u_{\max}$ under the action of relatively short pulses of focused ultrasound, the duration of which does not exceed the propagation time through the focal region, was obtained in [37]:

$$u_{\max} = \frac{\alpha a}{\rho c_l c_t} t_0 I \text{ for short pulses } (t_0 << a/c_t), \qquad (12)$$

where a is the radius of the sound beam (i.e., the radius of the focal spot); α is the absorption coefficient of ultrasound in the medium; t_0 is the duration of the action of the radiation force (i.e., the pulse duration); ρ is the density of the medium; c_t is the propagation speed of shear waves; c_l is the speed of longitudinal waves; and I and W are the intensity and acoustic power averaged over the pulse duration. From (12), it is evident that the displacement under the action of the radiation force is proportional to $t_0 I$, i.e., it depends on the pulse energy rather than on the intensity of ultrasound by itself. In [35], this expression was modified for long pulses, when the pulse duration is longer than the travel time through the focal region, which corresponds to the case under consideration. Then, the maximum amplitude displacement is

$$u_{\max} = \frac{\alpha}{\rho c_l c_t^2} a^2 I = \frac{\alpha}{c_l \mu} * W \text{ for long pulses } (t_0 >> a/c_t), \qquad (13)$$

where W is the acoustic power averaged over the pulse duration and μ is the shear modulus of the medium, $c_t = \sqrt{\mu/\rho}$.

A diagram illustrating the shape of an acoustic signal, the acoustic power, and shear displacement of the medium under the action of the ultrasonic pulse is shown in Figure 7 [35].

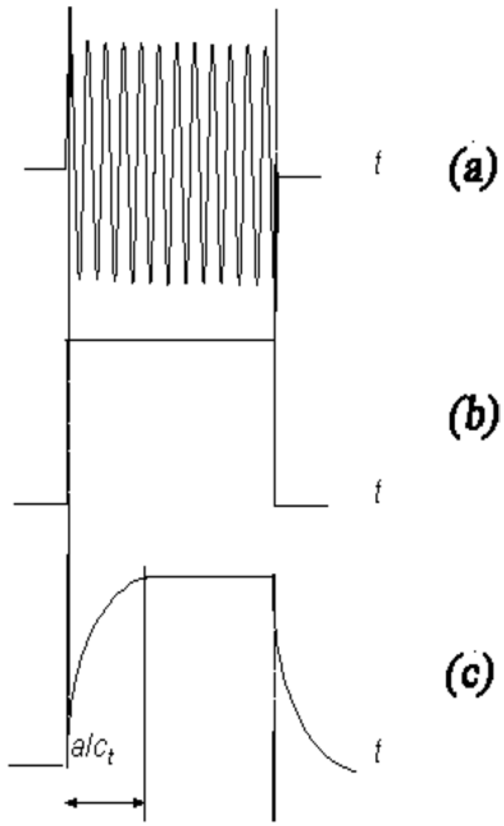


Figure 7. Diagram illustrating the shape of an acoustic signal (a), acoustic power (b), and shear displacement of the medium (c) under the impact of an ultrasonic pulse [35].

One can see that the displacement of the medium (Figure 7(c)) does not reproduce the shape of an acoustic signal (Figure 7(a)), or the acoustic power (Figure 7(b)). The displacement reaches its maximum $u_{\max}$ after the time of propagation of shear waves through the focal region $(t_0 = a/c_t)$. This time is relatively small, for example, for a = 1mm and c_t = 3m/s, it is t_0 = 0.3ms, which is significantly shorter than the duration of an ultrasonic stimulus (usually from 1 to 100ms). After this time, the magnitude of the shear displacement remains constant until the end of the pulse. This is consistent with our observation that pulses of the duration from 5-10 to 400ms caused tactile sensations in response to the beginning and the end of a stimulus and that a testee could not distinguish one long pulse (for example, 400-ms duration) from two short pulses separated by the same time interval [10, 21, 30].

These data support a conclusion that stimulation of neural structures is caused by to the gradient of a stimulatory factor. In our case, it is a unidirectional displacement of the medium due to the radiation force. The proportionality between the maximal displacement of the medium and the acoustic power explains the presented above experimental data on the stimulation of structures by focused ultrasound of different frequencies. Thus, the radiation force may be one of the factors responsible for the stimulatory effect of short pulses of focused ultrasound. This is consistent with the conclusions of other authors on the factors responsible for the evoking of tactile sensations with the use of focused ultrasound [38].

The mechanism of stimulation effects of amplitude-modulated ultrasound at the neural structures of the labyrinth was also studied [10, 24, 27, 30]. First, it is worth to keep in the mind that the human hearing organ is an extremely sensitive device designed by nature to perceive auditory information. Therefore, it is necessary to take into account the effect on the labyrinth of sound arising as a result of the radiation pressure of amplitude-modulated ultrasound.

It is known that the radiation pressure S is related with the displacement amplitude A as $S = \frac{1}{2}\rho\omega^2 A^2$, where ρ is the density of the medium, ω is the angular frequency. If the high-frequency ultrasound signal with the frequency f is modulated by the low-frequency sound signal as $a\mathrm{Cos}\Omega t$, where $\Omega = 2\pi F$ (F is the frequency of modulation), it is necessary to replace A by $A + \cos\Omega t$. Then [39]:

$$S = \tfrac{1}{2}\rho\omega^2(A + a\mathrm{Cos}\Omega t)^2$$

$$= \tfrac{1}{2}\rho\omega^2 A^2(1 + m^2/2 + 2m\mathrm{Cos}\Omega t + \tfrac{1}{2}m^2\cos 2\Omega t), \qquad (14)$$

where $m = a/A$ is the modulation index.

Thus, the signal of amplitude-modulated ultrasound includes three components: one constant and two variables. One of the latter changes with the modulation frequency, i.e., with the sonic frequency, and another with the double modulation frequency. Thus, the most probable factor inducing the auditory sensations in the hearing organ is the effect of sonic oscillations, arising due to the variable components of the radiation pressure, i.e., $P_F = 2m\,\mathrm{Cos}\Omega t$ and its second harmonic $P_{2F} = \frac{1}{2}m^2\cos 2\Omega t$, on the receptors in the labyrinth.

Thus, if the receptor system of the labyrinth in humans operates normally, the most probable factor inducing the auditory sensations near the threshold of perception is the effect of sonic oscillations arising due to the variable component of the radiation pressure on the receptors in the labyrinth. In this case, only sonic information is passing to the labyrinth, whereas ultrasound serves as a means of its delivery.

It is worth to notice that this mechanism, probably, is valid only for near-threshold ultrasound stimulations. For more intensive ultrasound impacts, in addition to this factor, the effects of sign-variable ultrasound oscillations can take place, i.e., thermal effects and mechanical actions of high-intensive ultrasound discussed above.

6. Ultrasound Stimulation of Auditory Nerve Fibers

One of the most substantial obtained result was that by means of focused ultrasound it is possible to stimulate through the skull not only the receptors (hair cells) of the internal ear, as with ordinary sound stimulation, but also auditory nerve fibers which until recently could be stimulated only by implanted electrodes. This conclusion was supported by experiments on animals with previously destroyed receptor systems of the labyrinth.

It was found that after mechanical destruction of the receptor apparatus, application of single stimuli of focused ultrasound to the auditory fibers of the frog (Rana temporaria) elicited the electrical activity in the auditory midbrain centers (torus semicircularis) [40]. The frequency of ultrasound was 2.34MHz, duration of pulses 1ms, the maximal intensity used in experiments achieved 700W/cm^2. Action potentials evoked by focused ultrasound were similar to those evoked by activation of the intact contralateral labyrinth but with higher thresholds [40]. The threshold intensity without destruction of labyrinth was 3W/cm^2, but after destruction it became 80W/cm^2. In the case of the intact labyrinth, the amplitude of the responses was increased until 350W/cm^2 and then decreased. For the destroyed labyrinth, the amplitude increased until the maximal intensity (700W/cm^2). After introduction of horseradish peroxidase into the destroyed auditory capsule, fibers activated by focused ultrasound were detected. Therefore electrophysiological and histochemical experiments reveal stimulating effect of focused ultrasound on the fibers of the VIIIth nerve even without participation of the receptor apparatus [40].

Finally, the possibility of direct stimulation of auditory nerve fibers by ultrasound was supported by the fact that some completely deaf persons, whose receptor system was diagnosed to have been destroyed may perceive auditory information delivered by means of amplitude-modulated ultrasound whereas standard hearing aids cannot help them [10].

7. Practical Applications of Ultrasound Stimulation of Peripheral Nervous Structures

This section includes data on ultrasound diagnostics of neurological, dermatological and hearing diseases, on practicability to develop ultrasonic devices for diagnostics of hearing diseases and for hearing prosthetics, on estimation of the bone tissues regeneration after fractures, and evaluation of the efficiency of anesthetic and analgesic drugs, and on the possible use of phased arrays for stimulation of neural structures.

7.1. Diagnostics of neurological diseases

There are grounds to suppose that ultrasonic methods for stimulating neural structures should find wide and useful application in the diagnostics of various diseases related to a change of sensory perception from normal. For example, a number of neurological and skin diseases are accompanied by considerable differences in the skin or tissue sensitivity from normal. Thus, by measuring and comparing the thresholds of various sensations in normal and pathological states, one can prove the diagnosis, evaluate the extent of pathological processes, monitor the results of treatment and so forth [10, 30, 41].

As an implementation of this approach, some diagnostic methods for several diseases related to the changes in the sensitivity of skin and tissues have been developed. In particular, a study of the threshold sensitivity in the terminal phalanges of fingers in 30 neurological patients and 21 healthy humans (control group) was performed [41]. The diseases included syringomyelia (distortion of the sensitivity at one side of the body and its preservation at another side), spondylogenic cervical radiculitis, residual effects of cerebral stroke, polyneuritis, ulnar and radial neuritis, etc. In all patients, increased tactile thresholds compared to the normal (up to entire absence of tactile sensitivity under the maximum possible intensity of the stimulus) were found by means of studies with focused ultrasound. The observation of a number of patients with unilateral reduction in sensitivity is worth noting. They had increased thresholds not only in the damaged arm, but also in the symmetric parts of the skin of another arm that was considered healthy upon standard clinical examination. This effect was observed, in particular, in patients with syringomyelia.

For illustration, Figure 8 [30, 41] presents the results of an examination of the tactile sensitivity in one of the patients with syringomyelia.

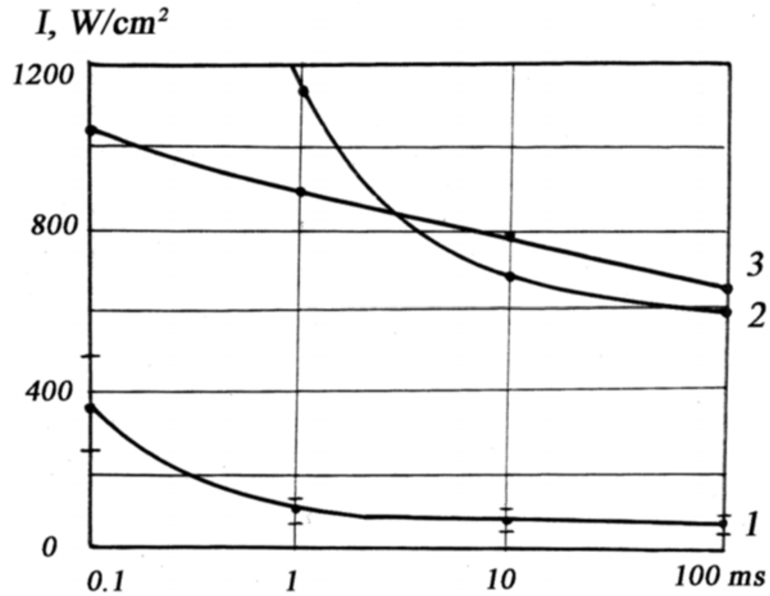


Figure 8. Thresholds of tactile sensitivity in a patient with syringomyelia: (1) thresholds in healthy persons (control group); (2) thresholds in the right (affected) hand; (3) thresholds in the left hand, where the standard neurological examination showed no sensitivity disorders [30, 41].

The frequency of focused ultrasound was 1.95MHz, the maximum intensity averaged over the area of the focal region was 1400W/cm^2 and the duration of single stimuli varied from 0.1 to 100ms. The vertical axis is the intensity of ultrasound in the focal region; the horizontal axis is the stimulus duration; points in the control group are the mean values from the measurements in 21 healthy patients. It can be seen that with a stimulus duration of 0.1ms the tactile sensitivity on the right (affected) hand was absent even at the maximum intensities (1400W/cm^2). It can also be seen that on the left hand where standard clinical examination showed no sensitivity disorders there was a considerable deviation of the tactile sensitivity from a normal one.

The results of studies using the ultrasonic method of diagnostics of neurological diseases show its usefulness for medical applications. It is important that the proposed method ensures detection of "subclinical" stages of disorders of tactile sensitivity that cannot be detected by other traditional methods. This possibility is particularly valuable for practical neurology.

The comparative study of the pain thresholds in 51 healthy persons and 64 patients with neurasthenia, which is a widespread kind of neurosis, was carried out [42]. This disease is a natural model of chronic psychoemotional stress. These patients show a distinct tendency to a decrease of pain thresholds, a lowered adaptation to repeated stimuli and a sensitization to pain perception.

7.2. Diagnostics of dermatological diseases

It is known that the state of the skin is related, in particular, with the function of subcutaneous neural structures and, first of all, of sensitive apparatus of the somatosensory system. Therefore, it was of interest to investigate thresholds of tactile sensitivity in patients with dermatosis on unaffected sites of the skin.

To elicit tactile sensations, single pulses of focused ultrasound (the frequency of 1.7MHz) with the duration of 1ms has been used [43]. If the sensation was absent, the duration was increased up to 6ms and sometimes up to 12ms. Measurements of sensitivity thresholds were carried out in 12 normal persons and 42 patients with dermatoses, e.g., psoriasis, neurodermitis, sclerodermia, lymphoma cutis and others [43]. Thresholds in patients were measured only in the visually unchanged, unaffected parts of the skin. It was found that tactile thresholds in all patients were higher than in healthy persons. In some patients, tactile sensations with the duration of stimuli of 1ms did not appear, in these cases the duration was increased up to 6 and 12ms. In the last cases, patients perceived not pure tactile but mixed sensation with thermal and even unpleasant pre-pain component. A patient with lymphoma cutis did not perceive a tactile sensation even after impact with the duration of 12ms. In some patients the sensitivity patterns have changed. Because the thresholds were measured at visually unaffected skin, their increase and change of sensitivity patterns could be evaluates as an indication of neurological disorders relating with the somatosensory system. Thus, the use of ultrasound method of stimulation of receptor structures allows revealing the degree of participation of somatosensory system in various pathological processes in skin.

7.3. Estimation the regeneration of bone tissues after fractures

The method was successfully tested also in orthopedics for estimating the regeneration of the bone tissues after fractures by measuring the dynamics of the pain thresholds in the periosteum (the data have not been published). The thresholds in the region of the fracture were essentially higher than at the normal periosteum. The normalization of the thresholds indicated successful bone regeneration.

7.4. Evaluation of the efficiency of anesthetic and analgesic drugs

An idea to use stimuli of focused ultrasound for evaluation of the efficiency of anesthetic and analgesic drugs by measuring pain threshold before and after drug administration seems self-evident [23, 30].

One example of application of this approach has been demonstrated [23, 25, 44]. The joint pain model was used in an assessment of the analgesic action of pethidine. The progressive diminution in the amplitude of the evoked potential over a period of 3.5h following the administration of the drug showed a significant correlation with the corresponding visual analogue scale score [44]. In view of this correlation, and since the intensity of the stimuli over the period of the trial was not altered, it was suggested that the evoked potential provided a reliable, objective measure of the perception of pain [44].

Restoration of the amplitude of the evoked potential to its original, pre-drug value by increasing parameters of a focused ultrasound stimulus would suggest that the value of those parameters that were required to achieve the threshold of pain had also increased. The measurement of those parameters in clinical environments could provide a quantitative measure of the sensitivity of diseased or damaged joints and could be used to follow the progress of ameliorative therapy [23, 25].

7.5. Effects on human acupuncture points

The accumulated knowledge of somatic receptive function was a pre-requisite for studies in which the sensitivity of acupuncture points and arbitrarily chosen sites was compared [45]. Activation of these points was carried out by single rectangular pulses of focused ultrasound (frequency of 2.67MHz) with the duration of 1ms for the study of tactile sensitivity and 30ms for investigation of pain sensations. Since corporal acupuncture points are located at various subcutaneous distances, for comparison were also chosen deep-located regions. The comparison showed that acupuncture points displayed mainly the sensation of deep pain, and the other points demonstrated in addition deep tactile sensations [45]. Tactile sensations in acupuncture points arose with higher thresholds and had a light shade of pain. The differences in the sensitivity to pain between acupuncture points and arbitrarily chosen ones were not revealed. Only in acupuncture points pain has not any previous tactile sensations. It was assumed that in the acupuncture points there was a greater preponderance of receptor structures related with pain in comparison with usual points.

Similar comparative studies of the skin of the earlobe did not reveal different sensitivities in the acupuncture points in comparison with arbitrarily chosen skin sites. It could be concluded from the results that auricular acupuncture points are situated, just as corporal ones, subcutaneously. With an increase in the duration of the ultrasound stimulus, the threshold of the sensations evoked by them was decreased [46].

7.6. Principle of the development of an ultrasound tactile display

It is known that there are mechanical tactile displays allowing blind and even deaf-and-blind persons to perceive textual information displayed by a relief-dot font. Small pins which rise and fall in order to form a symbol are commonly used in these devices. Letters are depicted in Braille system, which allows creating the equivalent of a printed symbol using from six to eight dots. To depict Braille's symbols at a display is much easier than the letters of the Latin or Cyrillic alphabet. However, such displays have drawbacks and limitations. The devices in which the pins are moved mechanically are noisy and require a direct contact between the skin of the subject and the pins, and the speed of the change of "images" at the display is very limited.

In [47], a principle of the development of an ultrasound tactile display has been offered. A main idea was to use multi-elements two-dimensional phased arrays with random distribution of elements to stimulate receptor structures in human fingers. Ultrasonic tactile displays can have potential advantages: they are noiseless, contact less, and provide a high update rate on the display. Symbols with a complex configuration (letters, digits, punctuation marks, etc.) may be created on the display.

For confirmation of workability this approach the calculations have been carried with the use of a virtual 2D phased array with the surfaces in the shape of a part of a spherical shell with a curvature radius of 60mm. The array diameter was 65mm and the ultrasonic frequency was 3.0MHz. The arrays consisted of 256 flat elements in the form of discs with a diameter of 2.5mm which were randomly distributed on the array surface. The detailed analysis of methods of calculations of two-dimensional phased array is presented in a number of papers [9, 48, 49].

It was shown that the use of such arrays allows to synthesize focal regions with complex configurations, in particular, in the form of the letters of the alphabet. The distributions of the intensity in the focal plane corresponding to two letters (S and W) are shown in Figure 9 [47]. To create these symbols, 24 and 25 foci, respectively, were used. The sizes of the fields where the simulations were carried out were $4 \times 4\text{cm}$ (a) and $1 \times 1\text{cm}$ (b).

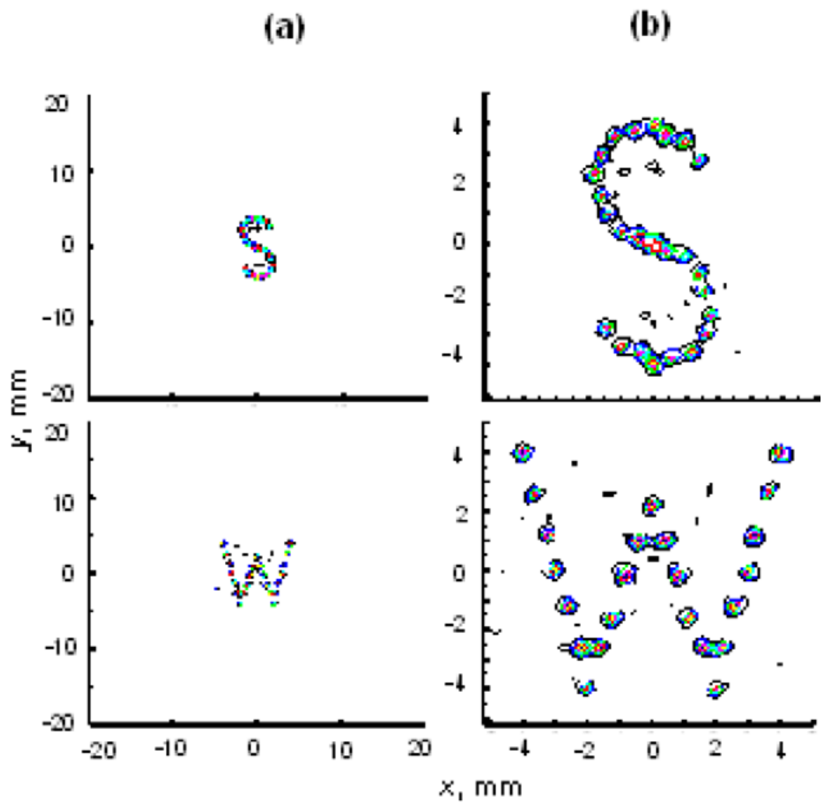


Figure 9. Synthesis of complex symbols in the form of some Latin letters, by a random array. The dimensions of the field under investigation are (a) $4 \times 4\text{cm}$ and (b) $1 \times 1\text{cm}$ [47].

Although the feasibility of implementing ultrasonic displays to image printed symbols on the display instead of their Braille equivalents is a subject of a separate study, the technical possibility of creating such devices has been proven [47].

Another possible application of such systems for realization of the stimulating effect of ultrasound can be its use for the development and implementation of new robotic techniques and systems, sensors, automated control systems, and also for creation of "human-machine" interfaces based on the use of tactile sensations. Such the systems, unconditionally, need in the development and improvement. Several authors suggest that the use of similar phased arrays is very promising for generating multiple-foci affecting regions with complex configurations in order to activate and change a functional state of neural structures [50, 51].

7.7. Diagnostics of hearing disorders

The diagnostics of hearing diseases now includes such useful and wide spread methods as pure-tone audiometry, investigation of the stapedial reflexes, brain stem evoked response audiometry, computed tomography and many others. The well-known audiometric methods based on the measurements of bone and air conductions are still playing a notable role in the complex diagnostics of hearing disorders. In the middle of 1980s, it was suggested a new modification of audiometric methods based on the use of amplitude-modulated focused ultrasound for stimulation of the ear labyrinth [27, 52, 53]. One of the main aims of this research was to compare in human clinical trials so called “ultrasonic audiograms” with the classical ones. It was supposed that the propagation paths of an auditory signal to the ear labyrinth in cases of the usual auditory stimulation and amplitude-modulated ultrasound stimulation may be essentially different. Consequently, the frequency-threshold curves obtained by these two methods should also differ. These differences could become a valid diagnostic indicator of various hearing diseases.

The clinical investigations were carried out in a long-term cooperation with the Leningrad Institute of the Ear, Throat, Nose, and Speech Disorders. In addition to study of fundamental problems of hearing physiology, some original, new methods of diagnostics, treatment and prognosis of the pathological process for different forms of hearing and vestibular function disorders were elaborated.

In this study, a single element focused radiators were used. The focal distance was 70mm, and the convergence angle was 30°. The operation frequency of different transducers was varied from 0.4 to 3.7MHz, although most of the studies, including those described below, employed 2.5MHz as the carrier frequency. As a matter of fact, similar results could be achieved using any other carrier frequency from the above-mentioned range. The diameter of the focal region in the main diffraction maximum of the beam for 2.5MHz was 1.5mm, the length of the focal region was 9mm and the area of the focal spot was $1.75 mm^2$.

The method has described in details in Section 3. Precise location and controllable acoustical matching of the focusing radiator to the ear labyrinth was ensured with the use of a coordination system (see Figure 5). The subject's head was placed on a pillow so that the acoustic axis of the radiator was perpendicular to the skin in front of the external ear. The focus pointer was set to a projection point which was marked at the middle of the distance between the posterosuperior edge of the mandibular joint and the vertex of the angle formed by the anterior and superior walls of the external auditory meatus. The pointer was then removed and the polyethylene bag was lowered until it was in tight contact with the skin. To improve the quality of the acoustic contact between the radiator and the target, the surface of the bag was smeared with a thin layer of Vaseline or a special gel. Thereafter, the radiator was moved 30-35mm closer to the skin by means of the coordination device. This depth of the focal region corresponds to the distance between the projection point and the cochlea as measured on human skull preparations.

It is clear however that in fact there is no precise focusing of ultrasound in the ear labyrinth because the skull bone is a very unfavorable medium for propagation of focused ultrasound, because the attenuation of ultrasound in bones is very high and the sound velocity in bone is more than twice as great as in water. Practically, it is important to bring in coincidence the direction of the acoustic axis of a focused radiator with the ear labyrinth. In this case the thresholds for hearing were minimal. Ultrasound was amplitude-modulated by a sinusoidal sound signals at standard audiometric octave frequencies in the range of 125-8000Hz, as well as by 50-100µs pulses with the same repetition frequencies.

At first, hearing thresholds were measured in a large group of people with normal hearing (50 persons aged from 20 to 45). An averaged ultrasonic frequency-threshold curve (threshold as a function of modulation frequency) is shown in Figure 10 [53].

As it known, a standard audiogram is presented in a form of chart that shows the audible thresholds for standardized frequencies as measured by an audiometer. The vertical axis represents the intensity measured in decibels and the horizontal axis represents different sound frequencies in Hz. Because one of the main aims of this study was to compare so called "ultrasonic audiograms" with the classical ones, it was necessary to define how the level of hearing loss should be expressed in the case of the use of ultrasound method. In Section 5, we have shown that the most probable factor responsible for inducing auditory sensations near the thresholds of perception was the radiation pressure of ultrasound which is proportional to the intensity of ultrasound. So, comparing in this case values of ultrasound intensities, one will compares, in fact, the values of the radiation pressure. Thus, the level of hearing loss was defined as 20 log of the ratio of the threshold intensity in patients with hearing disorders to the average threshold for persons with normal hearing, which has been shown in Figure 10.

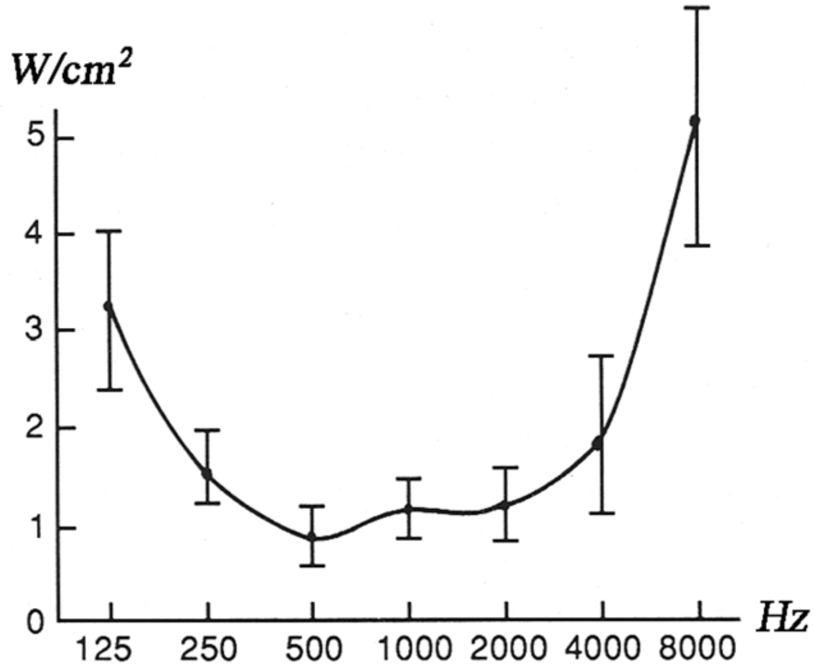


Figure 10. Averaged ultrasonic frequency-threshold curve for persons with normal hearing. The horizontal axis shows frequency of amplitude modulations in Hz, the vertical axis is the ultrasound intensity averaged over the focal spot area in W/cm^2 (according to measurements in water). Vertical lines correspond to mean square deviation of the thresholds [53].

Levels corresponding to degrees of hearing loss were marked on the same graph used to plot the conventional audiograms for air and bone conduction. In tonal audiograms, the tone frequency was plotted on the horizontal axis and the degree of hearing loss (in dB), on the vertical axis. In ultrasonic audiograms, the modulation frequency was plotted on the horizontal axis. Thus, three threshold-frequency curves were on the audiogram chart: two tonal audiograms (for the air and bone conduction) and an ultrasound audiogram. A comparison of tonal and ultrasonic audiograms for patients revealed some typical diagnostic indications of various hearing diseases.

The essences of the methods used in these studies, typical examples of tonal and ultrasonic audiograms for various hearing disorders, and also diagnostic criteria that allow diseases to be revealed by comparing and analyzing ultrasonic and tonal audiograms are presented in [53]. To the time of publishing of this work, over 500 patients with various peripheral hearing disorders have been examined with the use of this method, including 60 patients with sensorineural deafness, 40 with unilateral deafness and other cases of deafness. Of the 100 examined patients with otosclerosis and 40 patients with chronic otitis media, 36 and 12 patients, respectively, were diagnosed before the operation to improve hearing. The ultrasonic examination of those patients was repeated after the surgical intervention.

Among 250 patients suffering from usually unilateral hearing disturbances, suspected retrolabyrinthine hearing impairment and with an unclear diagnosis, 16 were selected as suspects for early acoustic neurinoma. Subsequent operations in all 16 cases confirmed the diagnosis [53]. In this work, typical tonal and ultrasonic audiograms for different hearing diseases (e.g., sensorineural hearing loss, otosclerosis, chronic otitis media, acoustic neurinoma, etc.) were presented. It was especially important that for different kinds of pathology the shape of the ultrasound frequency-threshold curves was essentially different, but for the same disorder the shape of ultrasound audiograms was quite similar.

As an example, the method of diagnostics of otosclerosis is described below. First of all, it was shown that ultrasonic audiograms of patients with this disease were different from their tonal audiograms [52, 53].

Thresholds in response to amplitude-modulated ultrasound were recorded when the air conduction was reduced by 45-50dB. In cases of greater hearing loss ultrasound stimuli caused no hearing sensations. A typical characteristic of otosclerosis was the absence of hearing sensation at one or several modulation frequencies of ultrasound, whereas at the other frequencies the sensitivity remained the same (Figure 11).

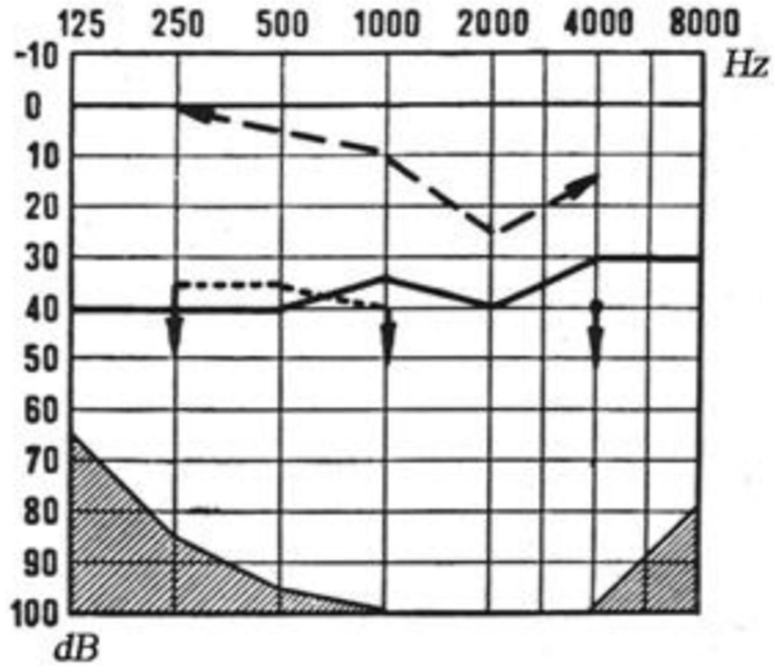


Figure 11. Tonal and ultrasonic audiograms of a female patient aged 45 with otosclerosis. Solid line is an air conduction audiogram; dashed line is a bone conduction audiogram, and dotted line is an ultrasonic audiogram of the patient. Horizontal axis is a frequency of tone or of ultrasound modulation in Hz; vertical axis is a degree of hearing loss relative to normal hearing in dB. Arrows mean that no measurements were carried out at the lower (or higher) frequencies. Hearing sensations at modulation frequencies of 125, 2000, and 8000Hz are absent [53].

The thresholds for ultrasound were usually between air and bone conduction thresholds or closer to air conduction thresholds. It is important to note that the absence of sensations in response to amplitude-modulated ultrasound at some modulation frequency did not depend on the degree of hearing loss for tonal stimuli at this frequency and, consequently, on the state of the receptor apparatus. After a successful surgical intervention, the sensitivity to modulated ultrasound was restored and the ultrasonic audiogram became more similar to the tonal audiogram.

These studies, as well as other ones with various diseases, have indicated that there are quite clear diagnostic signs or criteria of various hearing disorders based on the comparison of tonal and ultrasonic audiograms. Focused amplitude-modulated ultrasound allows to distinguish between conductive and perception hearing loss. Furthermore, it has become possible to make a differential diagnostics of damage to the receptors and to acoustic nerve structures, thereby broadening the diagnostic potential of audiometry. It has been shown that even in cases of conductive hearing loss when conventional audiometry is quite efficient as a diagnostic tool, the ultrasonic method improves reliability of audiometric examination.

As to the safety of application of ultrasonic method for diagnostics of hearing diseases, it should be noticed that for normal hearing persons the threshold values of the acoustic power of a transducer were not higher than 0.1-0.2W. Corresponding values of the intensity in the region of the projection point on the patient's skin were not higher than $0.02\text{-}0.05\mathrm{W/cm}^2$ [53]. The possible values of an ultrasonic intensity in deeper tissues could not be calculated even approximately due to uncontrolled defocusing of the ultrasonic beam in bone tissue and the very high attenuation of ultrasound in it (up to 20-30dB/cm at 2.5MHz). During the investigations of patients with hearing disorders, the ultrasound levels may be much higher than the above-mentioned values. But even in these cases, the intensity in the region of the projection point on skin was not higher than $1.5\mathrm{W/cm}^2$. This value of the intensity is known to be used widely in physiotherapy. The duration of our diagnostic tests is much shorter than those which are used in physiotherapy. So it may be assumed that ultrasound examination of hearing is essentially harmless for patients. At least our investigations concerning morphological, physiological and histochemical changes [27] under ultrasonic action showed that presently there are no insuperable obstacles on the way of the practical use of focused ultrasound for neural structures stimulation. At the same time, the potential hazards of prolonged exposure to ultrasound (e.g., in hearing training or prosthesis) should be a matter of further investigations.

To the end of 1980s, when these works were terminated, a number of diagnosed patients essentially increased and that allowed evaluating the diagnostic effectiveness of the developed method. Among the patients, 488 had chronic cochleaneural hearing loss, 122 had acute cochleaneural hearing loss, 230 had otosclerosis and 70 had Meniere's disease. For patients with cochleaneural hearing loss, the accuracy of diagnostic and prognostic tests approached the absolute level (near 100%), for otosclerosis 98% and for Meniere's disease 76%. If the ultrasonic tests were combined with complex clinical investigations, the trustworthiness of the diagnostics approached to 100%; i.e., the diagnosis was highly reliable. Thus, the preliminary results of the use of focused ultrasound in clinical practice for the stimulation of neural structures and diagnostics of hearing disorders look hopeful and promising.

7.8. Possibilities of ultrasound hearing prosthetics

It is worth to discuss the possibilities of audio prosthetics for the deaf people using amplitude-modulated ultrasound. It is well known that 3-5% of the population in developed countries suffer from deafness and impaired hearing.

To fight against this disease, in addition to new types of surgery and drug treatment, improved models of hearing aids based on the principle of amplification of sound signals are being developed. However, the existing tools cannot help or are insufficient for a large group of practically deaf people. For example, some patients with sudden bilateral deafness, whose receptor system is diagnosed to have been destroyed, perceived auditory information delivered by amplitude-modulated ultrasound, whereas standard hearing aids did not help them [10]. In order to help such patients, an attempt to use ultrasonic technique for audio prosthetics of the deaf people was undertaken at the Leningrad Institute of Ear, Nose, Throat, and Speech. In the late 1980s, a few promising experiments in this area were carried out. We believe that ultrasonic audio prosthetics may be effective in cases of hearing loss or deafness with partial or complete loss of receptor elements, when the auditory nerve fibers, by which the auditory information is usually transmitted from hair cells to the brain, remain intact. At present, auditory prosthesis of such patients is carried out by implanting stimulating electrodes in a region with surviving auditory nerve fibers. In contrast to this method, the input of auditory information to the deaf using focused ultrasound is noninvasive and does not require complicated surgery.

The proposed method may be also useful to reveal those patients whose hearing function might be successfully improved by electrical cochlear implants.

7.9. Obstacles and limitations in the practical applications of ultrasound stimulation of peripheral nervous structures

Possible practical applications of HIFU for stimulation of the peripheral nervous systems have no special obstacles or limitations hampering the use of focused-ultrasound stimulation technique for clinical applications. In fact, the limitations of focused ultrasound as artificial stimulus are defined by very general limitations of ultrasound: its inability to propagate through the media containing air and a risk of damage of biological tissues at wrong regimes of its application. In this regard, the technology of ultrasound stimulation of peripheral nervous structures differs favorably from well-known methods of ultrasonic neuromodulation of deep brain structures. Indeed, the possible sites of ultrasound stimulations (i.e., peripheral structures of humans or animals) do not have such vital importance for living beings as the brain structures. The only question that remains opened to date is whether there is any risk associated with long use of this method for diagnostic and therapeutic applications.

Some curious data on the long-term ultrasound irradiation of the hearing organ were obtained in the study of auditory perception of amplitude-modulated focused ultrasound carried out by a person with normal hearing (one of the leading participants of our studies). In this case, the ultrasonic intensity in the focal region was from units to tens of

W/cm^2 (data for water, i.e., without attenuation and defocusing), and the duration of exposure reached several minutes at each session. No changes in the hearing sensitivity of this person were observed after more than 10 years of his permanent participation in the experiments.

Qualitatively similar results were obtained when the same person several times a week measured thresholds of tactile and pain sensations in a specially marked point on the palm of his hand with the use of the method described in Section 3. The values of the thresholds of these sensations remained unchanged for almost 20 years such measurements.

However, it is still unknown whether a daily and long, sometimes lasting for many hours, action of focused ultrasound on ear labyrinth, necessary for hearing prosthetics, is harmless to the humans. It should be, however, noted that, during the first attempts to use electrode prosthesis for the deaf people, it was questionable how long patients could use the implanted electrodes. Now this is not disputed and the patients have been observed for decades. The problem of learning to use prosthesis or an ultrasonic device for the highest-quality reception of auditory information is common both to the electrode and ultrasonic prosthesis. Unfortunately, such studies have not been implemented.

8. Recent Studies of the Peripheral Nervous Structures Stimulation Carried out in Different Laboratories

During the last decade, investigations of ultrasound stimulation of peripheral nervous structures began to develop in a number of countries. Here a review of a part of them is presented. As already was mentioned, the works in the field of ultrasound neuromodulation of deep brain structures are out of the scope of this paper.

Several investigators studied nerve conduction block *in vitro* and *in vivo*. Researchers from Taiwan have studied the effect of ultrasound on the conductive properties of bullfrog sciatic nerve fibers *in vitro* [54]. Ultrasound with a frequency of 3.5MHz and an acoustic power of 1-5W was applied for 5min. The amplitude of evoked potentials and the velocity of nerve impulses before and after the ultrasound treatment were measured. The velocity of nerve impulses was increased by 5-20% at acoustic powers of 1-3W. The amplitude of evoked potentials increased by 8% during stimulation with an acoustic power of 1W; however, it significantly decreased at powers of 2 and 3W. The authors explained this inhibitory effect by the thermal action of ultrasound.

Foley et al. [55] used high-intensity focused ultrasound (HIFU) to treat spasticity (i.e., involuntary muscle tension) and pain associated with it. They produced a permanent conduction block of the nerve and suppression of its function by thermally coagulating sciatic nerves in rabbits *in vivo*. A 3.2-MHz focused transducer with a focal acoustic intensity of 1480 to 1850W/cm^2 and 36 ± 14 seconds of sonication was used in these experiments. Histologic observation of the nerve indicated the axonal demyelination and the necrosis of Schwann cells as probable mechanisms of nerve block. Authors believe that with accurate localization and targeting of peripheral nerves using ultrasound imaging, HIFU could become a promising tool for the suppression of spasticity.

In the following work [56], the influence of some parameters of focused ultrasound (intensity and duration of exposure) on the conductivity of the sciatic nerve in rats was studied *in vivo*. The goal was to determine the doses required for a partial and full blocking of the nerve conduction and, thus, to investigate the possibility of using ultrasound as an alternative to current clinical methods for blocking the nerve conductivity. Focused ultrasound with a frequency of 5.7MHz with peak intensities from 390 to 7890W/cm^2 was applied for 5s. Evoked responses of the muscles to the electrical stimulation of the nerve in combination with the ultrasonic effect were recorded in the muscle tissue before and immediately, 2, and 4 hours after ultrasonic treatment. In the range of intensities from 390 to 3300W/cm^2, the amplitude of the responses was reduced from 4h to 7 days after the exposure and returned to its original value 28 days after the treatment. For the maximal intensity of $7890 W/cm^2$, the responses were absent 28 days after the treatment. The authors believe that their data may be useful when using a powerful focused ultrasound for treatment of severe muscle pain and spasticity.

The possibility of using focused ultrasound for blocking nerve conductivity in order to control pain and create local anesthesia was studied by another group [57]. The sciatic nerve of a bullfrog *in vitro* in a Ringer's solution was used as an object of study. Action potentials were recorded during electrical stimulation of the nerve and repeatedly recorded after the impact of focused ultrasound with frequencies of 0.661 and 1.986MHz on the nerve. The action potential decreased, which correlated with an increase in temperature measured in the nerve. Depending on the parameters of the ultrasonic irradiation, action potentials recovered fully, partially, or not at all. Cooling of the liquid that surrounded the nerve did not prevent blocking the action potentials; however, more powerful ultrasound was needed to achieve the effect of blocking than that without cooling. This means that a constant or temporary thermal action is required for blocking of the nerve. According to the authors, when focused ultrasound of high frequencies is used, there is no need to search for explanations of the obtained effects any other reasons, besides the heat. However, when lower frequencies are used, some other non-thermal mechanism is expected to participate. This mechanism is not completely clear; it may be related to the activity of cavitation bubbles in that particular experimental conditions. In the conditions *in vivo*, this effect may not occur.

It is known that patients with diabetic neuropathy often suffer from neuropathic pain. Lee et al. [58, 59] investigated recently the effects of HIFU on the conduction blockade of normal and neuropathic nerves *in vitro*. These works were the first to study the possibility to use ultrasound for nerve conduction blocking in diabetic nerves. The focused radiator was a spherical bowl with an aperture of 6cm, radius of curvature of 5cm and the frequency of 2.68MHz. Adult male Sprague-Dawley rats were used, and diabetes was induced by streptozotocin injection. Diabetic neuropathy was evaluated with animal behavior tests. Stimulation and recording of the compound action potentials (CAPs) and sensory action potentials (SAPs) were performed. It was shown that the sensory conduction of sciatic nerves excised from rats with diabetic polyneuropathy can be blocked partially and temporarily by HIFU treatment. For continuous wave exposure, the intensity I_{SATA} at the focal region was equal to $1210\mathrm{W/cm}^2$. In burst sonications, the burst had a length of 50ms and a repetition frequency of 10Hz (duty cycle = 50%); I_{SATA} values of 3160 and $1850\mathrm{W/cm}^2$ were used for the 4- and 8-wk after injections, respectively. The duration of sonications was typically 3-10s. For the same nerve, CAPs and SAPs were recorded once per second during the sonication. After the sonication, CAPs and SAPs were acquired once every 2 min in the early phase, then every 5min in the middle phase and every 10 min in the last phase. The total recording time was 2h. For the control and neuropathic nerves, suppression of CAPs and SAPs started 2 min after HIFU treatment. Maximum suppression of SAPs was 34.4 ± 3.2% for the control rats and 11.6 ± 2.0% and 9.8 ± 3.0% for rats 4wk post-injection and 8wk post-injection, respectively. Time to full recovery was 25, 70, and 80 min, respectively. The blocking effect increases gradually as time increases until the minimum degree of suppression was reached. Then, the blockade effect disappears gradually until the 100% of the degree of recovery. The suppression lasts at least 1h. Control and neuropathic nerves exposed or not exposed to HIFU were submitted to a histologic analysis. It revealed, for example, that the nerves in which CAPs and SAPs did not fully recover were damaged thermally or mechanically by HIFU. It was obtained that sensory fibers were more vulnerable to the thermal effects of HIFU than the motor myelinated fibers; thus, the energy should be selected carefully. The results suggest that HIFU may have potential to block sensory nerves reversibly and provide peripheral pain relief. The results revealed several possibilities for future research. For example, an *in vivo* study is required to confirm the *in vitro* findings. Then, it is important to perform a reversible blockade of sensory fibers without damaging motor fibers.

Alhamami et al. [60] studied effects of HIFU with different acoustic doses on neural tissues *in vitro*. Focused transducer with a resonance frequency of 2.2MHz was used. The diameter of the transducer was 5cm and its radius of curvature was 7.5cm. For low- and medium-level acoustic doses treatments, a ventral nerve cord was excised from a marine lobster and placed on a special chamber to measure its compound action potential before and after exposure to HIFU using an electrophysiology system. In the low-level acoustic dose treatment, the spatial-peak temporal average intensity (I_{SPTA}) was around 3.3W/cm^2 and the sonication time was 10 seconds. In the medium-level acoustic dose treatment, the intensity (I_{SPTA}) was around 13.3W/cm^2 and the sonication time was 10 seconds. For treatments with high levels of acoustic dose, the lobster's ventral nerve cord was sandwiched between the nerve chamber and an *in vitro* chicken breast tissue, a scenario that resembles *in vivo* experiments. The sonication time for high-level acoustic dose therapy was 5 seconds and the I_{SPTA} was around 5500W/cm^2. At low-level acoustic dose, the amplitude of the action potential increased by 18.0% after a 10-second HIFU exposure of about 3.3W/cm^2. At a medium-level acoustic dose, the amplitude of the action potential decreased by 5.4% following a 10-second HIFU exposure of 13.3W/cm^2. A greater suppression in the nerve action potential amplitude was achieved at the high-level acoustic dose. A 5-second HIFU exposure of 5500W/cm^2 resulted in a 57.8% decrease in the amplitude of the action potential. Moreover, an examination of the chicken breast and neural tissues subjected to the high-level acoustic dose reveals a discoloration and coagulative necrosis in a localized volume where both tissues meet. Decrease in the nerve action potential amplitude after HIFU treatment at high and medium acoustic dose levels demonstrates the ability of HIFU to induce a nerve conduction block primarily due to its thermal mechanism, which is more pronounced at high acoustic dose levels. On the other hand, increase in the nerve action potential amplitude after HIFU treatment at low-level acoustic dose demonstrates the ability of

HIFU to stimulate neural tissues primarily due to its mechanical mechanism (non-thermal effect). Results of this study demonstrate the great advantage of HIFU as a non-invasive and localized acoustic therapy with promising applications in neurology, neurosurgery, anesthesiology and pain management [60].

Wahab et al. [61] studied how ultrasound pulses affect nerves through mechanisms that are neither thermal nor cavitational, and investigate how the effects are related to a radiation force impulse. A simple neural model consisting of the giant axon of a live earthworm was exposed to trains of pressure pulses produced by an 825kHz focused ultrasound transducer. The peak negative pressure of the pulses and duty cycle of the pulse train were controlled so that neither cavitation nor significant temperature rise occurred. The amplitude and conduction velocity of action-potentials triggered in the worm were measured as the magnitude of the pulses and a number of pulses in the pulse trains were varied. It was obtained that the functionality of the axons decreased due to noncavitational mechanical effects. The radiation force, possibly by inducing changes in ion-channel permeability, appears to be a possible mechanism for explaining the observed degradation. The radiation force impulse is also a promising parameter for quantifying neural bioeffects during exposure, and for predicting axon functionality.

Kim et al. [62] showed the opportunity of application of low-intensity transcranial focused ultrasound to selectively stimulate the rat abducens nerve located above the base of the skull. Focused ultrasound (frequencies of 350kHz and 650kHz) operating in a pulsed mode was applied to the abducens nerve of Sprague-Dawley rats under stereotactic guidance. The abductive eyeball movement ipsilateral to the side of sonication was observed at 350kHz, using the 0.36-ms tone burst duration, 1.5-kHz pulse repetition frequency, and the overall sonication duration of 200ms. Histologic and behavioural monitoring showed no signs of disruption in the blood brain barrier, as well as no damage to the nerves and adjacent brain tissue resulting from the sonication. As a novel functional neuromodulatory modality, the pulsed application of focused ultrasound has potential for diagnostic and therapeutic applications in diseases of the peripheral nervous system.

A series of following works were fulfilled at the University of Washington, Seattle, by the group led by Prof. Mourad. Dickey et al. [63] have demonstrated that intense focused ultrasound can reliably induce sensations in human test subjects in a manner correlated with the density of their mechanoreceptors. Referring to the previous works of our group [22-25, 30], investigators checked and proved the hypothesis that the intensity of focused ultrasound necessary to induce sensations should depend on the density of mechanoreceptors. They applied focused ultrasound at 1.1MHz for 0.1s to the fingertip pads of 17 testees in a blinded fashion and escalated intensities until they consistently observed ultrasound-induced sensations. Most test subjects achieved high values of sensitivity and specificity, doing so at values of spatially and temporally averaged intensity 100W/cm^2. Of interest for future research may be an obtained correlation of the density of mechanoreceptors and the intensity of ultrasound necessary to generate sensations with high sensitivity and specificity, given the variable density of mechanoreceptors throughout the human body, both cutaneous and at depth.

In the second work [64], authors tested the hypothesis that neuropathic tissue is more sensitive to stimulation by intense focused ultrasound than control tissue. They created a diffusely neuropathic paw in rats via partial ligation of the sciatic nerve, whose sensitivity to ultrasound stimulation was compared with sham-surgery and normal control paws. Then increasing amounts of focused ultrasound (individual pulses of 0.2s at 1.15MHz) was applied to the rats' paws, assaying for their reliable withdrawal from that stimulation. Neuropathic rats preferentially withdrew their injured paw from focused ultrasound at smaller values of the intensity $(84.2\text{W/cm}^2 \pm 25.5)$ than did sham surgery $(97.7\text{W/cm}^2 \pm 11.9)$ and normal control (223W/cm^2) animals, with greater sensitivity and specificity (85% for neuropathic rats and 50% each of sham surgery and normal control rats). Authors consider that "these results directly support our hypothesis as well as Gavrilov's idea that doctors may someday use focused ultrasound stimulation to diagnose patients with neuropathies".

It will be shown in Subsections 7.1 and 7.2 that focused ultrasound actually has been used successfully in medical practice for diagnostics of different neurological and skin diseases.

In the next study [65], authors evaluated the usefulness of intense focused ultrasound, already shown to generate sensations and other biological effects deep to the skin, as a means of quantifying deep diurnal pain using a standard animal model of inflammation. It was found that the night group's threshold for reliable withdrawal of the stimulated hind paw to be significantly higher than that of the day group as assayed by each focused ultrasound protocol. These results are consistent with the observation that the responses to mechanical stimuli by humans and rodents display diurnal variations, as well as the ability of focused ultrasound to generate sensations via mechanical stimulation. Since focused ultrasound can provide a consistent method to quantify pain from deep, inflamed tissue, it may represent a useful adjunct to those studying diurnal pain associated with deep tissue as well as chronotherapeutics targeting that pain.

The same group of investigators [66] has tested the hypothesis that focused ultrasound can differentiate focal and subcutaneous neuropathic tissue from control tissue using a rat model of a neuroma. The work constitutes the first of several steps necessary for achieving the goal of using focused ultrasound to localize focal painful tissue pathology within patients. Authors used intense focused ultrasound (2MHz, with individual pulses of 0.1s in duration) for irradiation of the rat's neuroma while the rat was under light anesthesia. They started with low values of the intensity, which were increased until intense focused ultrasound stimulation caused the rat to reliably flick its paw. Then the same intense focused ultrasound was applied to control tissue away from the neuroma and assayed for the rat's response to that stimulation. Intense focused ultrasound of sufficient intensity (I_{SATA} of $600 +/- 160\text{W/cm}^2$) applied to the neuroma caused the rat to flick its paw, while the same intense focused ultrasound applied millimeters to a centimeter away failed to induce a paw flick. The results suggest that using focused ultrasound under image guidance to locate neuropathic damage may one day help guide diagnoses, hence therapy, for patients with chronic back pain, amputees, and cancer patients for tracking the efficacy of pain management.

In the following paper [67], the same group of investigators tested an idea that successively applied pulses of intensive focused ultrasound will generate sensation in inflamed tissue at a lower intensity and dose than application of a single pulse. In the studies, they used individual pulses of intense focused ultrasound with the duration of 75ms and also a train consisted of five pulses, each with the duration of 75ms separated by 75ms during which ultrasound was off. The animal model of chronic inflammatory pain, created by injecting an irritant into the rat hind paw and applied in the previous paper [66], was used in these experiments. Focused ultrasound protocols consisting of successively and rapidly applied pulses elicited inflamed paw withdrawal at lower intensity and estimated tissue displacement values than single pulse protocols. However, both successively applied pulses and single pulses produced comparable threshold acoustic dose values and estimates of temperature increases. This raises the possibility that temperature increase contributed to paw withdrawal after ultrasound stimulation. Thus, the observed effects may be caused, at least for inflamed tissue, by a combination of mechanical effects via focal palpation and heat induction due to attenuation of ultrasound.

The studies of Muratore et al. are devoted to the possibility of inducing functional changes in the neuronal cells using small doses of ultrasound [68-70]. The objects of studies were cells of PC12 line *in vitro*. To test morphological changes in the culture of cells, pulses of focused ultrasound with a frequency of 4.67MHz, duration of 30ms, and amplitude of 100kPa were used. To control the functional changes, the cell culture from the hippocampus of the rat was excited by the pulses of focused ultrasound with a frequency of 4.04MHz, duration of 0.1ms, and amplitude of 77kPa. Before and after ultrasound treatment, the culture was stimulated using biphasic stimuli of electric current (100μA) with a duration of 0.1ms. Optical microscopy showed that, under the effect of ultrasound, cells of PC12 cultures that were clustered near the focal region elongated by approximately 2μm and, after the termination of the effect of ultrasound, they returned to their original shape. The authors suggest that a deformation of cells in the culture is caused by the action of the acoustic radiation force. In the rat hippocampal cultures, both electrical and ultrasonic stimuli exhibited similar biphasic waveforms. Furthermore, according to the electrical responses after ultrasound treatment, the culture remained viable. The authors believe that their experiments showed that ultrasound in small doses can stimulate the neurons; however, the mechanism of the effect remains unknown. In [70], it is noted that the threshold amplitude of the ultrasonic stimulus necessary for the emergence of this effect lies between a sound pressure of 20 and 48kPa. A possible fatigue was observed at the highest pressure (77kPa ≈ 0.8atm), which was the most intensive stimulus [70].

In the review of biological effects of ultrasound on peripheral nerves, Muratore and Vaitekunas [71] discussed the following general statements about the response of peripheral nerves to acoustic irradiation:

- The nervous system responds to irradiation over a wide range of acoustic parameters.
- It is not necessary to irradiate the entire length of a nerve and nerve fibers to evoke a response.
- Nerves exhibit a spectrum of responses to varying ultrasonic dose.
- Different fibers within a nerve respond differently to the same incident ultrasound beam.
- The mechanisms of biologically effective ultrasound irradiation of nerves at sub-ablation doses are not fully characterized.

Authors concluded that the sensitivity of the peripheral nervous system to ultrasound is remarkable. That sensitivity, which varies among the fibers within a nerve, and the ability to influence a nerve by affecting just a small portion of it, and to stimulate, inhibit, or irreversibly damage a nerve by changing the parameters of exposure, provide a complex set of possible clinical applications.

The group at Virginia Tech Carilion Research Institute carried out detailed research into somatosensory sensations induced by ultrasound [72]. They used an unfocused ultrasound transducer with a frequency of 0.35MHz and two regimes of ultrasound irradiation. The first one was intended to evoke a slight mechanical sensation, and another to elicit a thermal sensation. The parameters of irradiation in the first case were: the intensity I_{SPTA} was 11.8W/cm^2, pulse duration is 2ms, pulse repetition frequency is 70Hz and their number is 35 to produce the total time for stimulus duration of 0.5s. In the second case the parameters were: the intensity I_{SPTA} was 54.8W/cm^2, pulse duration is 10ms, pulse repetition frequency is 100Hz, a number of pulses is 100 to produce the total time for stimulus duration of 1.0s. Stimuli were delivered in alternating 50 event blocks to the right and left index fingers and counterbalanced across participants (20 subjects). Three blocks were delivered to each finger for a total of 150 stimulations per finger. Testing lasted approximately 1 hour and 15 minutes. Authors recorded evoked potentials using electroencephalography and also applied magnetic resonance imaging of blood oxygen level-dependent responses to fingertip stimulation with pulsed ultrasound. By changing the energy of the pulsed ultrasound stimulus, it was obtained that pulsed ultrasound can differentially activate somatosensory circuits. Authors concluded that an ability of pulsed ultrasound to functionally stimulate different somatosensory fibers and receptors may permit new approaches to the study and diagnostics of peripheral nerve injury, dysfunction, and disease.

British scientists used focused ultrasound for stimulation of peripheral nervous tissue [73]. An isolated *in vitro* peripheral nerve of a crab leg nerve bundle was chosen as a simple model to demonstrate and investigate the neurostimulatory effect. All the fibers in the bundle were unmyelinated. Authors used focused ultrasound with the frequency of 0.67MHz in a regime of short pulses with the repetition frequency of 10kHz, 50% duty cycle (i.e., the duration of a single pulse was 0.05ms), and with total stimulus duration of 8ms (80 pulses) with 2 seconds between each recording. The maximum intensity in the pulse was 260W/cm^2 I_{SPTP}. It is interesting that delivering a rapid pulse train of focused ultrasound directly into axonal tissue was able to produce significant excitation that only occurred in the first of 5 repeat stimuli. Authors suggest that their research has demonstrated the sensitivity of the *ex-vivo* peripheral nerve model to ultrasound stimulation, confirming that ultrasound neurostimulation mechanisms are not restricted to the structures of the CNS. An important characteristic of the crab leg nerve model is that all the fibers in the bundle are unmyelinated which may make them more susceptible to ultrasound stimulation than equivalent myelinated nerve fibers that have been tested previously in mammals and amphibians.

The separate direction of the research is related with the attempts to develop methods and device for artificial prosthesis of a degenerating retina. Scientists from Israel suggest [50] that the use of multi-element phased arrays is very promising for generating multi-foci affecting regions with complex configurations in order to activate and change the functional state of cortical and sub-cortical neural structures. To create appropriate devices and systems, the authors developed effective and fast algorithms for calculating the phase of the array elements necessary for the generation of multiple foci ultrasonic fields with given parameters of the foci. By combining the phased array and magnetic-resonance thermometry, it was experimentally demonstrated the simultaneous generation of tightly focused multifocal distributions in a tissue phantom. That is a first step towards the development of patterned ultrasound neuromodulation systems and devices. Authors believe that focused ultrasound directed onto neural structures is able to dynamically modulate their neural activity and excitability, opening up a range of possible systems and applications where the non-invasiveness, safety, mm-range resolution and other favourable characteristics of focused ultrasound are advantageous [50].

The goal of the following work of the same group of researchers [51] was to examine the general feasibility and properties of an acoustic retinal prosthesis, a new vision restoration strategy that will combine ultrasonic neurostimulation and ultrasonic field sculpting technology towards non-invasive artificial stimulation of surviving neurons in a degenerating retina. Authors applied the approach developed in the previous paper and used a miniature two-dimensional phased array for creation of an artificial prosthesis of a degenerating retina. A main unit of the device was a flat phased array (company InSightec LTD, Israel) with the sizes of $2 \times 4\text{cm}^2$, consisting of 987 elements. The array was positioned above a cornea, and affecting by ultrasound with the frequency of 0.5 and 1MHz was executed on a retina. The experiments carried out on rats have shown an occurrence of the visually evoked potentials in response to the pulsed ultrasonic stimulation. The range of parameters of the ultrasound stimulation used in animal experiments for the frequency of 1MHz was: the burst train duration of 10-20ms, single burst duration of 100μs, repetition frequency of 1667Hz, the peak instantaneous intensity of 10-17W/cm^2 and I_{SPPA} of 5.15-8.5W/cm^2. A device that is aimed at non-invasive patterned excitation of populations of retinal neurons using acoustic interference patterns projected from a multi-element phased ultrasonic array has been conceptually validated and analyzed. Authors believe that by means of the developed software and device, and also having in mind that the duration of a series of impulses for the effective stimulation of a retina is rather insignificant (approximately 10ms), it is possible to reach the speed of updating of the information on a retina of the order of tens images in a second. Although many questions about this technological framework remain open, the *in vivo* experiments and fulfilled analysis suggest that a low-acuity the acoustic retinal prosthesis with sub-mm resolution and intensities that comply with international ophthalmic safety guidelines appears to be feasible using frequencies in the lowMHz range. Moreover, a preliminary assessment showed no short-term damage to the retina, which appeared to remain functionally and morphologically intact. A prosthesis operating in the 2-10MHz range could potentially become an external, implant-less, alternative to existing implantable systems with a similar spatial resolution. Further research is clearly required to expand understanding of the mechanisms of ultrasound neuroactivation.

Menz et al. [74] from Stanford University used the isolated salamander retina to characterize the effect of ultrasound on an intact neural circuit and compared these effects with those of visual stimulation of the same retinal ganglion cells. Ultrasound stimuli at an acoustic frequency of 43MHz and a focal spot diameter of 90μm delivered from a piezoelectric transducer evoked stable responses with a temporal precision equal to strong visual responses but with shorter latency. The calculated time-averaged acoustic intensity was 10-30W/cm^2 for 50% duty cycle stimulus (1s on, 1s off) for most experiments. The 43MHz carrier frequency was modulated at low frequencies (0.5-15Hz) to match the temporal pattern used for visual stimulation. For most experiments, this consisted of 1s of stimulus on and 1s of stimulus off, repeated for many cycles, for a total duration of 1–5 min. By presenting ultrasound and visual stimulation together, authors found that ultrasonic stimulation rapidly modulated visual sensitivity but did not change visual temporal filtering. By combining pharmacology with ultrasound stimulation, it was found that ultrasound did not directly activate retinal ganglion cells but did in part activate interneurons beyond photoreceptors. These results suggest that, under conditions of strong localized stimulation, timing variability is largely influenced by cells beyond photoreceptors. It was concluded that ultrasonic stimulation is an effective and spatiotemporally precise method to activate the retina. Because the retina is the most accessible part of the central nervous system *in vivo*, ultrasonic stimulation may have diagnostic potential to probe remaining retinal function in cases of photoreceptor degeneration, and therapeutic potential for use in a retinal prosthesis. Authors consider that ultrasound neurostimulation, because of its noninvasive properties and spatiotemporal resolution, promises to be a useful tool to understand dynamic activity of neural pathways in the retina.

There are several comprehensive reviews of current states of the researches of focused ultrasound neuromodulation of neural structures [4-7] but all of them are devoted mainly to acoustic neuromodulation of structures of the central nervous system rather than to peripheral structures.

9. Conclusion

Let us discuss common and different features of adequate and ultrasound stimuli. In fact, the presence of sensations of cold produced by focused ultrasound stimulation allowed assuming that the nature of sensations resulting from ultrasonic stimulation of receptor structures is not always related with the action of adequate stimuli, because ultrasound obviously does not carry cold in itself. The above-mentioned data on taste sensations and reactions in the nerve fibers of skates obtained using focused ultrasound [33] also lead to the conclusion that activation of neural structures occurs due to the actions of one or several stimuli, which are not adequate for many sensations.

The studies of the mechanisms of stimulation effects of focused ultrasound (see Section 5) have led to a suggestion of rather simple, evident and reasonable mechanisms. In the case of inducing of hearing sensations such mechanism is based on arising of demodulated sounds oscillations in an amplitude-modulated ultrasound wave. For stimulation of tactile and temperature sensations (warmth, cold), the mechanism is probably related with unidirectional effect caused by the gradient of the mechanical displacement of the medium due to the radiation force. However, experimental facts show that only these factors could not explain clearly and uniquely all variety of observed effects of ultrasound stimulation.

An existence of significant difference between the adequate and ultrasound stimuli support the fact that clinical observations of some patients with full bilateral hearing loss confirmed audiologically were able to perceive auditory information transmitted by the amplitude-modulated focused ultrasound, while the usual hearing aids did not help them to hear [10] (see Section 6). Stimulating effect of the auditory fibers by means of amplitude-modulated focused ultrasound was confirmed by experiments with grass frogs (Rana temporaria) after destruction of their ear labyrinth receptor apparatus [40] (see Section 6). Usual sound signals, even of high intensity, are not able to induce such the effects.

In the case of stimulation of tactile and thermal sensations, there was suggested the mechanism of unidirectional action on neural structures caused by the gradient of the mechanical displacement of the medium due to the radiation force (see Section 5). In essence, this effect reduced to a deformation of a medium under the action of the gradient of the mechanical force. In some relations this effect is similar to one described for hearing stimulation. Because the effect reveals under the action of the gradient of the applied force, it is also appeared in the beginning and end of ultrasound stimulus. It was shown, for example, that investigated subjects could not distinguish one long pulse (for example, 400-ms duration) directed to a hand from two short pulses separated by the same time interval (see Section 5) [10, 30]. Thus, again the filling of a stimulus by oscillations of MHz-frequency did not influence on the character of the sensation if the duration of the stimulus was not too long. If, for example in the study of thermal sensations, the duration of stimulus was 100-500 ms and even more, the subject could perceive the sensation of warmth induced by temperature rise due to absorption of ultrasound energy in tissues.

The conclusion about a significant difference between the adequate and ultrasound stimuli agrees with the data of the study of ultrasound-induced temperature sensations (warmth, cold) (see Section 3). For example, estimations fulfilled with the use of Equation (11) and data from Table 3 shows that for the duration of ultrasound stimuli of 1ms in the frequency range of 0.48-2.67MHz, the temperature rises corresponding to induce in a person warmth and cold sensation are equal to 0.003-0.39°C and 0.007-0.39°C, respectively. It is evident that such the temperature rise is too insignificant to induce a warmth sensation and definitely has no relation to the origin of a cold sensation. So, in these cases ultrasound-induced sensations have nothing common with adequate stimuli. The main effective factors in these cases is probably related with the mentioned above mechanical displacement of the medium due to the radiation force (see Section 5), or, in the other words, with deformation of tissues under the action of the gradient of the mechanical force.

All the reasons mentioned above mean that a simple explanation of mechanisms of stimulating effects of focused ultrasound does not exist, and there is a necessity to continue research into this problem. Another conclusion is that ultrasonic and adequate stimulations have sometimes some common features (for example, in the case of stimulation of mechanoreceptors under the action of mechanical stimulus independently on their nature), but in majority of real applications these two kinds of stimuli have much more differences. By the way, an additional properties of ultrasound stimulation in comparison with an adequate one, gives to ultrasound methods of treatment and stimulation new perspective and possibilities of practical applications unknown till now.

In conclusion, it is worth to formulate the basic physiological findings obtained in studies using focused ultrasound as a tool for stimulation of neural structures [26].

- Ultrasound can activate not only peripheral receptor structures, but also nerve fibers.
- Temperature, tactile, and aural reception should be regarded as mechanoreception.
- Sensations of heat and cold depend on the activation of the same nerve fibers.

● Cutaneous pain is related to specific afferent nerve fibers (mostly for pain) or to nonspecific ones (for pain and other skin sensations).

● Studies show that the implementation of focused ultrasound as a stimulator of receptor structures is most promising in the following fields:

- Diagnostics based on the measurements of thresholds of sensations.

- Prosthesis of various sensory functions, such as auditory function.

- Selection of patients, the auditory function of which can be substantially improved by means of electrical implant prosthesis.

- Pain relief and therapy of various diseases by ultrasound effects on neural structures.

- Evaluation of the efficiency of anesthetic and analgesic drugs by measuring pain threshold before and after drug administrations.

- Investigation of the temperature sensitivity and the diagnosis of its disorders.

- Using two-dimensional phased arrays for activation of neural structures.

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