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USE OF FOCUSED ULTRASOUND FOR STIMULATION OF VARIOUS NEURAL STRUCTURES

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Contents

INTRODUCTION

An important task of physiology and medicine was and is searching for artificial stimuli that can activate neural structures noninvasively and locally and induce different sensations. 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 couldn't be considered as a noninvasive one because the electrodes need to be in a direct contact with an activated structure. The subject of this book is the description and analysis of research on application of focused ultrasound for stimulation of somatosensory, hearing and other neural structures.

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. When for guidance of ultrasound treatment the magnetic resonance imaging (or MRI) is used, the method is called Magnetic Resonance-guided Focused Ultrasound (MRgFUS or MRgHIFU). When for such guidance an ultrasound diagnostic technique is preferable, the method is called Ultrasound-guided Focused Ultrasound (USgFUS or USgHIFU). MRgHIFU is approved for the treatment of uterine fibroids in the USA, Canada, Europe, Israel, Asia, etc. The devices based on the application of focused ultrasound are used in dozens of medical centers for minimally invasive treatment of prostate. In several countries, first of all in China, tens of thousands clinical trials were carried out for the treatment of cancers of the liver and kidney, breast, pancreas, bone, etc. Wide experiments were carried out to investigate possibilities of applications of focused ultrasound for control bleeding, targeted drug delivery to specific sites of the body, liposuction (removal of unwanted fat), for neurosurgery, including brain therapy through an intact skull, for destruction of tissues located behind the rib cage, for cardiology, etc. All these possibilities of focused ultrasound have been described in details in numerous books and reviews.

The studies analyzed in this book are related mainly with research into application of focused ultrasound for activation of peripheral receptor structures. Pulses (stimuli) of focused ultrasound in the frequency range from 0.5 up to 3.5 MHz 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: (1) the method is noninvasive, since it doesn't require a destruction of tissues to access deep structures; (2) 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; (3) 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; and (4) it is possible to affect not only the superficial structures (e.g. located in the skin), but also tissues deeply located in the body. It was shown that 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 the book is investigations of the main affecting factors of focused ultrasound and mechanisms of its stimulating effects. The possibilities of the practical use of these effects including their applications in clinical medicine for diagnostics of different neurological, dermatological and hearing diseases are also presented in this book.

The results of all these studies, carried out in Russia from the beginning of 1970s and lasting up to nowadays, were published in several books and in dozens of articles and naturally, first of all, in Russian. So, at the moment, these publications are practically unknown to investigators in other countries and some of them have become a bibliographic rarity even in

Russia. This book allows to present at least a part of the obtained results and to acquaint with them the colleagues and other readers interested in this field of the science. One of the aims of this book was also to submit a review and analysis of numerous works carried out in many countries and, first of all, in the USA, regarding the use of focused ultrasound for reversible effects on different neural structures, including the brain structures. Such well-known effects as so called ultrasound neurostimulation and neuromodulation of the brain structures will be discussed, as well as advantages and limitations of focused ultrasound as a tool for neuromodulation of the central nervous system.

An essential part of Chapters 2-5 of this book is based on the results obtained with many colleagues and, first of all, with a long-term co-author Dr. Efim M. Tsirulnikov, Leading Research Scientist at the Sechenov Institute of Evolutionary Physiology and Biochemistry of the Russian Academy of Sciences. Author expresses his sincere appreciation and gratitude to him.

The book is intended, first of all, for specialists in medical ultrasound and physiology, particularly in research of somatosensory, hearing and other neural structures, in clinical and experimental medicine, and also in biophysics, medical physics, and ultrasound applications. In the modern scientific literature there was no book, especially devoted to applications of focused ultrasound for stimulation of neural structures.

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Chapter 1

BIOLOGICAL EFFECTS OF FOCUSED ULTRASOUND

The content of this chapter is a discussion of biological effects of focused ultrasound and their mechanisms. These effects include heating, cavitation, and mechanical interactions of the acoustic field with the tissue due to the radiation force and acoustic streaming. Knowledge of basic mechanisms for the production of biological effects with ultrasound is critically important to the development of new ultrasonic methods for medical applications and for their safe and effective implementation in clinical practice. The reversible effects on brain and other neural structures, as well as effects of neurostimulation and neuromodulation of brain tissues will be discussed. In conclusion, advantages and limitation of focused ultrasound as an artificial stimulus will be considered.

1.1. Main Physical Factors of Focused Ultrasound

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 (Rosenberg 1969) with a concave spherical surface. Other 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.

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 (Rosenberg 1969), the intensity in the center of the focal region does not achieve an infinity, and has a quite certain size.

Figure 1 (Gavrilov, Tsirulnikov 1980) presents the main geometrical characteristics of a spherical focusing radiator: R is the radius of a radiator; F is the focal length; a_m is the angle of convergence; h is the depth; r_0 is the radius and l is the length of the focal region.

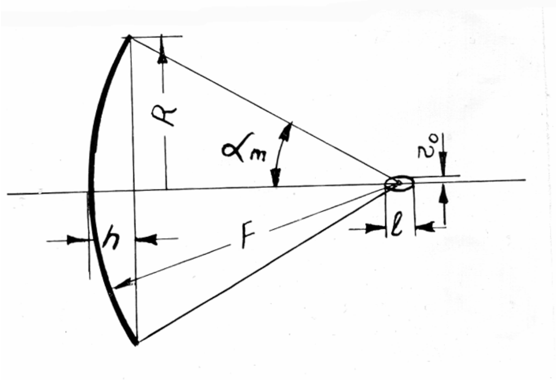

Figure 1. Main geometrical characteristics of a spherical focusing radiator (Gavrilov, Tsirulnikov 1980).

Let's presents the main relations for a single spherical focusing radiator (Rosenberg 1969). 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

$$r_o = 0.61\frac{\lambda F}{R}, \quad (1)$$

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

$$l = \frac{2\lambda}{1-\cos\alpha_m}. \quad (2)$$

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

The maximal intensity in the center of the focal region with a not very large angle of convergence a_m (a_m <45°) is equal (Rosenberg 1969)

$$I_F = 3.7 I_o \frac{\pi R^2}{\pi r_o^{\ 2}}, \quad (3)$$

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 (Rosenberg 1969).

The pressure gain due to focusing is

$$K_p = 2\pi\frac{h}{\lambda}, \quad (4)$$

the oscillatory velocity gain is

$$K_v = K_p \cos^2\frac{\alpha_m}{2}, \quad (5)$$

the intensity gain is

$$K_I = K_p^{\ 2}\cos^2\frac{\alpha_m}{2}. \quad (6)$$

These simple relations allow defining the sizes of the focal region and values of the gain of a single focusing radiator with the accuracy applicable for practical purposes. In the majority of medical implications of focused ultrasound, when it is used for active action on a medium, the radiators with the diameter which is approximately equal to the radius of curvature of a radiating surface are applied; i.e. the angle of convergence is equal approximately 30^o. In this case, the length of the focal region is approximately 5-6 times of its diameter. If the angle of convergence is smaller, the ratio of the diameter of the focal region to its length decreases. Therefore the locality of the effect on biological media and objects degrades, which, as a rule, is not appropriate for the users of this technique.

The geometry of a field of a spherical focusing radiator is shown in Figure 2 (Gavrilov, Tsirulnikov 1980). To the left from the focal plane a converging wave is shown, on the right a divergent beam is seen. Thus, through the focal region within the limits of the main diffraction maximum a plane wave is passing. Therefore, for calculations of parameters of the sound field in the focal region, the plane wave approach is used (Bergmann 1954):

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

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, ρc is the characteristic acoustic impedance of a medium with the density ρ and velocity of ultrasound c.

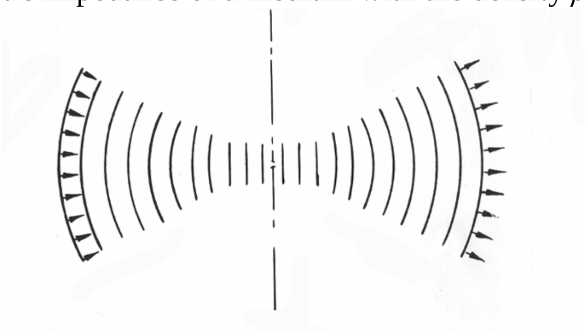

Figure 2. Geometry of a field of a spherical focusing radiator (Gavrilov, Tsirulnikov 1980)

When a plane ultrasonic wave is passing through a homogeneous medium, the intensity of ultrasound decreases with the distance exponentially (Bergmann 1954, NCRP Report № 74 1983):

$$I = I_o e^{-2\alpha x}, \quad (8)$$

where I_0 is the initial intensity, x is the distance from a source, 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 in dependence on the used unit of length. The attenuation coefficient represents the sum of the absorption coefficient α_o 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.

The attenuation coefficient in biological tissues is related to the frequency of ultrasound as $\alpha = af^b$. Table 1 expresses attenuation in tissues and biological fluids and coefficients a and b in normal human tissues for different temperatures of measurements (Duck 1990).

Table 1. Ultrasound amplitude attenuation coefficient for normal tissues of humans: $\alpha = af^b$ (Duck 1990).

Biological medium	Temperature °C	Frequency MHz	*a*	*a*	*b*	Reference
			Np cm^{-1} MHz^{-b}	dB cm^{-1} MHz^{-b}		
Blood	22	4.8/ pulse.	0.014-0.018	0.12-0.16	1.19-1.23	Narayana *et al.* 1984
Plasma	25	1.7-15	0.0066	0.057	1.41	Lang *et al.* 1978
Brain	Room	1-6	0.067-0.069 mean 0.067	0.58-0.60 mean 0.58	1.20-1.46 mean 1.3	Bamber 1981
-, white matter	Room	1-6	0.083-0.11 mean 0.09	0.72-0.96 mean 0.8	0.99-1.16 mean 1.1	Bamber 1981
-	37	1-5	0.050	0.435	1.08	Kremkau *et al.* 1981
Breast	25, 37	0.5-6	0.086	0.75±0.3	1.5	Foster, Hunt 1979
Fat, stomach	Room	1-6	0.07-0.6	0.6-5.2	0.4-1.4	Bamber 1981
Liver	35.5	1.25-8	0.0459	0.399	1.139	Lin *et al.* 1987
-	*in vivo*	2.5/ pulse.	0.041-0.070 mean 0.052	0.36-0.61 mean 0.45	1.05±0.25	Parker *et al.* 1988
Spleen	Room	1-6	0.036-0.062 mean 0.046	0.31-0.54 mean 0.4	1.14-1.47 mean 1.3	Bamber 1981
Testis	Room	3-7	0.012-0.029	0.01-0.25	1.26-2.04	Bamber 1981

It is seen, that for the majority of tissues the value of *b* is not equal 1, although not strongly differs from it. Therefore a widespread assumption of an availability of a linear frequency dependence of attenuation and absorption in soft tissues in not very wide frequency range (part and units of MHz) in some cases looks quite justified.

Thermal effects of focused ultrasound

One of the most important action caused biological effects of focused ultrasound is a heating of tissues due to absorption of ultrasound energy in them. The review of biological effects caused by thermal effects of ultrasound contains in a number of publications (Nyborg 1977; NCRP Report 74 1983; Nyborg, Steele 1983; AIUM 1993, 1994; Hill *et al.* 2004; O'Brien 2007, 2011).

If the plane wave is completely absorbed in the medium, the temperature *T* after time *t* will be (Nyborg 1977; NCRP Report 74 1983)

$$T = \frac{2\alpha_o It}{\rho c_m} + T_0, \qquad (9)$$

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

$T - T_0 = 0.014\ It$. (I in W/cm^2) . (10)

So, if I = 1 W/cm^2, the temperature rise is 0.014 ^{o}C/s or 0.8 ^{o}C/min. This rate of temperature rise is usual in ultrasound physiotherapy for the first minutes after application of ultrasound (NCRP Report 74 1983).

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 doesn't decrease due to heat conduction (also called diffusion).

An equation for the temperature rise ΔT in the center of a focal region taking into account thermal conductivity of brain tissues was obtained by Pond (1970)

$$\Delta T = \frac{2\alpha_o I_o}{J\rho c_p} e^{-2\alpha x} t \cdot A(t) \quad , \qquad (11)$$

where α и α_o are the attenuation and absorption coefficients of ultrasound in the medium; I_o is the maximum value of the unattenuated intensity in the center of a focal region (W/cm^2); x is the depth of location of an irradiated site from a surface of a tissue; t – the duration of ultrasound action; J is the mechanical equivalent of heat (J =4.18 J/cal and therefore 1 W = 0.239 cal/s); $A(t)$ is the function characterizing influence of heat conductivity of the medium; ρ и c_p are the density and specific heat capacity, often equated to values for water, i.e. 1.

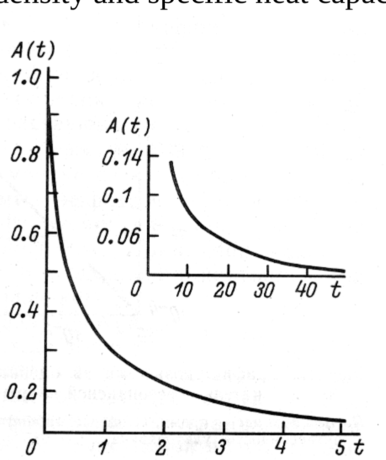

Figure 3. Function *A(t)* for a focusing radiator with the frequency of 3 MHz and an angle of convergence = 50° (after Pond 1970)

Function $A(t)$, representing the ratio of the actual temperature rise to that which would have occurred without heat leakage at the focus, is shown in Figure 3 (Pond 1970) for a focusing radiator with the frequency of 3 MHz and an angle of convergence = 50°. It is seen that the value of this function starts to differ noticeably from 1 at the duration of action exceeding 0.1-0.2 s. It means that, starting from these time durations, the account of influence of the medium heat conductivity becomes absolutely necessary. The dependence presented in Figure 3 can be used not only for estimation of $A(t)$ in the focal region of a radiator with the characteristics specified above, but also for focusing radiators with any other parameters. In particular, calculation $A(t)$ can be executed for such cases (Pond 1970):

a) for any frequency of ultrasound nf (n - any number), distinct from the specified above resonant frequency of a radiator f = 3 MHz, in geometrically similar focusing systems; thus $A_f(t) = A_{nf}\ (t/n^2)$;

b) for any values of the radius of a focal region nr_o, distinct from r_o for the specified radiator, thus $A_{ro}(t) = A_{nro}\ (n^2 t)$;

c) for any diffusivity of tissues k' by the use of a reduced time $t' = t\ k/k'$, where k is the diffusivity of water.

Because ΔT is proportional to the intensity of ultrasound I, switching off ultrasound at the time t_1 is equivalent to an addition of a source of heat with a "negative sign". Thus, for a time t after switching on ultrasound and at its switching off at a time t_1, one will have

$$\Delta T = \Delta T\ (t) - \Delta T\ (t - t_1). \qquad (12)$$

Though the method of calculations offered by Pond has been executed with a number of simplifying assumptions (for example, with an assumption about an absence of a dependence of acoustic properties of a tissue from temperature), this method of calculation, nevertheless, gives a good correlation with experimental data (Pond 1970).

The temperature field in a tissue can be calculated on the basis of the Pennes bio-heat transfer equation (Pennes 1948):

$$\frac{\partial T}{\partial t} = \kappa\, \Delta T - \frac{T - T_0}{\tau_p} + \frac{q_v}{c_v}, \qquad (13)$$

Here $T = T(\vec{r}, t)$ is the temperature in the tissue; T_0 is the equilibrium temperature of a tissue; c_v is the volumetric heat capacity of the medium; k is the thermal diffusivity; τ_p is the perfusion time. The first term in the equation (13) describes the process of cooling caused by diffusion of heat. The second one is the cooling related with an intensive heat exchange in blood vessels, being both in the heated volume and outside it. The third term describes a spatial distribution of a field of thermal sources $q_v(z, r)$. In a number of practical situations the axisymmetric 3-D geometry of a temperature field can be reduced to a two-dimensional problem (Kolios *et al.* 1996; Mahoney *et al.* 2001) which allows simplifying essentially calculations.

In many cases, especially in hyperthermia, a concept of a thermal dose is applied (Sapareto, Dewey 1984). Its mathematical expression looks as:

$$\text{Thermal dose} = \int_0^t R^{(T_{ref} - T_t)}\, dt, \qquad (14)$$

where integration is performed over the total time of heating and subsequent cooling and T_{ref} is the temperature with respect to which the thermal dose necessary for thermal ablation of the tissue is calculated; R = 0.5 for T_t > 43°C and 0.25 for T_t < 43°C. According to Sapareto and Dewey (1984), the thermal dose required to create a thermal lesion is 240 equivalent minutes. In the hyperthermia regimes, the thermal dose usually corresponds to the temperature $Tref$ = 43°C maintained in the tissue during 120–240 min (Daum, Hynynen 1999). For heating regimes used in acoustic surgery, it is convenient to use $Tref$ = 56°C; then, the thermal dose = 1 s is equivalent to = 140 min at a temperature of 43°C (Fan, Hynynen 1996, Sapareto, Dewey 1984). The detailed discussion of the concept of a thermal dose is presented in the review (O'Brien 2011).

Threshold values of the temperature needed for a destruction of a biological tissue essentially depends on the time of its heating. Thus it has not been observed any appreciable dependence of a thermal effect on the way of heating (usual, electric, laser, ultrasonic, etc.). The corresponding threshold curve is presented in Figure 4 for a case of destruction of grey and white matters of cat brain and monkey brain (Lele 1979).

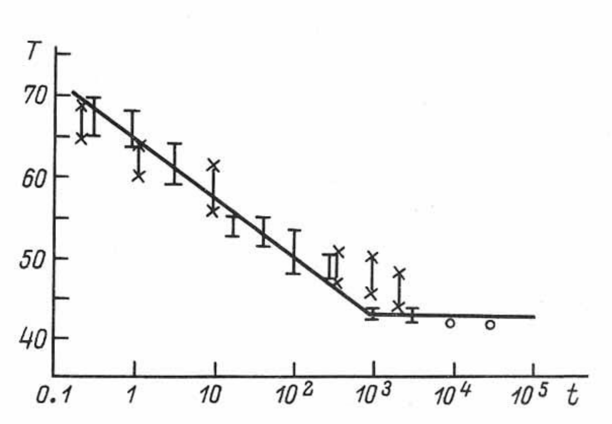

Figure 4. Dependence of threshold values of temperature needed for the destruction of brain structures of animals on the heating time (after Lele 1979). Circles show the lack of destructions, crosses are destruction with usual sources of heating, horizontal dashes are the same but with ultrasound.

It is seen that the rise of temperature up to 42°C does not lead to any destructions even after 8 hours of continuous ultrasonic irradiation. Almost similar results were obtained in other tissues of mammals: a muscle, liver, kidney, etc. (Lele 1979).

Data on the relation of the temperature needed for a thermal destruction of a biological tissue, and the duration of its heating are presented in Table 2 (O'Brien 2011).

Table 2. Temperature-time threshold-based data for various biological materials (O'Brien 2011)

Time, s	Temperature (^{o}C)	Material	Reference(s)
1	69.1	Cat brain *in vivo*	Lerner *et al*. 1973
10	53.4	Cat brain *in vivo*	Lerner *et al*. 1973
100	44.6	Cat brain *in vivo*	Lerner *et al*. 1973
1.4	65	Cat brain *in vivo*	Lele 1977
1.8	64	Cat brain *in vivo*	Lele 1977
2.5	63	Cat brain *in vivo*	Lele 1977
3	65	Cat brain i*n vivo*	Lele 1977
10	53	Rabbit brain *in vivo*	Vykhodtseva *et al*. 2000
30	48	Rabbit brain *in vivo*	McDannold *et al*. 2004
9	60.2	Rabbit brain *in vivo*	Pond 1970
3	63.7	Rabbit brain *in vivo*	Pond 1970
30	47.2	Rabbit muscle *in vivo*	McDannold *et al.* 2000

Non-thermal effects of focused ultrasound. Cavitation

The second factor defining biological action of ultrasound is its mechanical effects. In Table 3 (Vartanyan *et al.* 1985), the peak values of ultrasound parameters (the displacement amplitude A, particle velocity V, acceleration a, sound pressure P, sound pressure gradient ΔP over the distance between its maximum and minimum, radiation pressure S) are presented for some often used frequencies and intensities of ultrasound. It is seen, for example, that at the frequency of 1 MHz and the intensity of 1000 W/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 *A* is 0.6 microns, particle velocity *V* is 3.6 m/s, acceleration *a* is $2.3\cdot10^9$ cm/s^2 (that exceeds the acceleration of terrestrial gravitation in $2\cdot10^6$ times), sound pressure P = 55 atm, pressure gradient along the distance of one-half wavelength ΔP = 1500 atm/cm, radiation pressure at the action of ultrasound on a completely reflecting obstacle S = 0.13 atm. It is clear, that so intensive mechanical action on the medium can lead to various effects, including destroying, even without any thermal action of ultrasound. From Table 3 it follows that 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).

Table 3. Peak values of ultrasound parameters in a plane wave (Vartanyan *et al*. 1985).

f, MHz	*I*, W/cm^2	*A*, µm	*V*, cm/s	*a* 10^6 cm/s^2	*P*, atm	$\Delta P = \frac{2P}{\lambda/2}$, atm cm^{-1}	*S= 2I/c*, atm
0.5	0.1	$1.16\cdot10^{-2}$	3.65	11.5	0.55	7.5	$1.3\cdot10^{-5}$
	1	$3.7\cdot10^{-2}$	11.6	36.5	1.7	23	$1.3\cdot10^{-4}$
	10	0.116	36.5	$1.15\cdot10^2$	5.5	75	$1.3\cdot10^{-3}$
	100	0.37	$1.16\cdot10^2$	$3.6\cdot10^2$	17	$2.3\cdot10^2$	$1.3\cdot10^{-2}$
	1000	1.16	$3.65\cdot10^2$	$1.1\cdot10^3$	55	$0.75\cdot10^3$	0.13
	10000	3.7	$1.16\cdot10^3$	$3.6\cdot10^3$	170	$2.3\cdot10^3$	1.3
1	0.1	$5.8\cdot10^{-3}$	3.65	23	0.55	15	$1.3\cdot10^{-5}$
	1	$1.85\cdot10^{-2}$	11.6	73	1.7	46	$1.3\cdot10^{-4}$
	10	$5.8\cdot10^{-2}$	36.5	$2.3\cdot10^2$	5.5	$1.5\cdot10^2$	$1.3\cdot10^{-3}$
	100	0.185	$1.16\cdot10^2$	$7.3\cdot10^2$	17	$4.6\cdot10^2$	$1.3\cdot10^{-2}$
	1000	0.58	$3.65\cdot10^2$	$2.3\cdot10^3$	55	$1.5\cdot10^3$	0.13
	10000	1.85	$1.16\cdot10^3$	$7.3\cdot10^3$	170	$4.6\cdot10^3$	1.3
3	0.1	$1.9\cdot10^{-3}$	3.65	69	0.55	45	$1.3\cdot10^{-5}$
	1	$6.2\cdot10^{-3}$	11.6	$2.2\cdot10^2$	1.7	$1.4\cdot10^2$	$1.3\cdot10^{-4}$
	10	$1.9\cdot10^{-2}$	36.5	$6.9\cdot10^2$	5.5	$4.5\cdot10^2$	$1.3\cdot10^{-3}$
	100	$6.2\cdot10^{-2}$	$1.16\cdot10^2$	$2.2\cdot10^3$	17	$1.4\cdot10^3$	$1.3\cdot10^{-2}$
	1000	0.19	$3.65\cdot10^2$	$6.9\cdot10^3$	55	$4.5\cdot10^3$	0.13
	10000	0.62	$1.16\cdot10^3$	$2.2\cdot10^4$	170	$1.4\cdot10^4$	1.3

It is pertinently to compare the mechanical action of ultrasound with the action of an 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 rigidly connected 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 at an occurrence in the biological medium ultrasonic cavitation which physical nature is considered in a number of reviews and books (Flynn 1964; Lauterborn 1980; Neppiras 1980; Apfel 1981; Prosperetti 1982; NCRP Report 74 1983; Young 1989; Leighton 1994; Hill *et al*. 2004). Ultrasound cavitation is the formation and activity of gas or steam bubbles (cavities) in the medium irradiated by ultrasound.

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”, “vapor”, “true”, “real” or “collapsing”. Non-inertial cavitation is the process where a bubble in a fluid is forced to oscillate due to action of ultrasound. Such type of cavitation formerly was called “stable” cavitation and sometimes even “degasation”.

Inertial collapse of a spherical gas bubble in a liquid is characterized by a relatively slow (i.e. 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 (Leighton 1998). 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 an influencing the inertial cavitation and its consequences, but also producing effects of its own. They are related, first of all, with secondary mechanical effects of ultrasound (acoustic streaming, radiation force; see below).

To indicate the likelihood of exceeding the threshold required for the occurrence of cavitation, a measure called the “mechanical index” (MI) has been introduced (Leighton 1998). Mathematically, it can be defined as

$$MI = \frac{p\_[MPa]}{\sqrt{f[MHz]}}, \qquad (15)$$

where $p\_$ is a maximum negative sound pressure and f is a frequency. A higher mechanical index means a larger bio-effect. If MI ≤ 0.7, the likelihood for the occurrence of cavitation is very small. The FDA limit for diagnostic ultrasound scanners is that MI cannot exceed of 1.9.

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 (Tran *et al.* 2003, 2005; Vykhodtseva *et al.* 2004).

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 several 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, a number of researchers sometimes 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 action 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 (Beier, Dörner 1954; Bergmann 1954; Fry, Dunn 1972; Wells 1977; Dunn, Pond 1978; NCRP Report 74 1983; Rooney 1988, Duck 1998).

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 normal falling a sound can be found as (Hueter, Bolt 1955)

$$F = D\frac{IS}{c}, \qquad (16)$$

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, $D = 2\ Cos^2\theta$, 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 $D = 2\alpha$ (Duck 1998). For example, if α = 0.01 mm^{-1} at the thickness of a layer of 1 mm, 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 (Duck 1998). This gradient is the main reason of the occurrence of acoustic streaming in the liquid medium.

In Table 4 (Duck 1998) 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 1 W/cm^2, the peak intensity is 500 W/cm^2, the beam area is 10 мм^2, the frequency is 3 MHz, and the attenuation coefficient is 0.015 mm^{-1}.

Table 4. Estimated radiation forces and pressures in a typical diagnostic beam (Duck 1998).

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

With the use of lithotripters, the order of values is another (Starritt *et al.* 1991). At the intensity of 10^5 W/cm^2 and the average frequency of 0.5 MHz the gradient of the radiation pressure was 10 kPa mm^{-1} that is equivalent to 0.1 atm mm^{-1}. At the impact of such pulse on completely absorbing target, the radiation force will be 67 N, that is equivalent to the gravitational force acting on mass of 6.7 kg.

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. This mechanism will be discussed in details in Chapter 3 (Section 3.3). In brief, it is known that with the propagation of amplitude-modulated ultrasonic oscillations, the radiation pressure originates, presenting the sum of three components: one constant and two variables (Altenberg, Kästner 1952). One of the variable components changes with the modulation frequency and the other 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 on the receptors in the labyrinth.

As it has been shown above, acoustic waves are capable of generating radiation pressure gradients within the exposed medium. If the medium is a fluid, and therefore free to move, it will move. The result bulk flow of fluid is called acoustic streaming (Duck 1998; Hill *et al*. 2004). The following equation allows estimating the streaming velocity *u* in a liquid:

$$u = (2\alpha\, I/c\upsilon)\, d^{\,2}G, \qquad (17)$$

where *I* is the intensity, *α* is the attenuation coefficient, *c* is the acoustic speed, *υ* is the kinematic viscosity, i.e. the attitude of the dynamic viscosity to the density, *d* is the diameter of ultrasound beam, *G* is the geometrical factor depending on the sizes of a beam and the container in which the liquid is concluded. It is seen, that the streaming velocity *u* is proportional to the intensity of ultrasound. So, in the field of a focusing radiator the streaming velocity tends to be maximal in the region of the focus, but not at the source (Duck 1998). It has been shown that at diagnostic intensities of ultrasound, the streaming velocity could vary from 1 cm/s in the beam of usual B-scanner up to 14 cm/s in the beams of pulsed Doppler scanner (Starritt *et al.* 1989). It is considered, that the streaming velocity in blood is approximately the same order as in water, despite of an essential difference in attenuation (100 times) and viscosity (5 times). The scale of the acoustic streaming is very different, beginning from the sizes of a tank with water in which measurements of an acoustic field of a radiator carried out, and ending the sizes of the order of micron.

In conclusion of the discussion of biological effects of focused ultrasound, it is necessary to emphasize that such action is caused usually by joint action of several factors having various physical nature. Thus, as a rule, the attempts to explain all the consequences of biological effects of the focused ultrasound by any single factor, for example a thermal one (Lele 1971; Lele, Pierce 1972), are useless. It is possible to speak only about a prevailing action of some factor at a quite certain regime of ultrasonic action on biological media and objects. In these cases it is possible sometimes to reveal the main effecting factor responsible for the observed biological effect, and thus to obtain data regarding its physical mechanism.

1.2. Destructive Effects

One of the most known applications of focused ultrasound in medicine and physiological research is its using for a local and noninvasive destruction of predetermined deep structures of a human organism. Such opportunity represents a special practical interest, e.g., for a local destruction of deep structures of a brain. The curve for the results of threshold lesions obtained by different authors at various frequencies in white matter of the mammalian brain is presented in Figure 5 (F. Fry *et al.* 1970). Here *I* is the intensity in W/cm^2, *t* is the single pulse time duration in seconds; 1 shows data of authors on cat brain, 2- data on rat brain (Warwick, Pond 1968) and 3- on cat brain (Basauri, Lele1962). The figures near the curve are the frequencies of ultrasound in MHz.

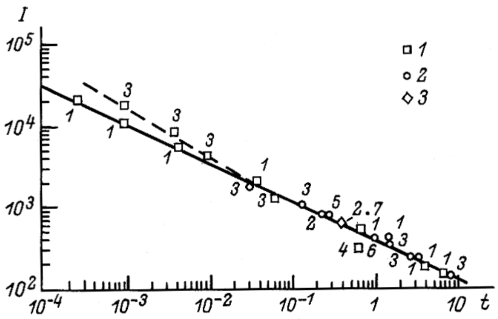

Figure 5. The doses curve for threshold lesions produced in white matter of the mammalian brain (after F. Fry *et al.* 1970).

On the basis of a histological analysis of ultrasonic lesions in the brain, three areas of the intensities and time durations have been defined (F. Fry *et al.* 1970; Dunn, Fry 1971):

1. At the intensities less than 150 W/cm^2 and time duration of the order of several seconds, the lesions are considered to be produced by heat. The corresponding lesions were called thermal ones.

2. At the intensity range from several hundred to approximately 1500 W/cm^2 and time durations more than 50 ms, thermal mechanisms do not account responsible for the lesions; ultrasound is considered to disrupt biological structure subtly by mechanical action. The corresponding lesions were named focal ones.

3. At the intensities above about 2000 W/cm^2 and time durations less than 40 ms the threshold lesions are produced by a cavitation mechanism. The corresponding lesions were named cavitation-mediated.

This research was supplemented by measurements of cavitation thresholds in the brain of animals *in vivo*, which allowed updating the given boundaries between different regimes of ultrasound destructions of tissues (Gavrilov 1974).

The long-term applications of focused ultrasound for tissue destruction allowed stating that for pure thermal mechanisms the lesions are the most predictable, reproducible and controlled. Their shape reminds an ellipsoid of revolution which length is in several times (usually in 5-7) more than the diameter. At the cavitation mechanism, the destructions occur on some "weak" points, which distribution in irradiated volume of tissue is, usually, random and chaotic. It was considered until recently, that with the use of high intensity ultrasound in medicine, it is necessary to avoid the cavitation regime because of specific features of the physical nature of cavitation (e.g., random and stochastic character of its origination, weak reproducibility of localization, the shape of lesions in tissues, etc.).

Nevertheless, the results of investigations carried out in different laboratories for the last years, demonstrate an opportunity of application in medicine of new, non-traditional methods based just on the use of ultrasound cavitation. For example, one of the methods of ultrasound surgery can be based on a preliminary creation in tissues gas bubbles decreasing cavitation thresholds and, therefore, a threshold of tissues ablation. That can be achieved by two ways. The first of them is a generation of the bubbles by means of a preliminary action on the tissue of an intensive, but short ultrasound pulse with rather small energy which doesn't induce thermal effects in tissues. For example, in the research of destructive effects of lithotripters it was shown (Tavakkoli *et al*. 1997) that if before a positive peak of the pressure, the negative pressure, increasing a number of cavitation bubbles in the tissue, was generated, the destructions or lesions became much more extensive and well reproducible.

The second method for changing the cavitation properties of a tissue is an administration into it microscopic gas bubbles, for example, ultrasound contrast agents applied to enhance the efficacy of ultrasound visualization. Tran *et al.* (2003) showed that after the administration of commercial ultrasound contrast agents into tissues, the cavitation thresholds of a dog's kidney were reduced from 2500 to 1000 W/cm^2. The threshold of the ablative effect of ultrasound was also essentially decreased (in 100 times for the duration and in 2 times for the intensity). Authors suggested that the reducing of the threshold by means of the administration of microbubbles, acting as cavitation nuclei, can make ultrasonic cavitation a more predictable, and, therefore, more appropriate modality for ultrasound surgery.

Several years ago, Prof. C. Cain from the University of Michigan has formulated scientific grounds of a scientific direction which, by analogy with lithotripsy, has been called "histotripsy" (Cain 2005, Xu *et al.* 2006). The main idea of this method was that microbubbles, irrespectively on the methods of their generation, ensure reproducible cavitation thresholds, reduce considerably the thresholds of ablation and provide lesions in tissues which are much more reproducible in shape. The use of a pulsed mode allowed to change the parameters of action over a wide range and therefore to achieve an optimal therapeutic effect. A special feature of the lesions obtained by the given method of soft tissues ablation is that all the cellular structures in the lesion can be destroyed completely if necessary, and that the borders of such lesions are rather precise and smooth. Cavitation effects provide mechanical, not thermal lesions of tissues at the averaged in time intensities which are unable to cause essential heating of the given volume. It is important that any complicated, expensive methods of noninvasive control of the temperature are not required in this case.

The cavitation regime can be used, for example, for ultrasonic ablation of deep-located brain structures through an intact skull to prevent excessive heating of skull bones. Application of a traditional thermal mode of insonation would inevitably lead to thermal damages of a skull bone due to the very high ultrasound absorption in it, whereas the regime of ultrasound cavitation could appear quite appropriate for the achievement of this aim.

Cavitation can be used as an effective means of increasing of acoustic absorption in tissues, and, hence, intensifying of the thermal effect of ultrasound due to creation gas bubbles in tissues. Therefore, it is expedient to use a regime when a short pulse of high intensity ultrasound which aim is a generation of gas bubbles in tissues is followed by a long-duration ultrasound pulse inducing an enhancement of heating of the given site of the tissue. Melodelima *et al*. (2004) presented an experimental verification of the workability of the given approach. A method of the essential increasing of the ablation depth in the tissues has been offered, based on the use of a combination of thermal and cavitation effects in the field of a plane ultrasound transducer. It was found that a preliminary, rather short treatment of tissues in the cavitation regime increased practically twice the depth of the destruction induced only by the following thermal action on the tissue. A mechanism of this effect is apparently related to the fact that bubbles, originating in the tissue due to cavitation, increase the sound attenuation and consequently the efficacy of the thermal effect of ultrasound.

Cavitation is considered to be one of the basic mechanisms of the enhancement of the efficiency of antitcancer chemical agents using for malignant tumor treatment. There is an evidence that cavitation is, probably, the basic mechanism of so-called sonodynamic effect of ultrasound, i.e. the enhancement of the efficacy of anticancer drugs. It has been found that the effects of acoustic cavitation can be an order of magnitude enhanced by superimposing the second harmonic of the fundamental frequency (Umemura 1997, Sasaki *et al.* 2004).

One of possible applications of cavitation in oncology can be based on the destruction of blood vessels, surrounding a tumor, which will block a blood flow in it and hence enhance a cytotoxic effect of ultrasound on tumor cells.

Cavitation can be used for the destruction of cellular membranes leading to the cell necrosis. This effect can be used in ultrasound surgery. Other fields of application are lithotripsy (accompanying by a relevant formation of the shape of the sound pressure pulse in the focal region) aiming, in particular, the destructions of cellular membranes and the following necrosis of cells, phonophoresis (or sonoporation) with participation of cavitation, and also creation of free radicals with the following biological consequences.

Thus, it is evident, that the continuation of scientific research, directed on the development of novel ultrasound methods for application in medicine, based on the use of cavitation, is very perspective. Use of this approach would allow implementation in practice of a number of medical applications having been considered impossible until recently.

In the last years, in medical acoustics appeared a new scientific direction related with the research of nonlinear effects in superpower and highly focused beams. In fact, in modern devices used in ultrasound surgery, the intensity in the focal region often reaches tens of kW/cm^2, which leads to the generation of higher harmonics in the propagating wave, distortion of its profile, formation of shocks and additional absorption of wave energy at the shock fronts. In these cases, local and very fast heating can occur up to temperatures higher than 100 ^oC, and the initiation of boiling within a few milliseconds is possible. The use of nonlinear regimes of sonication could lead to the development of new technologies for application in medicine (Khokhlova *et al.* 2006; Bessonova, Khokhlova 2009; Canney *et al.* 2008, 2010; T. Khokhlova *et al.* 2011).

In conclusion to this paragraph, Table 5 presents a summary of the most known applications of destructive effects of focused ultrasound in medicine, and also the list of references, where the details of these applications are presented.

Table 5. A summary of applications of destructive effects of focused ultrasound (FUS) in medicine and list of references

Neurosurgery and the action of FUS on the deep structures of brain	Fry *et al.* 1958; Fry, Fry 1960; Fry 1965
Transcranial irradiation of brain structures through the intact skull	Clement, Hynynen 2002; Aubry *et al.* 2003; Martin *et al.* 2009; McDannold *et al.* 2010
Surgery, oncology	Wu *et al.* 2004; Kennedy *et al.* 2004
Surgery with the action of FUS on tissues through the rib cage	Bobkova *et al.* 2010; Kim *et al.* 2011
Hyperthermia of tumors	Harari *et al.* 1991; Hand *et al.* 1992
Treatment of prostate cancer by FUS	Thuroff *et al.* 2003; Illing *et al.* 2006
Destruction of stones, lithotripters	Cleveland, Sapozhnikov 2005; Sapozhnikov *et al.* 2007
Control bleeding	Vaezy *et al.* 1999; Zderic *et al.* 2006
Vascular effects, thrombus ablation, targeted venous occlusion	Datta *et al.* 2006; Hwang *et al.* 2010

Ophthalmology, including maturing of cataracts and glaucoma treatment	Gavrilov *et al.* 1974; Silverman *et al.* 1991
Cardiology	Sanghvi *et al.* 1997
Uterine fibroids treatment	Hindley *et al.* 2004; Fennessy *et al.* 2007
Liposuction	Brown *et al.* 2009
Apoptosis	Vykhodtseva *et al.* 2006
Essential tremor	Lipsman *et al.* 2013

Noninvasive methods of tissue ablation based on the use of high-intensity focused ultrasound can in the near future successfully compete with the existing surgical methods.

1.3. Reversible Effects on Brain and Other Neural Structures (Early Studies)

Functional (reversible) effects of the action of ultrasound on neural structures have been well known since the middle of the last century. Interest in the possibility of a reversible inhibition of a functional activity of some brain structures using ultrasound was stimulated by a number of practical tasks in medicine. For example, it is possible to provide the high accuracy in irradiation of brain structures by high-intensity focused ultrasound during ultrasonic neurosurgical operations by means of a preliminary action of a deliberately nondestructive ultrasonic irradiation that could result in reversible changes in these structures. This would simplify complicated and time-consuming methods based on the finding of intracranial and cerebral reference points. According to Lele (1967), the problem of obtaining reversible changes in brain structures is crucial for successful implementation of focused ultrasound in clinical neurosurgery. Application of ultrasound would also be extremely useful for studying the functions of different parts of the brain and structural and functional relationships in the central nervous system.

W. Fry and his colleagues were among the first researchers to study the ability of focused ultrasound to induce reversible functional changes in nervous structures. They reported (Fry *et al*. 1950) a temporary inhibition of the spontaneous activity in the excised crayfish ventral nerve cord after the action of focused ultrasound with the intensity of 35 W/cm^2 (frequency of 0.98 MHz). The frequency of the spike potentials at first increased, then decreased and was followed by total disappearance of the large spike potential after 43.5 s exposure to the sound. Through 25 s after ultrasound was turned off, the large spike potentials reappeared, first slowly, then more rapidly, finally reaching a stable frequency.

This research has received the further development in a number of works (W. Fry *et al.* 1955; F. Fry *et al.* 1958; W. Fry 1958; W. Fry, F. Fry 1960). In particular, it was shown (F. Fry *et al.* 1958) that by irradiating with focused ultrasound of rather high intensity of the lateral geniculate nucleus of a cat it is possible to suppress temporarily the potential usually evoked in the visual cortex in response to light stimulus. Exposure time was 20-120 seconds. Complete recovery of the primary and secondary response was apparent 30 minutes after exposure. It should be noted that this effect is produced by an ultrasound dose which does not cause any histologically observable lesion in the tissue.

Lele (1963) studied the effects of irradiation of focused ultrasound at the frequencies of 0.6-2.7 MHz on nerve conduction in over 450 peripheral nerves of the cat, monkey and human. It was found that the ultrasonic dose required for blocking of conductivity of the fiber, decreases with growth temperature of the medium surrounding the nerve in the region of irradiation. According to Lele, all physiological effects connected with the influence of ultrasound on nerve conduction can be duplicated by an application of a graded amount of heat to the certain parts of nerves. In other words, Lele assumes the thermal mechanism of the effects of ultrasound on conductivity of nerves.

When affecting the sciatic frog nerve by focused ultrasound in a number of particular regimes of irradiation, the thin nerve fibers can be blocked without changing the conductivity of the thick fibers (Young, Henneman 1961 a,b). The peculiarity of this method was that the nerve was placed into a rubber block. As a result, the temperature of the nerve increased significantly more compared to the effect of ultrasound *in vivo,* as the absorption coefficient of ultrasound in rubber is very high.

Thus, the research of a number of authors showed that ultrasound does not cause the spread of excitation in the nerve or in individual nerve fibers, although it changes some of their functional properties. Neither local impulse activity, nor propagating excitation have been achieved by means of a direct action of ultrasound on brain structures.

In theoretical study of W.J. Fry (1968), a method of an electrical stimulation of the neural tissue in the deep structures of the brain without introduction of electrodes into the brain was suggested. The essence of the method is based on the interaction of an alternating electric field applied to the brain and the acoustic field generated by focused ultrasound and localized in the site of stimulation. In the simplest case, the electric and acoustic fields have the same frequency. Spreading of the acoustic oscillations in tissue is accompanied by temperature changes. Since the electrical conductivity of the tissue depends on the temperature, the acoustic field causes periodic changes in these parameters with maximal variations in the focal region. Tissues expand when heated, and contract when cooled. As a result, the electric current that flows in the tissue during the half-period when the pressure increases does not have the same value as the current that flows in the opposite direction during the negative half-period. Thus, “rectification” a small part of an alternating electric current, i.e., a unidirectional transfer of the charge takes place. Its magnitude depends on such factors as the parameters of acoustic and electric fields, electrical conductivity of the tissue, and the frequency-dependent absorption coefficient of tissue. However, calculations show that stimulation of nerve cells using this method requires very powerful acoustic and electric fields, the prolonged effect of which may lead to the destruction of nerve tissue.

Studies of the reversible changes in the brain structures of animals under the effect of focused ultrasound were carried out in the former USSR in the late 1970s. They were initiated by the Institute for Brain Research of the Soviet Academy of Medical Sciences together with the Acoustics Institute. The study was developed in two directions. The first of them was related to reversible interruption of the visual function in animals under the effect of focused ultrasound (Adrianov *et al.* 1984a,b,c). The changes in the evoked potentials recorded in the visual tract and visual cortex under the action of light stimulation of eyes were studied in cats. Focused ultrasound with a frequency of 1 MHz in the pulsed mode was used. The intensity of the ultrasound pulse was in the range from 7 to 63 W/cm^2, the duration was 5– 50 ms, the pulse repetition frequency was 0.5–50 Hz, and the total exposure time was 10–60 s. The nature and duration of the changes in the evoked potentials were determined by the parameters of the effect and varied widely in the dependence on these parameters.

In different cases suppression of the evoked potentials was full or partial. The changes of the potentials were reversible, partially reversible, or irreversible. Fully reversible suppression of the evoked potentials in the visual tract followed by the full restoration of their shape and amplitude appeared to be the most interesting for research. The suppression lasted from a few seconds to tens of minutes. The effect was achieved with nondestructive ultrasonic doses, which made it possible to affect the structure repeatedly without a risk of damage. The temperature increase did not exceed fractions of a degree, which excluded heat from the main factors affecting the visual tract. In one of experiments, a device with a built-in relatively small (diameter 18 mm) focusing transducer was fixed on the head of an animal in such a way that the focal region of the transducer was aligned with a targeted exposure site (Adrianov *et al.* 1984a). During suppression of the evoked potentials, the animal was not immobilized or anaesthetized. Its brain structures were exposed to focused ultrasound in the course of animal’s behavioral act.

The second branch of research was related to the study of the shifts of the constant potential in various brain structures of rats (in the cerebral cortex, hippocampus, optic thalamus, and caudate nucleus) under the action of ultrasound on these structures (Vykhodtseva, Koroleva 1986; Koroleva *et al.* 1986). The impact of focused ultrasound was realized with a frequency of 4.6 MHz and the intensity of 5–100 W/cm^2 in pulsed mode, with a pulse repetition frequency of 5– 200 Hz, pulse duration of 10–100 ms, and exposure time of 10–40 s. Special attention was paid to the study of the spreading depression that occurs in the brain in response to many types of stimuli, in particular, to ones induced by focused ultrasound.

In another study (Velling, Shklyaruk 1987, 1988) acute experiments were carried out on cats and chronic experiments on rabbits, in which irradiation of different cortical fields with focused ultrasound was performed in the intact skull or after craniotomy. Long-term irradiation by focused ultrasound with the intensities of 1–100 mW/cm^2 resulted in an increased excitability of the cortex to the action of other stimuli, such as light and electric current. It was reflected in an increase in the amplitude of potentials evoked by light stimuli, and a decrease in the threshold of a response to the direct electrical stimulation of the cortex. For low intensities (lower than 0.1 mW/cm^2) there was no change in biological activity. For higher intensities (1-100 W/cm^2) the inhibition of evoked potentials was observed. Affecting the cortex by single ultrasound pulses from 0.1 to 10 ms at the calculated intensities from 1 μW/cm^2 to 1400 W/cm^2 in the focal region did not lead to evoked potentials. Thus, all the observed effects were related with a modulation of the biological activity but not with the effects of its stimulation.

It was shown that single pulses of focused ultrasound can significantly modify the excitability of the myelinated nervous fibers of the sciatic nerve of a frog (*in vitro*) during 40–50 ms after the termination of the pulse (Mihran *et al*. 1990a,b). The modification consisted of both intensification and depression of excitability. Focused ultrasound with a frequency of 2.7 MHz was applied in a pulsed mode with the pulse duration of 0.5 ms and the peak intensity of 100–800 W/cm^2. The authors explained the changes in excitability of the fibers of the sciatic nerve by the action of the radiation pressure, but not by the action of the temperature, since the calculated temperature rise after each pulse was only 0.025°C.

Focused ultrasound was also used to modify evoked potentials and the functioning of local nerve circuits in the mammalian brain (Rinaldi *et al*. 1991; Bachtold *et al*. 1998). The samples of rat hippocampal preparations *in vitro* were used as an object of studies. The evoked potentials were studied by extracellular techniques. Under the effect of pulses of focused ultrasound with a frequency of 500 kHz at a repetition frequency of 200 kHz and intensities of 50–140 W/cm^2, the evoked potentials in some cases increased; in other cases, they were suppressed. According to the authors, this effect is caused by a superposition of mechanical and thermal effects. In their view, a clearer understanding of the true mechanism of stimulatory and inhibitory action of ultrasound on brain structures is very important for further development of the ultrasonic method in neurophysiological research and in clinics. If these mechanisms were to become clearer, then there would be the possibility of achieving the necessary effects in the living organism, as well as creating a reversible inhibition and temporary stimulation in the specified regions of the brain by means of manipulating the ultrasound parameters. According to the authors, it would be an invaluable noninvasive tool for studying the functions of the normal brain by the reversible suppression or stimulation of small parts of it.

Dalecki *et al*. (1995) studied tactile sensations evoked by a non-focusing ultrasonic radiator in the human fingers and forearm. A plastic disc was mounted on the skin and prevented the passing of ultrasound in tissues. A single ultrasonic stimulus with durations of 5–100 ms, and a series of pulses at repetition frequencies from 50 to 1000 Hz were used. The radiation force was considered the main factor responsible for producing tactile sensations. In the case of using single pulses with the durations equal to or larger than 1 ms, the threshold radiation forces necessary for the occurrence of tactile sensations in subjects with normal skin sensitivity were equal in fingers about 3 mN (or 0.3 g-force) and in a forearm about 10-20 mN, i.e. 1–2 g-force. In the case of applying series of pulses, the threshold forces were an order of magnitude smaller.

The optimal repetition frequency was about 200 Hz. The thresholds did not significantly change in the presence of the plastic disk and its absence, that is, with or without direct action of ultrasound on the skin.

The stimulatory effect of focused ultrasound and, in particular, its ability to cause threshold and near-threshold pain in the skin and underlying tissues of humans was studied at Queen's University in Belfast, United Kingdom. The records of brain evoked potentials resulting from intra-articular stimulations by focused ultrasound were studied and a new experimental method for investigating joint pain in human has been developed (Wright, Davies, 1989). In the mid-1990s, cooperation between the Sechenov Institute of Evolutionary Physiology and Biochemistry, the Acoustics Institute, and Queen's University in Belfast, was facilitated by two Grants of the Royal Society, which resulted in joint studies and publications on the use of focused ultrasound as a stimulator of peripheral receptor structures (Gavrilov *et al*. 1996, Davies *et al*. 1996).

1.4. Recent Studies of Activation Neural Structures

The boundary between the present and previous sections is rather conditional. In the previous one, the works fulfilled up to the end of the last century have been observed, in the following one the research carried out after 2000 will be discussed. During the last years, the investigations of reversible effects of focused ultrasound on brain and other neural structures were carried out very actively in various laboratories and in different countries.

Action on nerves and other neural structures.

The phenomenon of temporal summation of pain arising under the action of focused ultrasound in the skin, muscle tissue, and joints has been studied in humans (Wright *et al*. 2002). The ultrasound frequency was 1.66 MHz; the pulse duration varied from 25 to 100 ms, the intensity of ultrasound was estimated in arbitrary units. Single stimuli and a sequence of five stimuli at different repetition rates (from 0.5 to 5 Hz) were used. Temporal summation was found for skin, muscle, and joint pain; however, it was better expressed in muscle pain. A higher intensity of ultrasound was necessary to evoke pain in muscle tissue than for cutaneous and joint pain.

In was found in a completely different field of study that pulsed ultrasound generated by diagnostic ultrasonic systems can stimulate fetal movement (for 25–40 weeks of pregnancy) (Fatemi *et al*. 2001). When pulsed modes (Doppler and B-scan) of ultrasound were applied, a number of movements per minute was nine times more than in the control group (without an impact) and almost six times more than that for the Doppler scanning in a continuous mode. The authors suggested that this effect is caused by the action of the radiation force of ultrasound, because the pulse repetition frequency in most diagnostic systems ranges from 1 to 10 kHz, i.e., is in the audible frequency range. In addition to an increase in the frequency of fetal movements, the authors registered a slight increase in the fetal heart rate. In regard to this study, it is worth to note the studies on the sensitivity of the fetus to acoustic waves with frequencies from 4 to 24 kHz coming through the wall of the mother's abdomen that were carried out in the mid-1990s (Vartanyan *et al*. 1996). The similar reactions of the fetus in the above-described ultrasonic study (Fatemi *et al*. 2001) and in the vibroacoustic testing (Vartanyan *et al*. 1996) give reason to assume that the studies aimed to determine the presence of hearing in the fetus using amplitude-modulated ultrasound need in development. If these works are successful, then it will be possible to determine the condition of hearing in a fetus before birth during a routine ultrasonic examination of pregnant women simultaneously with visualization of a fetus, which is compulsory in many countries.

Khraiche with co-workers carefully studied an electrical excitability of single neurons with the application of high frequency ultrasound (Khraiche *et al*. 2008). High frequency tone bursts of ultrasound were applied to two neuron cultures using a transducer with an active area 1-1.5 mm in diameter. The ultrasound exposures consisted of one millisecond bursts of 7.75 MHz ultrasound delivered at a rate of 2.0 Hz for one minute. The temporal peak intensity of the ultrasound delivered to the cells was approximately 50-150 W/cm^2. It has been shown that high frequency tone bursts of ultrasound dramatically increase the spike frequency of primary hippocampal neurons in culture. In addition, these ultrasonic bursts also induce silent or still developing neurons to fire. Results indicate that the increase in excitability is largely mediated by mechanical effects, but not thermal effects of ultrasound. The researchers consider that future studies on neuron cultures exposed to ultrasound with varying protocols may provide insight into the feasibility of using ultrasound as a means for stimulation and neuromodulation in the central nervous system.

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* (Tsui *et al.* 2005). Ultrasound with a frequency of 3.5 MHz and an acoustic power of 1-5 W was applied for 5 min. 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 1–3 W. The amplitude of evoked potentials increased by 8% during stimulation with an acoustic power of 1 W; however, it significantly decreased at powers of 2 and 3 W. The authors explained this inhibitory effect by the thermal action of ultrasound.

Foley *et al*. (2004) 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 1850 W/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 (Foley *et al*. 2008) 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 5.7 MHz with peak intensities from 390 to 7890 W/cm^2 was applied for 5 s. 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 3300 W/cm^2, the amplitude of the responses was reduced from 4 h 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 (Colucci *et al.* 2009). 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 1.986 MHz 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.

Alhamami *et al*. (2011) studied effects of HIFU with different acoustic doses on neural tissues *in vitro*. Focused transducer with a resonance frequency of 2.2 MHz was used. The diameter of the transducer was 5 cm and its radius of curvature was 7.5 cm. 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 3.3 W/cm^2 and the sonication time was 10 seconds. In the medium-level acoustic dose treatment, the intensity ($I_{SPTA)}$ was around 13.3 W/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 5500 W/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 3.3 W/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 13.3 W/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 5500 W/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 (Alhamami *et al*. 2011).

Wahab *et al*. (2012) 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 825 kHz 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*. (2012) 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 350 kHz and 650 kHz) 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 350 kHz, using the 0.36-ms tone burst duration, 1.5-kHz pulse repetition frequency, and the overall sonication duration of 200 ms. Histologic and behavioral 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 neuro-modulatory 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 Dr. P. Mourad. Dickey *et al.* (2012) 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 (Davies *et al*. 1996; Gavrilov *et al.* 1977a,b; Gavrilov 1984; Gavrilov *et al.* 1996), 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 1.1 MHz for 0.1 s to the fingertip pads of 17 test subjects 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 100 W/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 (Tych *et al*. 2013) 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 0.2 s at 1.15 MHz) 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 W/cm^2 ± 25.5) than did sham surgery (97.7 W/cm^2 ± 11.9) and normal control (223 W/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 some day use focused ultrasound stimulation to diagnose patients with neuropathies".

It will be shown in Chapter 5 that focused ultrasound actually has been used successfully in medical practice for diagnostics of different neurological and skin diseases.

In the next study (Garcia *et al*. 2013), 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 (McClintic *et al.* 2013a) 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 (2 MHz, with individual pulses of 0.1 s 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 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 (McClintic *et al.* 2013b) 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 75 ms and also a train consisted of five pulses, each with the duration of 75 ms separated by 75 ms 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 (McClintic *et al.* 2013a), 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 R. Muratore are devoted to the possibility of inducing functional changes in the neuronal cells using small doses of ultrasound (Muratore *et al.* 2009a,b; 2012). 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 4.67 MHz, duration of 30 ms, and amplitude of 100 kPa 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 4.04 MHz, duration of 0.1 ms, and amplitude of 77 kPa. Before and after ultrasound treatment, the culture was stimulated using biphasic stimuli of electric current (100 μA) with a duration of 0.1 ms. Optical microscopy showed that, under the effect of ultrasound, cells of PC12 cultures that were clustered near the focal region elongated by approximately 2 μm 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 the paper of Muratore *et al.* (2009a; 2012), 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 48 kPa. A possible fatigue was observed at the highest pressure (77 kPa ≈ 0.8 atm), which was the most intensive stimulus (Muratore *et al.* 2012).

In the review of biological effects of ultrasound on peripheral nerves, Muratore and Vaitekunas (2012) 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 led recently by Dr. W. Tyler carried out detailed research into somatosensory sensations induced by ultrasound (Legon *et al*. 2013), which is the topic of Chapter 2. They used an unfocused ultrasound transducer with a frequency of 0.35 MHz 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 11.8 W/cm^2, pulse duration is 2 ms, pulse repetition frequency is 70 Hz and their number is 35 to produce the total time for stimulus duration of 0.5 s. In the second case the parameters were: the intensity I_{SPTA} was 54.8 W/cm^2, pulse duration is 10 ms, pulse repetition frequency is 100 Hz, a number of pulses is 100 to produce the total time for stimulus duration of 1.0 s. 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.

It will be shown in the next chapters that qualitatively similar effects were obtained several decades ago (naturally without their registration with MRI) and have been used for diagnostics of different diseases. The methods of electroencephalography for the research of somatosensory sensations were used in our group mainly in animal experiments, but in the case of need in studies in humans (Tsirulnikov *et al*. 2007).

Stimulation of acupuncture points

It is well-known that non-focused ultrasound radiators have been used from the end of 1960s in physiotherapy for stimulation of acupuncture points (acupoints). The typical parameters of flat ultrasound used in pulsed or continuous regime were: the frequency is about 1 MHz, area of a transducer is in the range from 0.5 to 2 cm^2, intensity usually 0.1-0.2 W/cm^2 (sometimes up to 0.3-0.5 W/cm^2), irradiation time for one point is about 0.5-2 min, the time of the procedure is about 10 min, number of procedures for total course of the treatment is about 10-15. Evidently, the use of focused ultrasound for this aim allows getting more local impact on acupuncture points. Due to specific of this book, a following review will contain a description of some works connected with the use of focused ultrasound for stimulation of acupoints.

An idea to utilize focused ultrasound for stimulation of acupuncture points was a subject of interests of investigators during a long time. Several interesting works in this field were fulfilled by the group at the University of California, Irvine headed by Prof. J.P. Jones (1941-2013). The results obtained were published in a number of papers (Cho et al. 1998, 2000; Jones 2002; Jones, Bae 2004, 2009). A transducer assembly used in these studies consisted of a focused piezoceramic ultrasonic radiator mounted in a water-filled flexible chamber that was placed on the skin overlying the acupoint of interest. The transducer, designed for high power applications, worked in a pulse mode, had a center frequency of 5 MHz, a diameter of 2 cm, and a focal length of 6 cm. Accurate positioning mechanisms allowed the focal point (a volume with a diameter of about 0.5 mm) to be moved in depth. Spatial-peak-temporal–averaged intensities in the range of from 5 to 8 W/cm^2 were required to produce an effect. These intensities are above those used in diagnostic ultrasound (in the range of hundreds mW/cm^2), they are well below the cavitation threshold and the range where irreversible effects occur (see Chapter 1). Optimal results were obtained when ultrasound was focused on the same position as the tip of the acupuncture needle during a conventional stimulation. In brief, the study showed that ultrasound pulses can stimulate an acupoint, eliciting a response similar to that produced by standard needling. Focusing of pulsed ultrasound on the surface of the skin or in depth beyond the acupoint produced no stimulation. No stimulations were observed at intensities below 5 W/cm^2.

In the first study of this group (Cho et al. 1998), it was shown that activation of an acupoint BL 67 (this acupoint is known in Oriental Medicine to be related to vision) in the foot along a specific meridian elicited increases in cortical blood flow in circumscribed regions of the visual cortex, comparable in magnitude and brain location to those obtained by stimulation of the visual cortex by flashes of light. Moreover, when the acupuncture needle was directed at a nearby site (nonacupoint), no such effects were obtained. Although the relationship between the lower limb acupoint and the visual cortex still remains to be explained, the fact that these responses were obtained at specific acupoints along a specific meridian, and not nearby, lends support to the meridian concept. The points stimulated along the urinary bladder meridian were those recorded in the ancient text for treatment of eye disorders and other unrelated matters. Following this initial study, it was confirmed a similar relationship between auditory-related acupoints and the auditory cortex (Cho et al. 2000).

A special feature of the method was that stimulation of acupoints was controlled by MRI technique (Cho et al. 1998, 2000). The acupoint was targeted and irradiated by ultrasonic pulses, and corresponding brain activity was recorded by MRI. For a wide range of ultrasound parameters, the MRI results were indistinguishable from those produced by conventional acupuncture needles. For example, MRI studies reconfirmed the close correlation between direct stimulation of the eye using light and stimulation of a vision-related acupoint using either a needle or pulses of ultrasound.

In addition, a quantitative ultrasound method for control of acupoints has been developed (Jones, Bae 2004). In fact, conventional ultrasonic imaging, operating at frequencies of 5 and 7.5 MHz, previously used by authors to monitor the placement and application of the acupuncture needle, was unable (because of low resolution) to identify any remarkable anatomical features associated with the acupoint. Therefore the authors implemented a simple pulse-echo data acquisition system using a 50 MHz hand-held transducer. Holding the small (1 mm diameter) transducer on the surface of the skin, a short (30 ns) ultrasound pulse was transmitted into the tissue, and the reflected signal known as an A-mode trace was recorded and stored in a standard personal computer. Ultrasonic power levels in an echo-location mode were well below those required to produce a stimulation of the acupoint. Moving the transducer in one-fourth diameter steps along a 2-dimensional rectangular grid, a series of A-lines were recorded which intersected and surrounded a specific acupuncture point. For the acupoint BL 67 in each foot, a 20-point by 20-point grid yielded 400 A-lines distributed evenly over a 5.75 x 5.75 mm square on the surface of the skin. Each A-line was a record of the reflected pressure waveform vs depth along a given direction. Due to very high attenuation at 50 MHz the depth of measurable reflected signals is limited by 1 cm.

After processing of obtained data, there were constructed cross-sections of the acupoint at its maximum diameter on the first day of the study, on the second day and so on over a 12-day period. It was shown that acupoint represents regions of enhanced ultrasonic attenuation, which change in size, shape, and even location in short periods of time (less than one day), and then over 12-day period.

One of the most interesting results was that stimulation of BL 67 with ultrasound equipment allowing to compare the speed of response in the visual cortex in the case of stimulation by ultrasound and adequate (i.e. light) stimuli. The amount of time registered between the start of flashing light into the eyes and brain activity was 180-200 milliseconds. The amount of time registered between the ultrasonic stimulation of acupoint BL 67 and brain activity was <0.8 milliseconds. This means that the signal from the acupuncture point comes to the brain two orders of magnitude faster than it does from the flashing light. Thus, the authors suggest that the pathway between the acupoint and the brain is different than the nerve pathway.

In the lecture of 2009 (Jones, Bay 2009) authors described a developed 2-D ultrasound transducer array system which enables to record three-dimensional data in real-time so that off-line analysis and reconstruction can produce a 3-D “real-time” attenuation image of the acupoint during stimulation. Such images clearly show the acupoint extending and twisting itself around the needle, confirming the feeling of “stickiness” felt by practitioners during the stimulation process. This rotation of an acupoint is consistent with the rotation of a chakra as described in the Oriental Medicine literature. Finally, using MRI to monitor the stimulation of acupoints by ultrasound, authors carried out precise measurements of the time between acupoint stimulation and brain activity. Using these methods they discovered three distinct pathways by which information is transmitted from the acupoint to the brain. The first, and previously well-documented pathway, is along the nerves where the brain activity was 180-200 milliseconds following acupoint stimulation. A second and very fast pathway shows brain activity less than 0.8 ms following acupoint stimulation (the measurement error) and may be instantaneous. This activity is two orders of magnitude faster than any known biological process and may be produced by an electromagnetic pulse generated by the acupoint. A third and very slow pathway shows brain activity 15 to 25 seconds following acupoint stimulation. This slow activity correlates precisely with the energy flow (“Qi”) along the meridians as observed by subjects sensitive to this process. Thereby the authors suggest that the pathway between the acupoint and the brain is different than the nerve pathway. Thus, the research of Prof Jones’ group not only confirms the reality of the existence of acupuncture points and channels, but also shows the fundamental difference between the processes in the acupuncture meridians from any known neuro-physiological processes in the physical body (Kronn 2006).

Ultrasonic stimulation of the acupoints offers many advantages over conventional methods and provides an extremely useful tool for the scientific study and the quantitative evaluation of acupuncture (Jones, Bae 2004, 2009). Since the subject experiences no pain or other sensation during ultrasonic stimulation, and no rise in temperature is observed, the method is well suited for sham experiments. Authors believe that in the future, a universal ultrasonic device could be developed that would locate the acupoint (using quantitative ultrasound methods), and then stimulate the acupoint (using pulses of higher ultrasonic energy).

Such a device would greatly increase the accuracy of acupuncture (today, even an experienced acupuncturist can easily place the needle in the wrong location, producing no stimulation and therefore no effect). It would also ensure that the stimulation was done at the correct location with the correct amplitude for the desired effect and would be a totally benign experience for the patient. Authors consider that ultrasonic acupuncture would seem to combine the best of Oriental medicine with the best of Western technology for the improvement of health care.

Scientists from Japan have developed an acupuncture point stimulation device using focused ultrasound (Tsurioka et al. 2010). The ultrasound frequency was 1.83 MHz and the intensity in the center of the focal region was almost the same as used in ultrasound physical therapy (1-3 W/cm^2). The continuous mode was used and the duration of ultrasound irradiation was 36 seconds. The diameter of a concave piezoceramic transducer was 5.5 mm and the focal distance was 9 mm. To confirm effectiveness of the device, acupoints LR 3 located on the foot were stimulated in fifty healthy volunteers and blood flow volume of brachial artery was measured. The volunteers were randomly distributed to two groups. In one of them the subjects were stimulated by conventional acupuncture; in the other group the subjects were stimulated by focused ultrasound. It was found that in both groups the blood flow volume had significantly increased (by more than 20%) at 180 seconds after stimulation. During 36-sec stimulation period, blood flow volume in the group where subjects were stimulated by conventional acupuncture was temporary decreased (about 30%). Although almost all subjects in this group didn't feel pain, nociceptive stimulation due to insertion of needle might cause transient contraction of brachial artery. But in the group where subjects were stimulated by focused ultrasound, no decrease of blood flow volume was observed during stimulation. It might suggest that focused ultrasound stimulation didn't induce nociceptive stimulation. As the device size is about 6 mm in diameter, it can be easily put on the skin during daily life. Because the device stimulates acupoints without any contact with tissues, the advantages of this device are impossibility of infection and the lack of feelings of fear and pain due to insertion of acupuncture needles.

The above-mentioned experiments were described and discussed in more detail in the later publication of the same group (Tsuruoka et al. 2013). According to their opinion, the results should be treated with caution, because that is a pilot study with no placebo control of intervention and with a short examination time. Therefore, the authors intend to compare the effects of ultrasound stimulation with placebo control in clinical conditions in a future study. In this method, the measurement time of blood flow volume was 180 seconds, which seems insufficient to evaluate the effects of ultrasound acupuncture. However, a significant increase of blood flow volume was shown. Additional research should be done to establish whether the blood flow volume at 30 minutes after ultrasound stimulation changes in the same way or differently as compared with acupuncture stimulation. Nevertheless authors consider that focused ultrasound stimulation has potential for use as noninvasive acupoint stimulation in clinical applications, because in healthy persons the effect of focused ultrasound stimulation on blood flow is similar to acupuncture. Further study will be needed to investigate the effects in clinical conditions.

Development of tactile displays

Japan scientists offered and developed an idea to use the ultrasound stimulating effect for the development of tactile displays (Iwamoto *et al.* 2001, Iwamoto, Shinoda 2005, 2006). Such a display has to create rapidly changing images, especially with a complex configuration (geometric figures, symbols, letters, etc.). They consider two possible approaches to solve this problem (Iwamoto *et al.* 2001). One of them, described by Dalecki *et al.* (1995), was based on the use for stimulation the radiation pressure of ultrasound. In their study, the subject's finger was exposed through water to 2.2 MHz unfocused ultrasound which was modulated by rectangular pulses with the frequency of 50, 100, 200, 500, and 1000 Hz (Dalecki *et al.* 1995). In most experiments, an acoustically reflecting target, Corpren™, was affixed to the tissue site. This material is a cork/robber compound that contains large amounts of trapped air and thus acts as an acoustic reflector. Since Corpren™ is an acoustic reflector, it maximizes the radiation force delivered to the tissue site. For the finger, maximum tactile sensitivity was obtained at 200 Hz. They also found that for single pulses of 1 to 100 ms at 2.2 MHz, the threshold radiation forces were an order of magnitude greater than for continuous exposure modulated at 200 Hz (Dalecki *et al.* 1995).

The essence of another approach the researchers from Japan found in the review (Gavrilov *et al.* 1996) where our works of 1970-80s were summarized. That approach was based on the direct activation of skin and deeper located receptors by focused ultrasound.

Iwamoto *et al.* (2001) choose the first approach to avoid the direct action of ultrasound to neural structures. A cap made of foamy silicon rubber was put on a subject's finger. According to the data available to the authors, this rubber almost totally reflected ultrasound. As a source of ultrasound, they used single element focused transducers with operating frequencies of 1 and 5 MHz. The radiation pressure force generated by the apparatus was 0.23 gf/cm^2 per power of 1 W and frequency 1 MHz. On the other hand, the obtained thresholds were close to earlier presented (Gavrilov *et al.* 1996).

The second design of a tactile display applying the effect of the acoustic radiation pressure was based on the use of a linear phased array (Iwamoto, Shinoda 2005). The frequency was 3 MHz and a number of elements was 60. The display can produce a 1 mm diameter focal spot and 2 gf total radiation force. By steering the focal region using a linear phased array, the display creates various precise spatiotemporal patterns of the pressure distribution on the skin.

At last, the next improvement of a tactile display was based on the use a two-dimensional phased array (Iwamoto, Shinoda 2006); an operating frequency was 3 MHz. To generate a focus and move it along the display plane, a focusing system was used, which was a combination of eight linear phased arrays. The angle of convergence of all linear arrays with respect to the system axis was 70 degrees. As in the previous cases, the propagation of the ultrasonic energy was implemented through water. The maximum dimension of the housing, in which all arrays were located, was 8 cm, and the focal distance was 3 cm. Each array has a shape of a trapezium and consisted of 40 piezoceramic elements with different lengths. Thus, a total number of individually controlled electronic channels was 320. The distance between the element centers was fixed and equal to 0.5 mm. The required size of the tactile display was 1 x 1 cm. The acoustic field measurements performed with a hydrophone demonstrated (Iwamoto, Shinoda 2006) that the focus diameter at the intensity level of 25% of the maximum value at the center of focus was 9λ, where λ=0.5 mm is the wavelength of ultrasound, and, at the level of 50%, it was about 5λ, which is an evidence of a very low spatial resolution of the system. The results of computer simulation of the acoustic field generated by this system demonstrated that the intensity in the secondary intensity maxima was 13%, even for the case where the focus was located at the acoustic axis of the system. For the case of steering the focus off the system axis, the intensity in the secondary maxima would be much greater. Thus, the quality of the acoustic field generated by the system needs considerable improvement. Another essential drawback of the system is that it is intended for moving only a single focus along the display area at a given time moment.

During last years scientists from Japan tried to develop tactile displays able to produce tactile sensations and to work in air (Iwamoto *et al*. 2008; Hoshi *et al*. 2009, 2010; Shinoda 2010; Hoshi 2012). This task is much more complicated rather than the work in water because attenuation in air is extremely high. Meanwhile, Iwamoto *et al*. (2008) developed a tactile device which produces stress fields in 3D space. Combined with 3D stereoscopic displays, this device is expected to provide high-fidelity tactile feedback for the interaction with 3D visual objects. The principle is based again on the use of the acoustic radiation pressure. Authors fabricated a prototype device consisting of a 12 channel annular array, a 12 channel driving circuit, and a PC. The measured output radiation force within the focal region was 0.8 gf, and the spatial resolution was 20 mm. Though the produced radiation force was weak for users to feel constant pressure, it was sufficient to induce vibratory sensation up to 1 kHz.

The next version of the device consisted of 324 airborne ultrasound transducers with individual control of phases and amplitudes at each element. The measured output radiation force within the focal region is equal to 1.6 gf (16 mN) which provides suitable touch feeling. The spatial resolution (the diameter of the focal spot) was 20 mm (Hoshi *et al*. 2009, 2010). The developed interaction system enables users to see and touch virtual objects and therefore realize literally touchable 3D images (Hoshi *et al*. 2010, Shinoda 2010).

Recently Hoshi (Hoshi 2012) presented a system which transmits handwriting motion in a tactual manner. It records the writer's handwriting motion and reproduces it on the reader's palm. It enables them to share the handwriting motion and exchange non-verbal information in addition to the appearance of characters and graphics. An ultrasound two-dimensional phased array is used for tactile stimulation. Its characteristics are the following: 384 ultrasound transducers are arranged in a square area whose side length is 20 cm. The resonant frequency of the transducers is 40 kHz (i.e. λ = 8.5 mm). The width of the focal spot (i.e. the spatial resolution) is 13 mm and the focal length is set at 15 cm. The force of 18 mN is produced with all transducers at the maximum power. The ultrasound stimulation is combined with a graphic tablet, and the handwritten characters and graphics are displayed by moving the pressure spot according to the pen strokes. The spot moves at a 1 mm resolution. The experiments showed that users can identify the 26 capital alphabets at a 44-percent accuracy rate. The future works includes (1) increasing the output force of the ultrasound tactile display to make a sensation clearer, and (2) employing a hand-tracking technology to enable users to feel the sensation in an arbitrary position in midair.

Artificial prosthesis of a degenerating retina

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 (Hertzberg *et al*. 2010) 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 favorable characteristics of focused ultrasound are advantageous (Hertzberg *et al*. 2010).

The goal of the following work of the same group of researchers (Naor *et al.* 2012) 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 x 4 cm^2, consisting of 987 elements. The array was positioned above a cornea, and affecting by ultrasound with the frequency of 0.5 and 1 MHz 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 1 MHz was the following: the burst train duration was 10-20 ms, single burst duration was 100 μs, repetition frequency was 1667 Hz, the peak instantaneous intensity was 10-17 W/cm^2 and I_{SPPA} was 5.15-8.5 W/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 10 ms), 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 low MHz 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 2–10 MHz 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 neuro-activation.

Menz *et al.* (2013) 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 43 MHz and a focal spot diameter of 90 μm 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 10–30 W/cm^2 for 50% duty cycle stimulus (1 s on, 1 s off) for most experiments. The 43 MHz carrier frequency was modulated at low frequencies (0.5–15 Hz) to match the temporal pattern used for visual stimulation. For most experiments, this consisted of 1 s of stimulus on and 1 s 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.

1.5. Neurostimulation and Neuromodulation of Brain Structures

First of all, it is useful to clear up the terminology. As it is known, there are two various kinds of effects at the action of focused ultrasound on structures of the brain: ultrasonic neurostimulation and ultrasonic neuromodulation. Dr. Tsirulnikov (2011, unpublished lecture) proposed the following definitions allowed to separate distinctly these very different effects:

1. Let us call ultrasonic neurostimulation an occurrence of functional electric activity of neurons in the brain at direct influence on them ultrasonic stimulus (to our opinion, convincing and unambigiuous evidence of the existence such effects have not been presented until nowadays).
2. Let us call ultrasonic neuromodulation a change of functional activity neurons in the brain under direct or mediated influences on them of ultrasound (there are numerous experimental data demonstrating different examples of ultrasound neuromodulation).

It is worth to note, that in a number of publications of last years connected with the action of focused ultrasound on structures of the brain, effects which, as a matter of fact, were ultrasonic neuromodulation, frequently were named ultrasonic stimulation.

Although there are already many feasible and useful applications of focused ultrasound for functional effects on neural structures, “sensational” ideas and approaches that do not take into account the long-term experience of previous and present studies are increasingly appearing in the worldwide literature and in the mass media. Thus, in recent years, attempts to use a direct effect of focused ultrasound on the human brain to activate the central nervous structures significantly increased. For example, Sony Corporation and Sony Electronics have patented a method and a system for inputting information into the human brain (Dawson 2003). The invention consists of a method and apparatus for stimulating certain areas of the human brain by focused ultrasound to cause different sensations, such as tactile, hearing, visual, and taste. The device is based on the use of a phased array that stimulates certain areas of the cerebral cortex by focused ultrasound. Ultrasound transducers are located inside a helmet, following its configuration, and are in contact with the tissues of the head. According to the authors of the patent, ultrasonic pulses change the state of neural fields, causing users to experience tactile sensations, smell odors, hear sounds, and see graphic images synthesized by a computer. One of the goals of the patent was to return the vision and hearing to blind or deaf patients, respectively. It should be noted that a Sony Electronics spokeswoman has said (New Scientist, # 2494, April 7, 2005, p. 10) that this work is speculative. “There were not any experiments done,” she says. “This particular patent was a prophetic invention. It was based on an inspiration that this may someday be the direction that technology will take us”.

In the end of 2011 an inventor from California has submitted a number of applications for patents which essence is stimulation by focused ultrasound the deep structures of a human brain. It is possible to judge practicability of these inventions under the name of one of the declared patents: «Orgasmatron via deep-brain neuromodulation» (Mishelevich 2011).

The media has reported that researchers at Arizona State University are developing claiming to be sensational neuromodulation techniques for brain stimulation through the skull bone. It is assumed that the ultrasonic dose required for these effects should be significantly less than that ones used for ultrasonic imaging of tissues. According to the developers, the technology could be useful in treating various neurological diseases and for military purposes, such as pain relief immediately on the battlefield in the case of injuries, as well as in computer games, for memory control, entertainment, etc. It is clear that the path from the preliminary experiments described below to the final goal of the authors (managing the functions of the central nervous system) is very long and its eventual success is in doubt.

Some grounds for the proposed ideas and patents were given by experiments in which the cerebral cortex of animals was affected by short pulses of ultrasound. For example, the goal of the series works carried our in the laboratory at Arizona State University led by Dr. W. Tyler was to obtain the effect of neurostimulation of brain tissues with the use of safe regimes of ultrasound irradiation (Tyler *et al.* 2008; Tufail *et al.* 2010, 2011; Tyler 2011). First of all, it was shown using culture of hippocampal slices of a mouse brain that ultrasound with a low frequency (0.67 MHz or less) and low intensity (300 mW/cm^2) can cause the appearance of action potentials and synaptic excitation transfer (Tyler *et al.* 2008). In the following works of this group it was demonstrated that at irradiation of a cerebral cortex of a mice brain by short pulses of ultrasound, the motor reactions of animals were registered (Tufail *et al.* 2010, 2011; Tyler 2011). These effects were observed at very low intensities averaged in time (40– 60 mW/cm^2) in the frequency range from 0.35 to 0.5MHz (Tufail *et al.* 2010). Under the action of ultrasonic pulses (in unfocused beams with different diameters) on the motor area of the mice brain through an intact skull, there was an increase in the frequency of spontaneous impulse activity registered from a head. Tufail *et al.* (2011) presented a protocol of ultrasonic neuromodulation by brain stimulation with transcranial ultrasound. These effects could be hypothetically connected with an activity of nervous cells of the motor area of the brain, or with a pulse activity of muscles of the head (Tufail *et al.* 2010). It was impossible to separate reliably the activities related to the nerve cells and muscles or to determine the dependence of the activity on the diameter of an ultrasonic beam and on a place of ultrasound irradiation of the brain. Thus, no convincing evidence of the appearance of ultrasound-induced impulse activity of brain cells, i.e. ultrasonic neurostimulation of brain, was shown in this paper. It was also shown that in the motor cortex, ultrasound-stimulated neuronal activity was sufficient to evoke motor behaviors (Tufail *et al.* 2010). And again, this effect can be explained by the effect of neuromodulation rather than neurostimulation of brain, as we understand them in accordance to definitions of these terms given above.

The latest years are characterized by especially active work of a number of laboratories studying a functional action of ultrasound on the central nervous structures. That allows hoping for a successful development of this field of research in the future. Brief summaries of a few such works are presented below.

A group at Harvard Medical School led by Dr. S.S. Yoo carried out a series of interesting works in this field. The possibility of using focused ultrasound for treatment for epilepsy artificially induced in rats by means of special chemicals was studied in Min *et al.* (2011a). The diameter of the radiator was 6 cm, the radius of curvature was 7 cm, and the ultrasonic frequency was 690 kHz. Ultrasound pulses with duration of 0.5 ms, with a repetition frequency of 100 Hz and the intensity of 130 mW/cm^2 were used. The animal's brain was affected through the skull twice for 3 min, and an electroencephalogram was recorded. The deviations in the electroencephalogram related to epilepsy rapidly decreased after the effect of ultrasound; behavioral reactions changed in a positive direction as well. Histological analysis confirmed the absence of damage in the brain tissues. According to the authors, low-intensity ultrasound in a pulse mode can be used for a noninvasive treatment for epilepsy. A hypothetical possibility of the treatment for epilepsy in humans by using focused ultrasound, which the authors believe to be an ideal tool for neuromodulation of the brain structures, is considered in Yang *et al*. (2011).

Min *et al*. (2011b) investigated the possibility of the use of low-intensity, pulsed focused ultrasound to modulate the extracellular level of dopamine (DA) and serotonin (5-HT) in the brain of rats. An air-backed, spherical segment ultrasound transducer (6 cm in diameter; 7 cm in radius-of-curvature) operating at a fundamental frequency of 650 kHz was used in this study. The acoustic intensity at the focus was 175 mW/cm^2 (I_{SPTA}). This intensity value corresponded to 3.5 W/cm^2 in terms of spatial peak pulse-average intensity (I_{SPPA}). The following ultrasound parameters were used in this study: 0.5 ms for the tone burst duration and 100 Hz for the pulse repetition frequency. The sonication lasted for 20 min. Focused ultrasound was delivered to the thalamic areas of rats, and extracellular DA and 5-HT were sampled from the frontal lobe using the microdialysis technique. The concentration changes of the sampled DA and 5-HT were measured by high-performance liquid chromatography. A significant increase of the extracellular concentrations of DA and 5-HT in the ultrasound-treated group as compared with those in the unsonicated group was observed. The results showed that focused ultrasound alters the level of extracellular concentration of these monoamine neurotransmitters and has a potential modulatory effect on their local release, uptake, or degradation. Authors believed that the use of pulsed focused ultrasound offers new perspectives for a possible noninvasive modulation of neurotransmitters and may have diagnostic as well as therapeutic implications for DA/5-HT-mediated neurological and psychiatric disorders.

It was shown that pulsed application of focused ultrasound to the regional brain tissue alters the state of tissue excitability, and thus provides the means for non-invasive functional neuromodulation (Yoo *et al*. 2011a). Authors report that the application of transcranial focused ultrasound to the thalamus of anesthetized rats reduced the time to emergence of voluntary movement from intraperitoneal ketamine-xylazine anesthesia. Low intensity focused ultrasound was applied to the thalamus of anesthetized animals. The times required for the animals to show distinct physiological/behavioral changes were measured and compared to those times required in a control session without sonication. The sonication significantly reduced the time to show pinch response and voluntary movement. The modulatory effects of focused ultrasound on anesthesia suggest potential therapeutic applications for disorders of consciousness. Although the mechanism behind this observation is still unknown, the neuromodulatory potential of this non-invasive and spatially-specific tool warrants further investigation.

Yoo *et al*. (2011b) have demonstrated in experiments on rabbits *in vivo* feasibility of using focused ultrasound to transiently modulate (through either stimulation or suppression) the function of regional brain tissue. Focused ultrasound was delivered in a train of pulses at low acoustic energy, far below the cavitation threshold, to the animal's somatomotor and visual areas, as guided by anatomical and functional information from magnetic resonance imaging. A piece of skull was removed while leaving the dura intact and the skin was sutured back over it. The temporary alterations in the brain function affected by the sonication were characterized by both electrophysiological recordings and functional brain mapping with the use of MRI. The modulatory effects were bimodal, whereby the brain activity could either be stimulated or selectively suppressed. Parameters of ultrasound were the following: the diameter and radius of curvature of a focusing radiator were 10 cm and 8 cm, operating frequency was 690 kHz. The intensity of ultrasound, duration of pulses, pulse repetition frequency and number of tone bursts varied over a wide range to induce different physiological effects. For example, the minimal acoustic intensity needed to elicit an excitatory effect was 1.6 W/cm^2 I_{SPTA} (3.3 W/cm^2 I_{SPPA}), which was significantly greater than the range in which Tufail *et al*. (2010) elicited the motor response in mice (less than 180 mW/cm^2) using a non-focused pulsed application of ultrasound. In this case sonication was administered with the duration of 50 ms and repetition frequency of 10 Hz for duration equal to and greater than 1 s. In the other case, it was found that focused ultrasound with shorter pulses of 0.5 ms, repetition frequency of 100 Hz, duration of sonication of 9 s and peak intensity of 3.3 W/cm^2 (I_{SPTA}= 160 mW/cm^2; 5% duty cycle) suppressed the visual activity induced by the light stimulation, as estimated by the measurements of visual evoked potentials. The suppressive effect lasted a few minutes after the sonication. Histological analysis of the excised brain tissue after the sonication demonstrated that the focused ultrasound did not elicit any tissue damages. Unlike transcranial magnetic stimulation, focused ultrasound can be applied to deep structures in the brain with greater spatial precision. The ability to modulate localized neural activation can ultimately be used as a new functional brain mapping method that permits the study of brain connectivity in a much more elegant and non invasive way than it was possible until now. Authors consider that ultrasound-mediated modulation of neuronal activity may also open new avenues of clinical applications for the treatment of various neurological and psychiatric diseases.

Pulsed focused ultrasound applied for excitatory neuromodulation with subsequent small animal FDG-PET scanning has demonstrated localized ultrasound-mediated changes in glucose metabolism of the rat brain (Kim *et al*. 2013). Sprague–Dawley rats underwent sonication to a unilateral hemispheric area of the brain prior to PET scan. The pulsed sonication (3 W/cm^2 in I_{SPTA}, 350 kHz, tone burst duration of 0.5 ms, pulse repetition frequency of 1 kHz, and duration of 300 ms) was applied in 2 s intervals for 40 min immediately after the FDG injection *via* tail vein. Spatially distinct increases in the glucose metabolic activity in the rat brain was present only at the center of a sonication focus, suggesting localized functional neuromodulation mediated by the sonication. In addition to previously suggested evidence for the neuromodulatory effects of focused ultrasound (e.g., motion detection, MRI, electrophysiological recordings, and microdialysis of neurotransmitters), this offers further evidence of metabolic changes induced by focused ultrasound as a neuromodulator. Various neurological and psychiatric disorders are known to be related to aberrant local glucose metabolic activity in the brain. Thus, the authors consider that the modulatory effect of focused ultrasound in brain glucose metabolism may provide several potential clinical applications.

A comprehensive review of the current state of neuromodulation with the use of low-intensity focused ultrasound pulsations was provided by Bystritsky *et al*. (2011). The existing methods of neuromodulation used for the treatment of a number of neurological diseases have significant drawbacks (e.g., lack of special specificity and depth for the repetitive transcranial magnetic stimulation, and invasiveness and cumbersome maintenance for deep brain stimulation for Parkinson's disease). Authors reviewed the background, rationale, and pilot studies to date, using a new brain stimulation method, i.e. low-intensity focused ultrasound pulsation. The ability of ultrasound to be focused noninvasively through the skull anywhere within the brain, together with concurrent magnetic resonance imaging, may create an additional stimulus for research and clinical use of this method. This technique is still in preclinical testing and needs to be assessed thoroughly before being advanced to clinical trials. The review over 50 years of research data on the use of focused ultrasound in neuronal tissue and live brain was presented, and novel applications of this noninvasive neuromodulation method were proposed in this article. Authors believe that it is time to carefully precede to the first human use trials.

Another review article (Jun 2012) characterized ultrasound as a noninvasive neuromodulation tool. It is known that electrical stimulation has been the most common approach for the treatment of some neurological diseases and disorders. However, due to the risk of intrusive surgical procedure, the use of electrical stimulation is limited only to the subjects with profound symptoms. The transcranial magnetic stimulation with the minimum resolution of ~1 cm is currently used for human applications, but it still has the limitation of low focusing capacity and high attenuation though brain tissue. On the other hand, ultrasound is becoming a promising modality as a future neuromodulation approach due to its high spatial selectivity and high penetrating power into the brain. The current stage of the ultrasonic neural stimulation technique is reviewed and the future direction of investigations is discussed in the review.

A feasibility study on a Göttingen minipig, demonstrating reversible, targeted transcranial neuromodulation has been carried out by Mulgaonkar *et al*. (2012).

Surprising results were obtained at the University of Arizona Medical Center in Tucson (Hameroff *et al*. 2013). A possibility to use transcranial ultrasound for modulating mental states in human volunteers suffering from chronic pain was investigated. A clinical ultrasound imaging device was used, with the ultrasound probe applied at the scalp over posterior frontal cortex (visible on the imaging screen), contralateral to maximal pain. In random order, each subject received two 15 second exposures: sham/placebo (in a double blind crossover study), and 8 MHz ultrasound. Following exposure, subjects reported (by visual analog scales) significant improvement in mood both 10 minutes and 40 minutes after ultrasound, but not after sham/placebo. The mechanism by which ultrasound can affect mental states is unknown although authors believe that transcranial ultrasound acts via intra-neuronal microtubules, which resonate in ultrasound megahertz range. Authors plan further studies of ultrasound on traumatic brain injury, Alzheimer's disease and post-traumatic stress disorders.

Scientists from Stanford University (King *et al*. 2013) consider that the mechanisms of ultrasound-induced neurostimulation are not clearly understood as well as little is known about the range of acoustic parameters and stimulation protocols that elicit responses. Authors have established conditions for transcranial stimulation of the nervous system *in vivo*, using the mouse somatomotor response. They use a single element flat transducer with a diameter of 25.4 mm, a center frequency of 500 kHz. All experiments were conducted using continuous wave ultrasound sonications. Ultrasound intensities were ranged from 0.01 to 79 W/cm^2 (0.03 to 1.11 MPa); sonication durations varied from 20 to 480 ms. Authors obtained that continuous-wave stimuli are even more effective than pulsed stimuli in eliciting motor responses. They found also that the responses are elicited with stimulus onset rather than stimulus offset. The probability of transcranial stimulation increases as a function of both acoustic intensity and duration. The motor response elicited appears to be an all-or-nothing phenomenon, meaning that higher stimulus intensities and longer durations increase the probability of a motor response without affecting the duration or strength of the response. Authors confirmed findings of Tufail *et al*. (2010) that noninvasive neuromodulation can be accomplished reliably using ultrasound frequencies at about 500 kHz, however the obtained data that the efficacy increases with stimulus intensity is the most serious discrepancy between the results of the authors and those of Tufail *et al*. (2010), who reported that efficacy of this process decreases with increasing stimulus intensity.

The laboratory at the Institut Langevin (Paris, France) is well-known over the world by their significant works in so called "time reversal acoustics" but recently they began investigations in the field of application of focused ultrasound in neurophysiology. They investigated the role of the pressure field distribution in transcranial ultrasonic neurostimulation (Younan *et al*. 2013). They used in the article the term neurostimulation rather than neuromodulation (our understanding of these terms has been given above in this section), but there is no sense to argue here against terminology. The primary goal of the work was to investigate transcranial ultrasonic neurostimulation at low frequency on anesthetized rats for different acoustic pressures and estimate the *in situ* pressure field distribution and the corresponding motor threshold, if any. A single element focused transducer was used (diameter and radius of curvature of 64 mm). The detailed parameters are as follows: ultrasound frequency was 320 KHz, number of cycles was 75 per pulse (pulse duration = 230 μs), pulse repetition frequency was 2 kHz (duty-cycle = 50%), and the total burst duration was 250 ms. Only the pressure was changed in this study to identify the threshold, and ranged from 0.4 to 1 MPa peak pressure (≈ 4-10 atm). The corresponding acoustic pressure distribution inside the brain, which cannot be measured *in vivo*, is investigated based on numerical simulations of the ultrasound propagation inside the head cavity. A transient motor response has been elicited in anesthetized rats in more than 60% of the experimental sessions; 37 ultrasonic neurostimulation sessions were carried out in rats. The average acoustic pressure threshold was found to be 0.68 MPa (corresponding mechanical index, MI = 1.2 and spatial peak, pulse averaged intensity, I_{SPPA} = 7.5 W/cm^2), as calibrated in free water. Several kinds of motor responses were observed: movements of the tail, the hind legs, the forelimbs, and the eye were induced separately. In some cases, the stimulation of very specific structures such as the occulomotor system or a single whisker was observed, even though the wavelength at 320 kHz is approximately 5 mm. Numerical simulation using a finite-difference-time-domain software and CT scan shown ultrasound reverberations in the head cavity yielding a 1.8-fold increase of the spatial peak, time peak pressure compared to free water, and a 2.3-fold increase of spatial peak, pulse averaged intensity. Applying such corrections due to reverberations on the experimental results would yield a higher estimation for the average acoustic pressure threshold for motor neurostimulation at 320 kHz at 1.2 ± 0.3 MPa (MI = 2.2 ± 0.5 and I_{SPPA} = 17.5 ± 7.5 W/cm^2). At this low frequency, several subwavelength peaks are also created. The acoustic field resulting from the reverberations needs to be carefully taken into account for small animal studies at low frequencies.

1.6. Advantages and Limitations of Focused Ultrasound as a Tool for Neuromodulation

The main advantages of the using focused ultrasound for activation of neural structures are:
1. The technique is essentially noninvasive, i.e., it does not require a surgical intervention to stimulate deep-seated neural structures.
2. The locality of the stimulation can be controlled and changed by altering the resonant frequency of the ultrasound transducers to stimulate predetermined volumes of tissues and selected neural structures.
3. Precise control of stimulus parameters, e.g., intensity, duration of stimuli, their number, repetition frequency, volume of stimulated region and so forth, is possible.
4. The possibility of inducing a wide variety of different sensations, both superficially and at different depths within tissues, is available with the same equipment.

The limitations of focused ultrasound as artificial stimulus are defined by 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.

Let us summarize the results of this chapter.

(1) As follows from the presented materials, the opportunity of ultrasonic neurostimulation of brain structures in the form of spike activity under direct action of focused ultrasound on central neural structures has not proved yet, because it has not been received the convincing data confirming activation of cells of a brain of vertebrate animals under the action of ultrasound.

(2) At the same time it is shown, that effects of ultrasonic neuromodulation of brain structures are rather diverse, and many of them can be successfully used in the medical or diagnostic purposes.

(3) The recorded functional effects on the brain structures are expressed mainly in the form of suppression or reduction of functional activity, but not in the form of its initiation or increase.

(4) The ultrasonic doses required for obtaining the suppression effects sometimes are comparable to the threshold destructive doses. Therefore safety problems with the use of such effects arise.

(5) Neuromodulation of brain structures is advantageous because in principle it is possible to transmit and focus ultrasound into the deep brain sites through skull and brain tissues without essential distortions.

(6) In contrast to other methods (radioactive or chemical therapies) this approach has no any cumulative effects and is repeatable.

(7) The main problem in the wide-spread application of ultrasound methods of stimulation or modulation of neural structures is an unclear understanding of mechanisms responsible for their

activation effects. It is especially important because sometimes it is necessary to extend an ultrasonic application for long period of time to affect the nerve system chronically or repeatedly (Jun 2012). Therefore, the long-term effects of ultrasound on the tissues and the individual cells should be carefully examined and the effectiveness and the safety need to be verified before clinical use.

(8) One of the aims of nearest future is to elicit if the effects of ultrasound on brain tissue could be reliably visualized by MRI.

As already mentioned, not those ultrasonic stimuli that cause only one-time effects, but stimuli that induce long–term and repeated actions without the risk of injury for tissue and neural structures are promising in medicine and physiology. The content of the following chapters is devoted to research into using just this kind of ultrasound stimulation.

Chapter 2

SOMATIC SENSATIONS INDUCED BY FOCUSED ULTRASOUND

This Chapter includes methods of ultrasound stimulation of somatic sensory receptor structures, the description of different types of somatic sensations induced by focused ultrasound, and data on the mechanisms responsible for the eliciting of ultrasound-induced somatic sensations.

2.1. Methods of Ultrasound Stimulation of Somatic Sensory Receptor Structures

Studying the possibilities of stimulation of sensory receptor structures with pulses of focused ultrasound started at the early 1970s in the Acoustics Institute together with a laboratory at the Sechenov Institute of Evolutionary Physiology and Biochemistry of the USSR Academy of Sciences, headed by the famous Russian physiologist Prof. G.V. Gershuni (1905–1992). Almost immediately a laboratory at the I.P. Pavlov Institute of Physiology headed by Prof. O.B. Ilyinski joined to this research. The most outstanding role in all these studies played during several decades Dr. Efim M. Tsirulnikov, Sechenov Institute of Evolutionary Physiology and Biochemistry.

As proposed by Gershuni, the human hand became an initial object of study. The reasons of this choice were the following. Skin and tissues of the hand contain a large number of sensory receptor structures. Mechanical, thermal, and other irritants serve as adequate stimuli for them. Obviously, these studies were much safer than ones on the structures of the central nervous system. Other preliminary objects of the study were Pacinian corpuscles, single mechanoreceptors, isolated from the cat intestinal mesentery, as well as frog ear labyrinth (see Chapter 3).

In brief, 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 (Gavrilov *et al*. 1974, 1976a,b, 1977a; Gavrilov, Tsirulnikov 1980). 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.

An experimental set-up used for the research on the human's hand and forearm is shown in Figure 6 (Gavrilov *et al*. 1976a, 1977a). Ultrasonic frequency varied widely; however, in most experiments, the focusing transducers with the frequencies of 0.48, 0.87, 1.95, and 2.67 MHz were used (Gavrilov *et al*. 1974, 1976a,b, 1977a; Gavrilov, Tsirulnikov 1980). 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 6). 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 85 mm, the radius of curvature was 70 mm, 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 6 (Gavrilov *et al*. 1976b).

Table 6. 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
0.887	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 (Table 6) 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 6. Thus, using the focused ultrasound transducers of relatively high resonance ultrasound frequency, one can stimulate sensory receptor structures within relatively small volumes.

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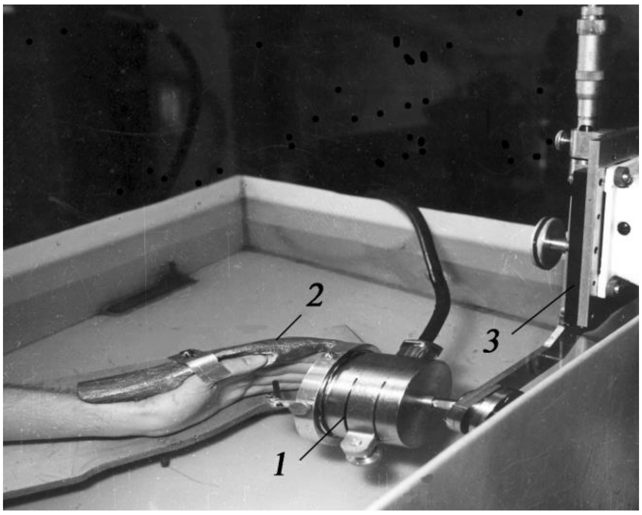


Figure 6. The focusing radiator (1) and casting (2) for fixation of the forearm in a water bath. A part of the coordinating system (3) is also seen (Gavrilov *et al*. 1976a, 1977a)/

The dimensions of focal regions of focused transducers, as well as the space distributions of their acoustic fields, were measured in water in free-field conditions by means of miniature hydrophones (Gavrilov and Tsirulnikov 1980). The measurements gave a good agreement between the theoretical data (see Eqs. 1 and 2 in Chapter 1) and experimental results.

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 7 (Gavrilov and Tsirulnikov 1980; Gavrilov *et al*. 1996). The focused transducer was mounted in a coordination system (positioner) such that a three-dimensional location of the transducer could be controlled with an accuracy of 0.1 mm. The hand and forearm of a human were constrained in a specially made casting of silumin (a light alloy of aluminum and silicon) and submerged in a bath with water of variable and thermostatically controlled temperature, from 15° to 45°C (Gavrilov *et al*. 1976a, b, 1977a; Gavrilov, Tsirulnikov 1980). The transducer focal distance (usually 70 mm) permitted the stimulation not only the skin and superficial neural structures, but also subcutaneous or deep-seated sites of the hand and forearm.

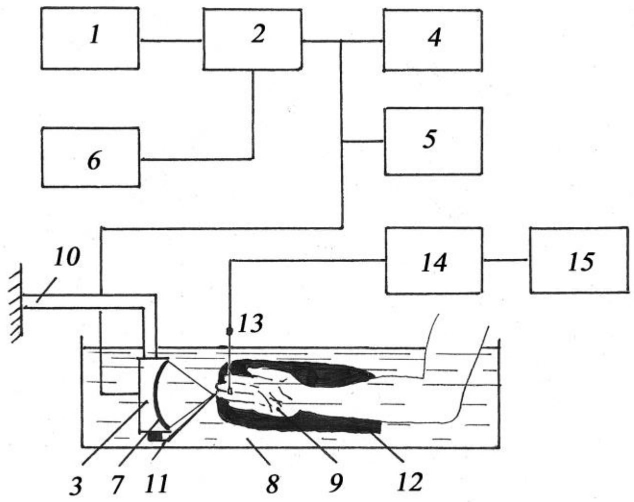


Figure 7. A schematic drawing of the set-up for the study of the stimulation effects of focused ultrasound on the sensory receptor structures of the hand and forearm. 1 - ultrasound generator; 2- modulator and power amplifier; 3- housing of the focused transducer; 4- voltmeter; 5- oscilloscope; 6- generator 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- coordination system (positioner); 11- removable pointer of the center of the focal region; 12- casting of silumin to keep a hand and forearm in physiologically convenient position; 13- miniature hydrophone for measurements of cavitation; 14- selective voltmeter-amplifier tuned to a frequency of one half the ultrasound frequency; 15- oscilloscope (Gavrilov and Tsirulnikov 1980).

Sensory receptor structures usually were stimulated by single pulses of specified durations (from 100 μs to 100 ms and, as an exception, up to 500 ms) with an intensity in the focal region from a few W/cm^2 to thousands of W/cm^2 (for the shortest pulses) (Gavrilov *et al.* 1976a,b, 1977a; Gavrilov, Tsirulnikov 1980). 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 person. The subject was asked to describe its character. On the bases of the reports of subjects 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 different sensations, and, for subjects with normal sensitivity, they were well reproducible. The variation of measured values did not exceed the data scattering in standard psychophysical experiments. From the values of 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 well-known relations for a plane wave (see Chapter 1).

The stimulated points were marked on the skin and these marks were redrawn onto the photograph or schematic drawing of the hand of the person. For example, a photograph of the hand with investigated points of a person is presented in Figure 8 (Gavrilov *et al.* 1977a). Such "maps" enabled the same points to be stimulated on subsequent occasions.

Figure 8. A "map" of the hand of one of the persons with spots examined (Gavrilov *et al*. 1977a).

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 7) placed in water 2-3 cm from the centre 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 40 dB the noise level was taken as a threshold to induce cavitation (Gavrilov *et al*. 1977a).

The values of tissue temperature rise due to absorption of ultrasound energy were calculated with the use of Eq. 11 (Pond 1970) presented in Chapter 1.

A modified focused transducer with a truncated cone-shaped housing filled with water was used on other occasions. The hand or forearm was placed above the open end of the truncated cone and the acoustic contact between the focused transducer and the hand was accomplished through water. For some applications, a thin ultrasound-transparent film was attached to the open end of the truncated cone and the acoustic contact with the skin was provided by means of a thin coating of Vaseline.

In the study of pain in joints, a focused ultrasonic transducer was mounted on a stereotaxic targeting system immersed in a water bath at 37°C and its position above the hand was adjusted so that the focal region of the ultrasound beam was projected into the proximal interphalangeal joint (Davies *et al*. 1996). A variable-voltage input signal was amplified using a broadband power amplifier, the output of which was applied to the ultrasound transducer to produce stimuli of different intensities. The ultrasound transducer had a diameter of 36 mm and a focal length of a 40 mm. The stimulus duration of 100 ms and the variable interval between stimuli were controlled from a microcomputer which also recorded the evoked potentials from the brain. These recording were obtained by the summation of 80 stimuli, 40 of which were at an intensity of 1.5 times that of the threshold of pain perception and 40 at twice the threshold intensity (Wright and Davies 1989).

For the analysis and description of studies of ultrasound-induced somatic sensations, the representations about the receptive field and receptive unit related with it were used. The receptive field implies the site of the skin including one receptive formation, i.e. a receptor or a free nerve ending. The receptor and/or the free nerve ending and related with them the afferent nerve fiber are named by a receptor unit. This concept is used widely in the present physiological literature and has been used long ago in a number of the works of our group (Enin *et al*. 1992, 1993; Tsirulnikov *et al*. 1993, 2000).

Other methodological peculiarities of the research into the stimulation effect of focused ultrasound have been described in a number of papers (Gavrilov *et al*. 1974, 1976b, 1977a,b, 1996; Davies *et al*. 1996; Gavrilov, Tsirulnikov 2012) and books (Gavrilov *et al*. 1976a; Gavrilov, Tsirulnikov 1980; Vartanyan *et al*. 1985).

2.2. Ultrasound-Induced Somatic Sensations

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 other: temperature, pain, tickling and itching.

When the centre 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. Such increased thresholds were apparently due to the tissue attenuation of ultrasound. In this case, the tactile sensations were localized in the place of projection of the centre of the focal region.

Figure 9 (Gavrilov *et al*. 1976a,b) illustrates changes in thresholds for tactile (triangles) and pain (squares) sensations when immersing the center of the focal region under the skin. Abscissa is the immersion depth; the ordinate is the thresholds of sensations expressed in the intensity. The frequency of ultrasound is 0.887 MHz; the duration of the stimuli is 1 ms. Note that tactile sensations were projected by an examinee into the skin whereas the pain sensations were projected into the soft tissues.

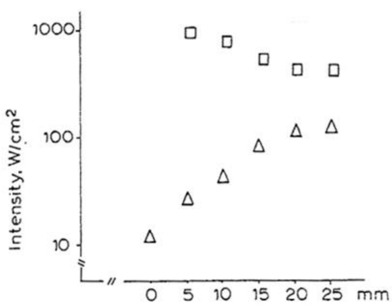


Figure 9. Changes in thresholds for tactile (triangles) and pain (squares) sensations when immersing the center of the focal region under the skin (Gavrilov *et al*. 1976a,b).

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 (2.67 MHz), 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.

A shift of the centre of the focal region with respect to a sensitive spot was accompanied by disappearance of sensations or by higher thresholds. As a rule, the tactile sensations were repeatedly produced with a repeated stimulation while temperature and pain sensations were not. When a sensitive spot was able to respond to ultrasound stimulus with several different sensations, the shortest latency was observed for tactile sensation and longer, respectively, for temperature and pain sensations.

Table 7 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 1 ms and different ultrasound frequencies (Gavrilov *et al*. 1976a,b). 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 Gavrilov *et al*. 1976a,b). 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 7. 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 1 ms and different ultrasound frequencies (Gavrilov *et al*. 1976a,b).

Sensation	Localization	Frequency (MHz)			
		0.48	0.887	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

The projection of the center of the focal region of an ultrasound radiator in soft tissues under a skin, led to occurrence sensations differing from skin tactile sensations and pain (Tsirulnikov, Gurgenitze *et al*. 1985). Deep tactile sensations even at the near-threshold intensity of ultrasonic stimuli had an unpleasant character. Similarly, pain in subcutaneous soft tissues at the threshold level of stimulation had subjectively more unpleasant character in comparison with different kinds of a skin pain (Gavrilov, Tsirulnikov 1980; Vartanyan *et al*. 1985).

Tactile thresholds and the threshold pain on the right and left hands had no reliable differences. It was not revealed also any distinctions of thresholds related with gender differences (Vartanyan *et al*. 1985).

2.3. Tactile Sensations

Dependence of tactile sensations on the duration of ultrasound stimuli

Amongst all somatic sensations induced by focused ultrasound, the tactile ones were associated with stimuli of the lowest intensity. Table 8 (Gavrilov *et al*. 1977a) presents the dependence of the thresholds of tactile sensations in a finger on the duration of stimuli and, simultaneously, on the temperature of water in the bath in which the measurements were carried out. The ultrasound frequency was 2.67 MHz; the results were obtained for two persons. It is seen from the Table that increasing stimuli duration from 1 to 100 ms had not changed reliably the values of associated thresholds. Increasing water temperature from 30 ^{o}C to 40°C resulted in a moderate decrease of the thresholds.

Table 8. The dependence of the thresholds of tactile sensations in a finger on the duration of stimuli and on the temperature of water in which the measurements were carried out (Gavrilov *et al*. 1977a)

Water temperature, ^{o}C	Intensity, W/cm^2		
	1 ms	10 ms	100 ms
	Subject 1		
30	208 ± 28	214 ± 30	240 ± 40
35	196 ± 22	213 ± 18	225 ± 54
40	160 ± 15	160 ± 15	203 ± 29
	Subject 2		
30	240 ± 33	260 ± 23	250 ± 30
35	210 ± 31	200 ± 10	210 ± 14
40	200± 16	197 ± 18	193 ± 19

But in the different parts of a hand and forearm the picture was very different. The work of Vartanyan *et al*. (1990) was carried out especially to study the effect of the stimulus duration on the skin sensitivity in various parts of a hand and forearm for different sensations, including the tactile one. Rectangular pulses of focused ultrasound (2 MHz frequency) with the duration of 1, 5, 50, 100 and 500 ms were used. The center of the focal region was directed to the sensitive points on the palm and back sides of four fields – fingers, hand, wrist and forearm. The results obtained for tactile sensations in 747 sensitive points are presented in Figure 10 (Vartanyan *et al*. 1990). The temperature of water in the bath, where the hand and forearm were located, was about 37 ^{o}C. The threshold intensities are expressed here in decibels; and the intensity of 11 W/cm^2 is corresponded to 0 dB.

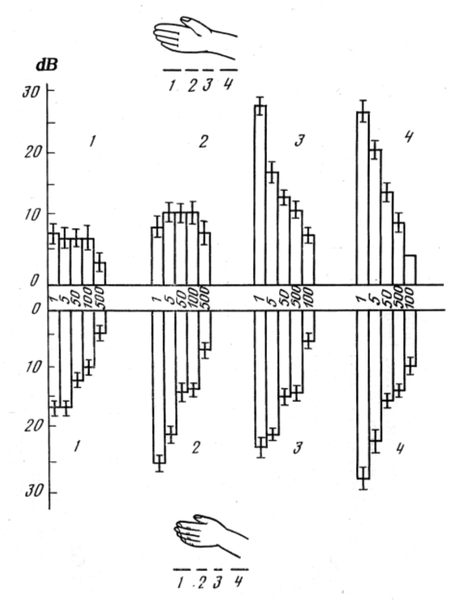


Figure 10. Threshold of tactile sensations in different parts of a hand and forearm on the palm (above) and back (below) sides of four fields – fingers (1), hand (2), wrist (3) and forearm (4). The intensity of 11 W/cm^2 is corresponded to 0 dB. The stimuli durations are changed from 1 to 500 ms (Vartanyan *et al*. 1990).

It is seen from the Figure that the thresholds on the palm side of the hand practically do not depend on the duration of stimuli, which is in agreement with the data in Table 8, but in the wrist and forearm they change significantly with the duration of stimuli.

It is known that the minimal duration of the stimulus causing sensation of a given modality with the least threshold of sensations is called a critical duration of stimulus or a critical time of summation. To measure this important parameter which allows functional comparisons of the integrating (summation) properties of different sensory systems, a special work has been carried out (Andreeva *et al*. 1991). Rectangular pulses of focused ultrasound at a frequency of 2.67 MHz and with the duration varied from 0.1 to 5 ms were used. Thresholds of tactile sensations were determined in 5 healthy subjects (age from 20 to 49 years) for stimulation by focused ultrasound and electric current. It was obtained that the critical duration of stimulus measured in fingers was the same for both kinds of stimuli but for ultrasound stimulation it increased from fingers to forearm. The data obtained suggest that the critical duration of the stimulus is related with the diameter of non-myelinated nerve fibers and increased as the diameter of fibers increased. For the investigated parts of the skin its value for tactile sensations was varied from 1.5 to 2.5 ms.

It was shown, that with the use of short ultrasound stimuli with the duration close to the critical duration of the stimulus, a human perceives a single stimulus. When the duration stimulus becomes $\geq$ 5 ms, sensation becomes “blurring”, or two stimuli are perceived. For stimulus of 10 ms or longer, two tactile sensations are appeared, always coinciding in time with the beginning and the termination of the stimulus. With the stimuli duration longer than 100, the tactile sensations appeared in response to both the beginning and the end of the stimulus and they gradually get an unpleasant character. Stimulation with the duration of 500 ms and more can be accompanied by a temperature sensation or an occurrence of pain. Experiments show that it was practically impossible to distinguish a sensation produced by two stimuli of 10 ms duration spaced by 380 ms from that produced by a threshold stimulus with the duration of 400 ms (Gavrilov *et al*. 1976).

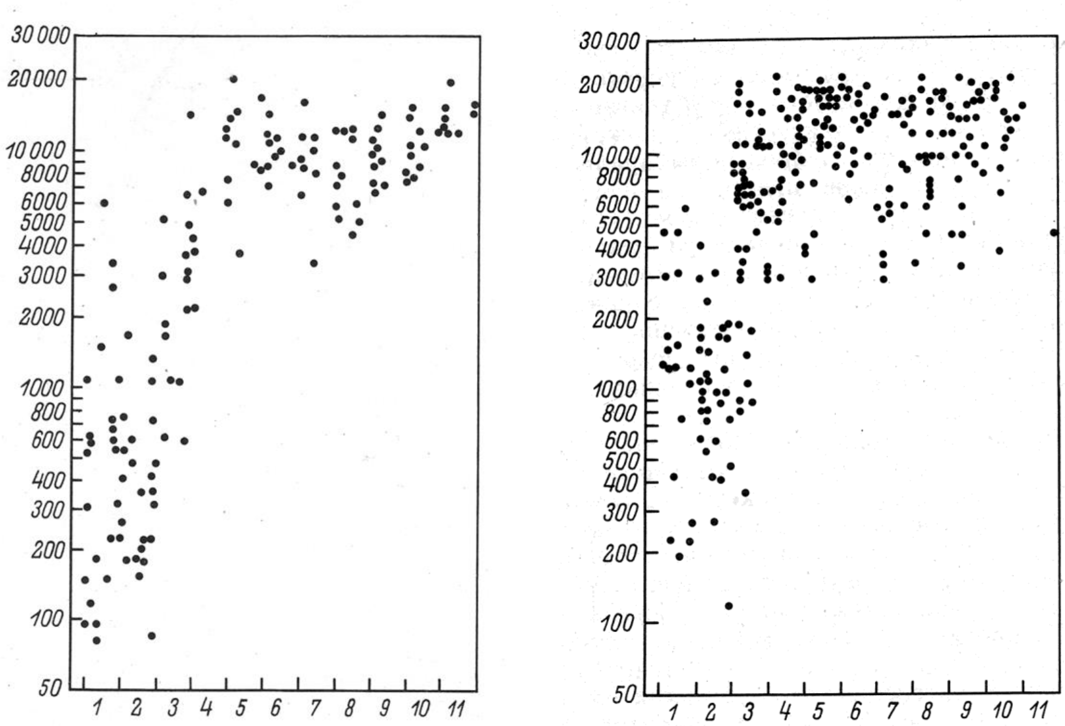
The thresholds measured in the values of the intensity of ultrasound become still lower if the stimulation is carried out not by a single pulse, but with the use of the series of such stimuli with the different repetition frequency of pulses.

Tactile sensitivity in different parts of a hand and forearm

Thresholds of tactile sensations in different parts of a hand and forearm have been investigated (Tsirulnikov, Gurgenidze *et al*. 1985; Vartanyan *et al*. 1985, 1990). The hand and forearm were immersed into a bath with water (Figure 6); the temperature of water was about 30^{o}C. After achievement by an examinee of a temperature adaptation, the action of ultrasound on arbitrary chosen points on the skin or in deep soft tissues of fingers, palms or forearms was carried out. The ultrasound frequency was 2.67 MHz. Single stimuli of a rectangular form with the duration of 1 ms were used. The center of a focal region of a focused radiator, where the intensity of ultrasound is maximal, was aligned with the chosen point on a skin or in subcutaneous soft tissues, on the depth of 5-15 mm from a surface of a skin.

There were tested 480 points on fingers, palms and forearms in 9 healthy persons aged from 18 till 45 years (2 women, 7 men). In 101 points (mainly in soft tissues of a forearm, and occasionally on the skin of the forearm), the sensations were not obtained. In dependence on a direction of the center of the focal region and, accordingly, on the projection of the sensations on the skin or in deep soft tissues, the data were divided to two groups: the first - with sensations projected on the skin (137 points), the second - with sensations projected in soft tissues (242 points).

Figure 11a (Tsirulnikov, Gurgenidze *et al*. 1985; Vartanyan *et al*. 1985) presents the results of measurements of thresholds of tactile sensations which are projected by a person on the skin (the first group). In spite of an essential scattering of the values of thresholds, an evident regularity is seen: tactile thresholds on a skin increase in a direction from fingers to an elbow. Figure 11b shows results of measurements of tactile thresholds in the second group when the focal region of an ultrasound radiator has been directed to the deep soft tissues and the sensations were projected to subcutaneous tissues. It is seen that on the forearm the tactile thresholds in soft tissues are higher, than on the fingers and palm. For both groups of data, i.e. for skin sensations and for sensations in the soft tissues, there were not found any sensitive points (sites 4-11 in Figure 11), in which the minimal thresholds would be equal to or smaller than the thresholds on fingers and a palm (sites 1-2). This result agrees with the published data on the structure of skin receptors and suggests that the threshold values depend on the density of the receptors distributed on the skin surface rather than on their morphological features (Tsirulnikov *et al*. 1982). It was obtained also that the thresholds of tactile skin sensations appeared to be higher on the dorsal surface of the hand, rather than on the palm side (see Figure 10).



(a) **(b)**

Figure 11. Thresholds of skin tactile sensations in different parts of the hand and forearm. a) the sensations are projected by a person on the skin; b) the sensations are projected by a person to the deep soft tissues. The horizontal axis is the place of measurements: 1- fingers; 2- a palm; 3 - a wrist; 4-11 – sites of the equal length on the forearm in a direction from a wrist to an elbow. The vertical axis is the threshold intensity of ultrasound in W/cm^2 (Tsirulnikov, Gurgenidze *et al*. 1985; Vartanyan *et al*. 1985).

These studies have shown that there are sensations in humans which can be considered as the deep tactile one. The prevailing quantity of points in which the tactile sensations were not obtained was registered in soft tissues, mainly on the forearm, where sensory receptor structures, possibly, are located more discretely than on fingers and a palm.

For deep sensations, as well as for the skin ones, the results are in agreement with the assumption that the quantity of perceiving structures in tissues decreases in the forearm in comparison with fingers and a palm. The data testify to a larger amount of the skin receptor structures in comparison with deep located ones, in particular on a forearm, where, as it was already noticed, in many deep points tactile sensations were absent. Therefore it is possible to conclude that deep tactile sensations are not related with the skin receptor structures or nerve fibers going from them.

The obtained results show that for the deep tactile reception, similarly the skin one, the value of a threshold is not defined by the morphological characteristic of perceiving structures, and depends on the density of their distribution in the subcutaneous soft tissues including muscles, tendons, vessels, and connecting or fatty tissue. The fact of the dependence of the thresholds of skin sensations on the density of the distribution of sensitive points in the skin is discussed in several works (Tsirulnikov *et al*. 1982, 1986, 1988; Tsirulnikov, Gavrilov *et al*. 1985; Tsirulnikov 1986). The sensitivity of subcutaneous soft tissues does not differ considerably from the sensitivity of skin, but are still higher than in skin. It increases more considerably for periosteum and synovial sheaths of joints, being characterized also by the increasing of an unpleasant shade (Gavrilov *et al*. 1976a; Gavrilov, Tsirulnikov 1980). In subcutaneous soft tissues, the deep tactile sensations are elicited with an unpleasant shade in comparison with tactile skin sensations. For periosteums and synovial sheaths of joints, only the pain arising without any previous sensations of a tactile character is typical (Tsirulnikov *et al*. 1982; Tsirulnikov, Gurgenidze *et al*. 1985).

2.4. Temperature Sensations (Warmth, Cold)

In some spots within the skin, increasing the intensity of focused ultrasound resulted in temperature sensations, when a person felt warmth or cold sensations (Tsirulnikov, Shchekanov 1975, 1976; Tsirulinkov 1977; Gavrilov, Tsirulnikov 1980; Vartanyan *et al*. 1985). The sensation is expressed mostly at the place of stimulation but could also spread. For example, a cold sensation evoked in a spot on the skin of a finger tip could irradiate along the finger skin, the palm and the distal third of the forearm. The temperature sensations disappear when the focal region was directed under the skin. Similar to the tactile sensations, the warmth ones could be evoked in the skin of the opposite side of the hand if the focal region was projected there through soft tissues. The cold sensations did not appear in this case.

The threshold temperature sensation was taken to be a sensation of warmth or cold reproduced by repeated presentation of the corresponding stimulus or a stimulus with a slightly higher intensity. The thresholds for temperature sensations, as those for tactile ones, increased with the moving of the focal region from fingers to forearm; they decreased with increasing of the stimulus duration. If stimuli of the same or higher intensity were repeatedly directed to the same spot, the temperature sensations, as a rule, were reproduced. On different days, however, repeated stimulation of the same spot could fail to result in temperature sensation.

Figure 12 (Tsirulnikov 1977; Gavrilov, Tsirulnikov 1980) presents the data of 18 experiments performed in different days on the same person and in the same spot on the skin of the tip of the index finger. The ultrasound frequency was 2.67 MHz; the stimulus duration was 1, 10 and 100 ms. The temperature was changed from 35^{o}C to 45 ^{o}C. It is seen that at the stimulus duration of 1 and 10 ms and the temperature of 30^{o}C the cold sensations appeared predominantly. At the temperature of 35 ^{o}C both cold and warmth sensations were elicited, and at the temperature of 40-45 ^{o}C only the warmth sensations were obtained.

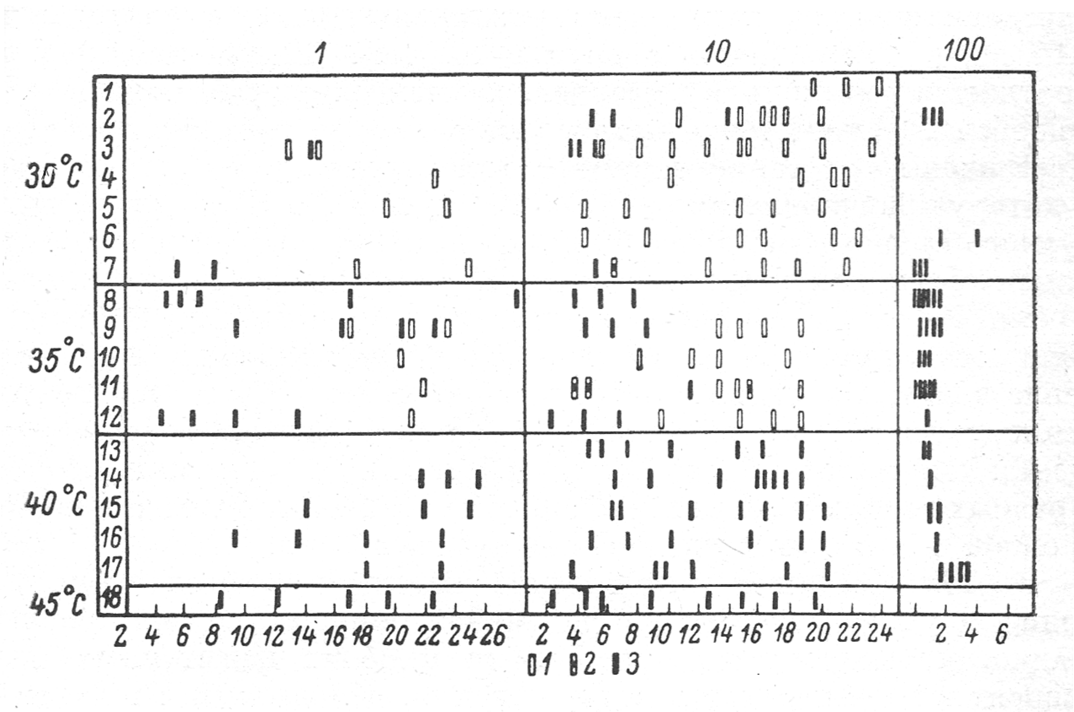


Figure 12. Change of the modality of temperature sensations at the action of focused ultrasound on the same sensitive point of a human index finger in depending on temperature of water in which the hand was immersed. Figures above are the duration (in ms) of action of focused ultrasound with the frequency of 2.67 MHz; figures below are the intensity of ultrasound in the focal region, W/cm^2 x 10^3; at the left - temperature of water in the bath (°C) and a number of experiment. *1* - sensation of cold; *2* - sensation of warmth, replaced by sensation of cold; *3* - sensation of warmth (Tsirulnikov 1977; Gavrilov, Tsirulnikov 1980)

Figure 13 (Gavrilov *et al.* 1976a, 1977a) illustrates the similar dependence more evidently and presents the data obtained in 17 experiments only for the stimulus with the duration of 10 ms and in the temperature range of 30-40 ^{o}C. If both types of sensations appeared, the warmth sensation always preceded the cold one.

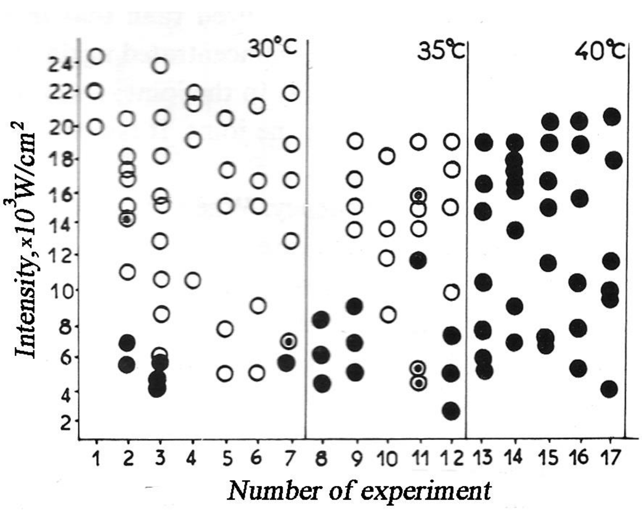


Figure 13. Relationship between the modality of the temperature sensation (cold-warmth) and the temperature of surrounding medium. ○ - cold sensation; ● - warmth sensation. At the top is the temperature of surrounding medium (water in the bath). Ultrasound frequency is 2.67 MHz; stimulus duration is 10 ms. Data from 17 experiments done in different days on the same spot of the index finger skin (Gavrilov *et al*. 1976a, 1977a).

A special series of experiments was done where a thermode, with a tip diameter of about 1 mm, was used to find “warm” and “cold” spots on the palm skin (Gavrilov *et al*. 1976a, 1977a). The temperature of water circulating in the thermode was, respectively, 50 or 10 ^{o}C. When the points found by the thermode were stimulated with ultrasound stimuli (frequency of 0.887, 1.95 and 2.67 MHz; duration of 1 and 10 ms; water temperature of 34-36 °C), the cold or warmth sensations appeared independently on whether a point under study was previously determined as “warm” or “cold”. For example, a “warm” point was determined on the palm skin of a subject with the use of a warm thermode. But stimulation with the ultrasound stimuli elicits sensations of both cold and warmth. In another case, a “cold” point determined on the palm skin by the thermode gave a warmth sensation when it was stimulated by ultrasound. Thus, irrespectively of whether a point determined with the thermode was “cold” or “warm”, it could generate either cold or warmth sensations in response to ultrasound stimuli. As in Figs. 2.8-2.9, the cold sensations appeared at a temperature of 30 °C, while at 40 °C the warmth sensations appeared.

Thus, the results of experiments carried out with the use of focused ultrasound have allowed to prove a hypothesis according to which the same perceiving nervous structures are responsible for sensations of warmth and a cold (Tsirulnikov, Shchekanov 1975, 1976; Tsirulnikov 1977; Vartanyan *et al*. 1985). Occurrence of both types of temperature sensations depends on a fixed relation of an internal body temperature and an ambient temperature (Tsirulnikov 1977). From Figure 12 and the results of many other 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 1 ms. Simple calculations fulfilled with the use of formulae for evaluation of the temperature rise due to absorption of ultrasound in tissues (see, e.g., Eqs. 10-11 in Chapter 1) showed that even for relatively high intensities of ultrasound the temperature rise ΔT will not exceed 0.5-1 °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 °C 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 10 ms the mechanical factor is prevailing, but for the durations of longer than 50 ms 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 (Enin *et al*. 1992). For example, pulse with the duration of 400 ms could consist of the elevating and falling off components with the duration 100 ms each and a plateau of 200 ms. 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 70-120 W/cm^2 (frequency of 2.6 MHz), which corresponded to the temperature rise of 0.9-1.4 ^{o}C.

2.5. Pain

With further increasing of the intensity of stimuli and action on some spots, a sensation of pain is appeared. It occurred more often with stimuli of 10-100 ms duration than with shorter stimuli (1 ms). 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 centre 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.

The threshold intensity was taken to be the intensity of a stimulus to produce, with gradually increasing intensity from stimulus to stimulus, the first sensation of pain. It was found that depending on the location of the centre of the focal region, pain sensations, namely pain in skin, in soft tissues, in bone and in the joint differed markedly in the intensity required to achieve thresholds, the area of the pain irradiation and in subjective characteristics of the response.

Thus, four types of the pain sensations were observed (Gavrilov *et al*. 1976a,b, 1977a; Gavrilov, Tsirulnikov 1980; Vartanyan *et al*. 1985; Tsirulnikov *et al*. 2000).

(1) Focal region center within the skin. Sensation of a sharp localized burning pain, similar to a sharp pain from pricking a hand with a needle but with a burning component.

(2) Focal region center in soft tissues beneath the skin. Sensation of pain at a defined depth within a tissue; radiated slightly around the point of stimulation but without a burning component. The sensation was "more unpleasant" and lasting longer than that obtained in the skin.

(3) Focal region center is projected into the bone. Such pain was named a periosteal pain because it is known from the surgical experience that, after removal of a periosteum, the bone at surgical interventions is painless. This sensation is even less localized than that in soft tissues, irradiates over a long distance along the bone and is concentrated again at the place of stimulation, with a "far more unpleasant feeling". Other sensations, except for a pain, were not obtained after an impact on a periosteum.

(4) Focal region center in a joint. Sensation is similar to the pain in the periosteum but irradiating only within the joint. This kind of pain was named intra-articular pain. It was also spread quickly and then also came back to the place of stimulation. A sensation is similar to the pain in the bone. It was assessed by the subject as the "most unpleasant" sensation.

Table 9 (Gavrilov *et al*. 1977a) gives presentation about the values of thresholds corresponding to all these types of pain sensations. Here the lowest thresholds obtained from two subjects (water temperature is 35 ^{o}C) are given. (It was assumed that the lowest thresholds are associated with the most optimal conditions for stimulation effect, due to a more exact coincidence of the centre of the focal region with a receptor structure. Because of this, the comparison of the lowest thresholds should be preferred to the procedure used in the conventional statistical treatment.) It will be seen that threshold intensities depend on the type of pain and the part of the extremity stimulated (fingers, palm, and forearm). It is seen also that the threshold intensities for pain decreased markedly when the duration of the stimulus was increased from 1 to 100 ms.

Table 9. Pain thresholds (in W/cm^2) with different location of the focal region and at the different frequencies (Gavrilov *et al*. 1977a)

Parameter	*I*, W/cm^2								
Duration, ms	1			10			100		
Frequency, MHz	0.48	0.887	2.67	0.48	0.887	2.67	0.48	0.887	2.67

Location of the focal region									
Skin									
Fingers	14	140	9000	12	100	3600	13	80	620
Palm	160	680	1700	50	140	3000	45	140	620
Forearm	700	-	-	65	-	11400	13	340	200
Soft tissues									
Fingers	120	100	13400	28	100	2200	14	80	220
Palm	27	820	17000	42	1380	-	42	220	1800
Forearm	225	x	-	120	x	x	350	x	1200
Bone									
Fingers	12	380	x	13	120	x	13	60	x
Palm	90	160	15000	60	-	11600	590	-	800
Articulatio interphalangea of the middle finger	12	36	3000	28	80	3000	x	x	1200
Articulatio metacarpophalangea of the thumb	200	x	18800	140	x	13000	130	x	700

- absence of pain sensation with maximum intensity of ultrasound stimulus; x - not studied.

The thresholds for all types of pain exhibit a tendency to increase with the moving of the focal region in a proximal direction, i.e. from fingers to forearm, both on the skin and in the deep tissues. It is illustrated by Figure 14 (Tsirulnikov, Gurgenidze *et al*. 1985; Vartanyan *et al*. 1985) where thresholds of pain sensations in subcutaneous soft tissues of different parts of the hand and forearm are shown. These data testify that values of pain thresholds depends on the density of distribution of receptors structures in tissues.

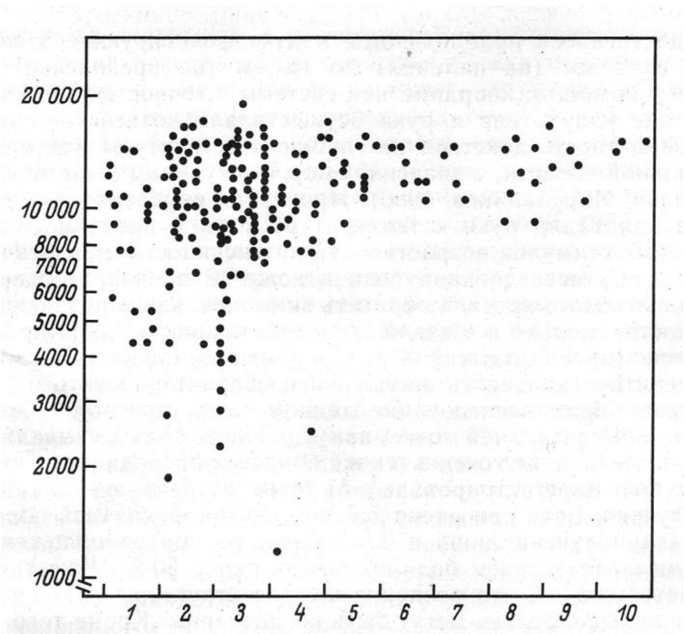


Figure 14. Thresholds of pain sensations in subcutaneous soft tissues of different parts of the hand and forearm. The horizontal axis is the place of measurements: 1- fingers; 2- a palm; 3 - a wrist; 4-10 – sites of the equal length on the forearm in the direction from a wrist to an elbow. The vertical axis is the threshold intensity of ultrasound in W/cm^2 (Tsirulnikov, Gurgenidze *et al*. 1985; Vartanyan *et al*. 1985).

When the focal region covered two or three types of tissue, as could easily be the case on the finger tip, there appeared a mixed pain sensation. The subjects trained to differentiate each type of pain sensations were able to sort out such a sensation into its separate components. Stimulation of the forearm, rather than the finger, proved superior for the selective study of pain sensations in skin and soft tissues.

The dependence of the thresholds of pain sensations on the duration of ultrasonic stimuli has been investigated in detail (Vartanyan *et al*. 1990). The thresholds of pain sensations were determined at 179 different points of the hand and forearm (finger, palm, wrist and forearm). The durations of stimuli were 1, 5, 50, 100 and 500 ms and the ultrasound frequency was about 2 MHz. The threshold intensity at the focal region of the ultrasound beam that was required to induce pain sensations decreased markedly when the duration of the stimulus increased from 50 to 500 ms.

A separate series of experiments was carried out to evaluate the possible influence of ultrasonically induced cavitation in soft tissues on the origin of pain sensations (Gavrilov, Tsirulnikov 1980). Cavitation was controlled by the measurement of the subharmonic components of the acoustic noise arising when cavitation appeared in the medium. The methodical details of such measurements have been given in Section 2.1.

With stimulus duration of 1 ms, no pain sensation, as a rule, was reported, although violent cavitation was registered. With stimulus duration of 10 ms, the threshold intensities for pain exceeded the intensities corresponding to cavitation thresholds. At stimulus duration of 100 ms, cavitation appeared at lower intensities than pain in some cases but, in other cases, when pain was experienced, cavitation was absent. Naturally, in these particular experiments, it was rather difficult to separate reliably cavitation occurring in the tissues from that in water; however, in terms of possible correlation between pain and cavitation, the most noteworthy cases were those in which pain was experienced but cavitation was not registered. Thus, in these experiments, there was no defined causal relationship between the appearance of cavitation and threshold sensations of pain (Gavrilov, Tsirulnikov 1980).

Naturally, such experiments could not give a direct and unambiguous explanation of the relationship between the ablation of the tissue and the induction of pain. From this point of view, another of our studies was more informative, in which it was found that pain in a defined sensitive point in one of the participants of the work could be reproduced many times over a number of years without a marked change in the pain threshold. Thus, at least in our experiments, threshold pain was not associated with the destruction of, or injury to tissues. This important conclusion suggests that it is not always correct to use the term “nociception” (from the Latin word “nocivus” meaning to hurt or to injure) for the characterization of a threshold sensation of pain.

The peculiarities of pain and tactile sensations induced by focused ultrasound in the acupuncture points and in arbitrary deep-seated sensitive points were investigated (Tsirulnikov *et al*. 1986; Vartanyan *et al*. 1985). It was presumed that in the acupuncture points there was a greater preponderance of receptor structures related with pain. The sensations after ultrasonic stimulation of the acupuncture points were similar to so-called “foreseen” sensations induced by the use of needles for acupuncture.

A detailed investigation of the subjective characteristics and the threshold intensity for skin pain sensations in the different parts of the human body was carried out (Tsirulnikov, Gurgenidze 1990). Single pulses of focused ultrasound with the frequency of 1.98 MHz and duration of stimuli of 500 ms were used in this study. Maps of the distribution of the thresholds of specific skin pain in the human body were drawn. In the majority (up to 90%) of the 3750 points investigated, the pain sensations in superficial, cutaneous layers had relatively low thresholds and similar qualitative characteristics in the different parts of the body, which suggests that specialized receptors had been stimulated. The thresholds of such kind of pain were identical over all the surface of the body.

Such pain has been classified as “specific pain” to distinguish it from a qualitatively and quantitatively different pain sensation, which has been classified as “nonspecific” pain. Subjectively, this “nonspecific” pain was far more “unpleasant” and frequently associated with a “burning” sensation. The intensities of focused ultrasound required to induce “nonspecific” pain differed markedly in various parts of the body and the pain probably resulted from a super-intense stimulation of tactile or temperature receptors. The threshold intensity for “nonspecific” pain increased from the fingers to the elbow (Tsirulnikov, Gurgenidze *et al*. 1985).

Pain produced by projecting a focused ultrasound stimulus at 0.5 MHz for the duration of 100 ms into the interphalangeal joint of the hand in human persons was monitored by recording the brain-evoked potential (Davies *et al*. 1996, Wright and Davies 1989). These recordings were obtained by the summation of 80 stimuli, 40 of which were at an intensity of 1.5 times that of the threshold of pain perception and 40 at twice the threshold intensity.

From the collected medical experience, it is known about the existence of other kinds of pain differing from ones described above: pleural, peritoneal, etc., however not all of them can be referring to somatic pains because of the location of their origin.

2.6. Other Somatic Sensations

Aside from tactile, temperature and pain sensations, focused ultrasound was capable to evoke the feeling of itching and tickling (Gavrilov *et al*. 1976a,b, 1977a; Gavrilov, Tsirulnikov 1980) . 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.

2.7. Mechanisms of Some Ultrasound-Induced Somatic Sensations

Obviously, understanding the mechanisms of activating action of focused ultrasound on the structures responsible for skin and tissue sensitivity, as well as 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 (Gavrilov 1984; Gavrilov *et al*. 1996; Gavrilov, Tsirulnikov 2002).

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 in Chapter 4) and reactions in the nerve fibers of skates obtained using focused ultrasound (Broun *et al*. 1980) (see in Chapter 4) 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 our studies (Gavrilov *et al*. 1976a,b, 1977a, 1996; Gavrilov, Tsirulnikov 1980, 2002; Gavrilov 1984). The goal of these studies 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 when ultrasound of different frequencies was used.

The frequencies were ranged from 0.48 to 2.67 MHz. 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 are shown in Figure 15 (Gavrilov *et al*. 1996).

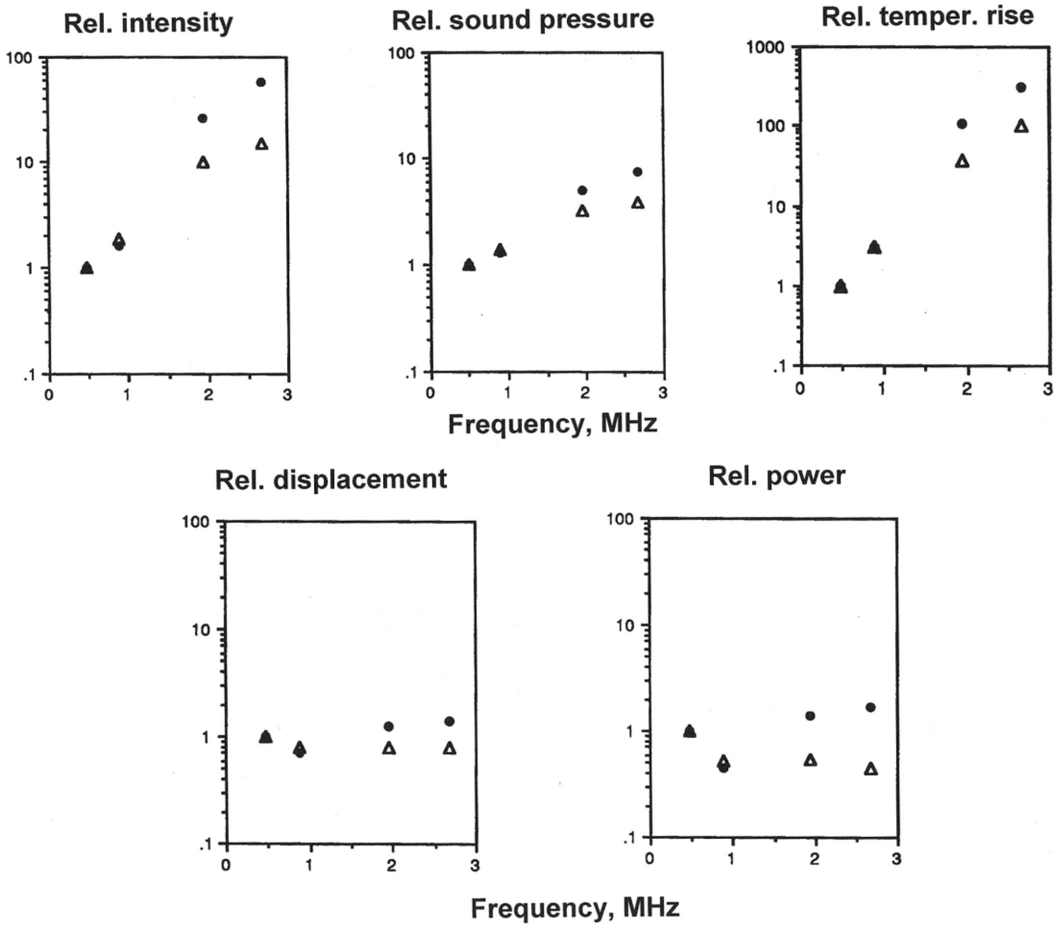


Figure 15. Relative changes in the parameters of focused ultrasound (intensity, sound pressure, temperature rise, displacement amplitude, and acoustic power) when the frequency changed from 0.48 to 2.67 MHz for tactile (triangles) and thermal (points) sensations in the human fingers (Gavrilov *et al.* 1996)

The figure shows 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.

It is seen from the Figure that, formally, the amplitude of displacement 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 (Gavrilov *et al*. 1976a,b, 1977, 1996; Gavrilov, Tsirulnikov 1980, 2002; Gavrilov 1984). 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 application. Indeed, the focal spot area (32 mm^2 for the frequency of 0.48 MHz and 1 mm^2 for the frequency of 2.67 MHz) 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*. (1998) it was shown that amplitude-modulated ultrasound with a carrier frequency of 3 MHz, modulation frequency of 1 kHz, the speed of shear waves in tissues of 3 m/s, and the intensity on the axis of ultrasonic beam of 10 W/cm^2, produced a displacement of about 30–40 μm 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 Pishchalnikov *et al*. (2002):

$$u_{\max} = \frac{\alpha a}{\rho c_l c_t} t_0 I \qquad \text{for short pulses } (t_0 << a / c_t) \; , \qquad (18)$$

where a is the radius of the sound beam (i.e., the radius of the focal region), 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 (1) 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 more than on the intensity of ultrasound by itself. In Gavrilov, Tsirulnikov (2002), 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

$$u_{\max} = \frac{\alpha}{\rho c_l c_t^{\,2}} a^2 I = \frac{\alpha}{c_l \mu} * W \qquad \text{for long pulses } (t_0 >> a / c_t) \; , \quad (19)$$

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 several years later Myers (2006) obtained the following formula for calculation of $u_{\max}$:

$$u_{\max} \approx \frac{4\pi \alpha I z^2}{c_l \mu (kR)^2} \quad . \qquad (20)$$

It is easy to show that this equation can be transformed into

$$u_{\max} \approx \frac{\alpha I a^2}{1.168 c_l \mu} \quad . \qquad (21)$$

Thus, it is seen that Eqs. 19 and 21 are similar and differ only by a coefficient 1.168 in the denominator of (21), i.e., taking into account an approximate character of Eq. 21, they are qualitatively equivalent. Thus, the maximum amplitude of the displacement is proportional to the acoustic power and, hence, to the radiation force.

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 16 (Gavrilov, Tsirulnikov 2002). One can see that the displacement of the medium (Figure 16c) does not reproduce the shape of an acoustic signal (Figure 16a), or the acoustic power (Figure 16b). 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 = 1 mm and c_t = 3 m/s, it is t_0 = 0.3 ms, which is significantly shorter than the duration of an ultrasonic stimulus (usually from 1 to 100 ms). 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 400 ms caused tactile sensations in response to the beginning and the end of a stimulus and that a tested person could not distinguish one long pulse (for example, 400-ms duration) from two short pulses separated by the same time interval (Gavrilov *et al*. 1976; Gavrilov, Tsirulnikov 1980; Gavrilov 1984).

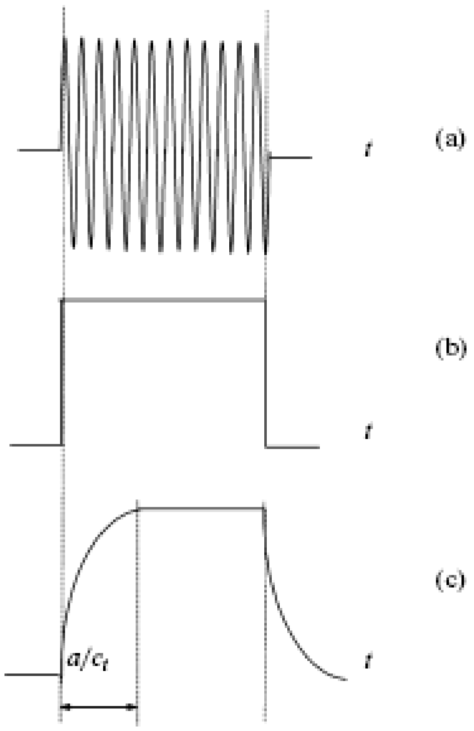


Figure 16. Diagram illustrating the shape of an acoustic signal (a), acoustic power (b), and shear displacement of the medium (c) under the effect of an ultrasonic pulse (Gavrilov, Tsirulnikov 2002).

These data support a conclusion that stimulation of neural structures is caused by 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 focused ultrasound. It is agree with the conclusions of other authors on the factors responsible for the evoking of tactile sensations with the use of focused ultrasound (Dalecki *et al*. 1995).

Thus, studies show that there are at least two factors responsible for stimulation by focused ultrasound of peripheral structures related to tactile and thermal sensations. The first one is a unidirectional effect caused by the gradient of the mechanical displacement of the medium due to the radiation force. The second possible factor is the direct action of focused ultrasound on biological media, which has been discussed in details in Chapter 1. For example, it follows from Table 3 (Section 1.1) that, e.g., at the frequency of 3 MHz and intensity of 10 W/cm^2 (combination, which is often used for stimulation of receptor structures), the displacement amplitude A is 0.02 microns, acceleration a is $7\cdot10^8$ cm/s^2 (that exceeds the acceleration of terrestrial gravitation in about$\cdot10^6$ times), sound pressure P = 5.5 atm, and the pressure gradient over the distance of one-half wavelength ΔP = 450 atm/cm. Because for a given frequency of ultrasound, a half wavelength ($\lambda/2$) is equal to 250 μm, the pressure gradient at the cellular structure with the size of, e.g., 50 μm will be equal to about 10 atm/mm. So, such intensive mechanical action on the tissue elements can potentially lead to various physical and physiological effects, e.g., oscillations of gas bubbles in biological media, an increase in membrane permeability, etc.

It has been shown in Section 1.1 that the use of relatively long pulses of focused ultrasound of rather high intensity is accompanied by the essential heating of tissues due to absorption of ultrasound energy in them. Evidently, the thermal factor is able to influence to an occurrence and formation of some sensory sensations and, first of all, temperature and pain sensations.

Mechanisms of pain sensations appearing under the effect of focused ultrasound include some combination of the effective factors and require further studies.

2.8. General Comments on the Use of Focused Ultrasound in Studies of Somatic Reception

Thus, it has been shown that with the use of short stimuli of focused ultrasound different somatosensory sensations could be induced corresponding to a projection of the center of the focal region in the skin, subcutaneous soft tissues, bones, joints, etc. Focused ultrasound could evoke practically all sensations to which a human is subjected under natural conditions, i.e. tactile, thermal (warmth and cold), pain, tickling and itching. It was important that there was a possibility to elicit monomodal sensations and their various combinations (Tsirulnikov *et al.* 1993). To obtain monomodal sensations (touch, warmth, cold and various kinds of pain), it was necessary to use rectangular ultrasound stimuli of duration from 1 to 10 ms. In such pulses, the main effective factor in evoking the sensations was the mechanical displacement of the medium in the focal region of the radiator. With the use of longer pulses, e.g. with the duration of 500 ms or so, together with the mechanical displacement at the beginning and end of the pulse, additional factors as the temperature rise in the focal region and also the effect of mechanical oscillations of the ultrasound frequency were added.

When the focal region was localized in soft subcutaneous tissues, deep tactile and deep pain sensations were evoked. In bones and joints there appeared only periosteum or joint pain, which differed from other types of pain in their threshold values, subjective characteristic, irradiation, concentration and duration of the sensation after the action of the stimulus. Most likely bone and joint pain are associated with the action of the ultrasound stimuli on the periosteums and synovial sheaths of joints respectively.

Two varieties of post-stimulus pain were evoked: nonspecific, replacing tactile or thermal sensations after an extra-strong action on the corresponding structures in the skin, and specific, most likely associated with stimulation of specialized receptive structures in the skin (Tsirulnikov, Gurgenidze 1990). The characteristics of specific skin pain and their thresholds were identical over all the surface of the body. Nonspecific pain differs from specific pain by the character of the sensation, and the threshold value in different regions of the skin.

The studies of thermal effects induced by stimuli of focused ultrasound led to the conclusion that one and the same receptor structures are, under different conditions, responsible for the sensations of warmth or cold. It has been found (Gavrilov *et al.* 1976a; Tsurilnikov 1977) that the modality of the thermal sensation (warmth or cold) depends on a fixed relation of the internal temperature of the organism and temperature of the surrounding environment. It is interesting that the stimulus of a trapezoid form acting on the skin of human fingers did not elicit tactile sensations, but induced the warmth sensation changing by the burn pain (Enin *et al.* 1992)

The results of the studies showing a close connection of deep and skin sensations elicited by ultrasound with the mechanical displacement of the medium in the focal region of the ultrasound radiator, allowed to conclude that, physiologically, somatic receptors may be classified as mechanoreceptors (Vartanyan et al. 1985; Tsirulnikov 1992). For tactile reception this conclusion does not disagree with accumulated empirical and scientific data. For thermal reception this conclusion seemed, at first sight, rather unexpected, and required further studies to elucidate a possibility of transformation of the thermal action into mechanical one in natural conditions (Tsurilnikov 1977).

The use of local action of ultrasound not only on skin receptor structures, but also on subcutaneous ones, opens the long awaited possibility of noninvasive action on deep structures under physiological conditions, and without the involvement of cutaneous structures. The results confirm and widen the data on the function of deep receptive structures, differing from the cutaneous in the sensitivity thresholds and modality of the sensations.

Thus, it was shown in this Chapter that focused ultrasound has been used to elicit cutaneous tactile, thermal, specific and nonspecific pain sensations, and also subcutaneous (deep) sensations which included tactile and some pain sensations (muscular and joint, etc.). The clinical application of this effect for diagnosis of different diseases will be discussed in Chapter 5.

Chapter 3

FOCUSED ULTRASOUND AND HEARING

This chapter contains mainly the summary of research into implementation of focused ultrasound to induce hearing sensations, which was carried out from the mid-1970s until the end of 1980s at Sechenov Institute of Evolutionary Physiology and Biochemistry Russian Academy of Sciences; the Leningrad Institute of Ear, Nose, Throat, and Speech; and the Acoustics Institute, Moscow.

3.1. Early Research in Animals

Preliminary research was carried out on grass frogs *Rana temporaria* (Gavrilov *et al*. 1975 a,b; 1977a). It was shown that pulses of focused ultrasound stimulated the auditory receptors of the labyrinth of a frog. The scheme of experiments is presented in Figure 17 (Gavrilov *et al.* 1975a).

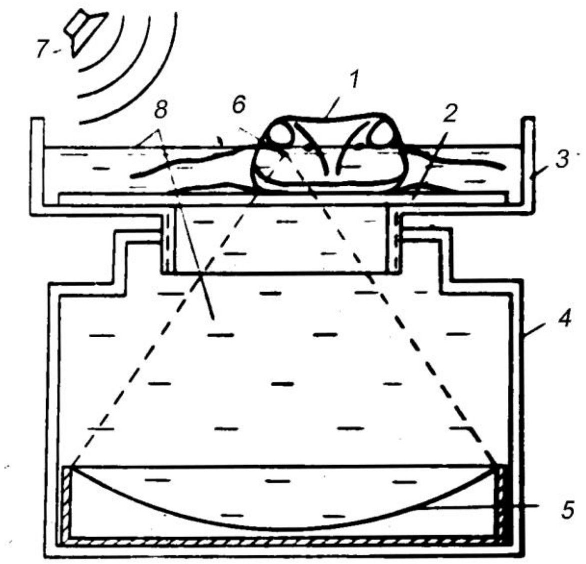


Figure 17. A schematic drawing of a setup for the study of the stimulation effects of focused ultrasound on the ear labyrinth of an animal: 1 - an experimental animal; 2 - a plate for fixing an animal; 3 - a tray; 4 - a housing for fastening a tray and a focusing radiator; 5 - a focusing radiator; 6 - the center of the focal region; 7 - a loudspeaker; 8 – water (Gavrilov *et al*. 1975a).

The evoked responses at sound and ultrasonic stimulation were compared. The frequency of ultrasound was 480 kHz, and the duration of stimulus was 0.1-100 ms. The bioelectric responses were detected in the auditory part of the midbrain (*torus semicircularis*). It was obtained, that the labyrinth stimulation by pulses of focused ultrasound produced electrical responses which were similar in shape, amplitude and latency to ones arising in response to sound stimuli (Figure 18).

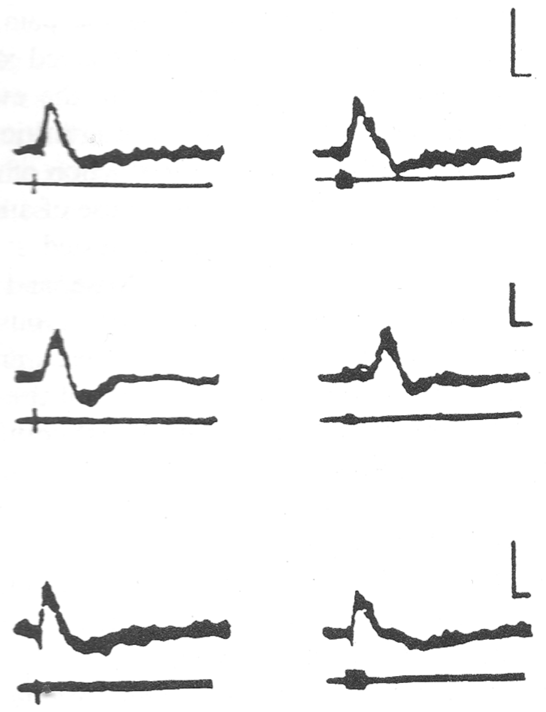


Figure 18. Evoked responses in the midbrain auditory area of a frog. The left column: stimulation of the labyrinth with focused ultrasound (stimulus duration of 1 ms). The right column: responses to sonic stimuli of optimal frequency (100-1600 Hz), 20 ms duration. Stimulus intensity: 20 dB above threshold. Top tracing: the oscillogram of responses; bottom tracing: the stimulus. Calibration: 100 μV, 25 ms. The results in three animals are presented (Gavrilov *et al*. 1975a,b, 1977a).

At a combination of sound and ultrasonic stimulus, an interaction between responses was observed. Thresholds of excitation of receptors by focused ultrasound depend on the duration of stimulus. For example, at the frequency of ultrasound of 0.5 MHz and the duration of stimulus of 0.1 ms the threshold intensity in the focal region was approximately 1 W/cm^2, and at the duration of 100 ms it was 0.01 W/cm^2 (Gavrilov *et al.* 1975 b). Thus, excitation of receptors occurred at rather small values of the intensity of ultrasound that excludes an opportunity of destruction of receptor structures.

Comparative study of characteristics of the responses to ultrasonic and sonic stimulation was undertaken to be certain that the responses to ultrasound are caused really by stimulation of receptor structures of the hearing organ. Responses to suprathreshold (estimated in dB with respect to the threshold) ultrasonic and sonic stimuli of equal magnitude were found to be of about the same size; the latency associated with ultrasound was, in the majority of experiments, somewhat shorter than the latency of responses to sonic stimuli.

Switching off of a long stimulus (100 msec and longer) resulted in some experiments in appearance of an additional off-response similar in shape and other characteristics with responses to onset of the stimulus. The off-responses were observed with both ultrasonic and sonic stimuli.

The full restoration time of the amplitude of responses to a test ultrasonic or sonic stimulus after, respectively, ultrasonic or sonic stimuli was the same (250-300 ms) for both types of stimulation.

When one of the stimuli was ultrasonic and the other was sonic and *vice versa*, the amplitude of a response to the stimulus depended on a time interval between stimulations (Figure 19) (Gavrilov *et al*. 1975a; 1977a). The restoration of the amplitude of responses to the test stimulus was in both cases complete in 200-250 ms.

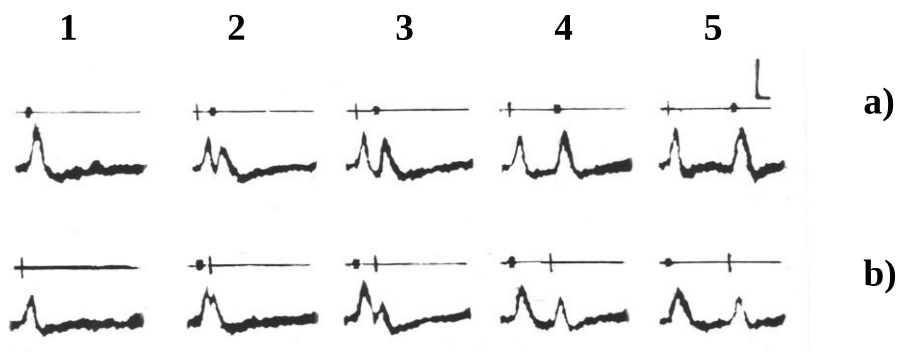


Figure 19. Interaction between responses to sonic and ultrasonic stimuli at consecutive presentation. Bottom tracing: the oscillogram of potentials; top tracing: the stimulus. a: the first stimulus is ultrasound; the second is sound. b: the first stimulus is sound; the second is ultrasound. 1: response to a single test stimulus; 2-5: double stimuli. Time intervals between the stimuli: 2- 50 ms; 3 - 100 ms; 4- 200 ms; 5 - 300 ms. Calibration: 100 μV**,** 50 ms (Gavrilov *et al*. 1975a; 1977a).

In the later work of the group in the USA similar experiments were fulfilled in cats (Foster, Wiederhold 1978). Auditory-nerve responses and cochlear microphonics were produced in cats by pulsed 5 MHz ultrasound using a flat transducer with a diameter of 0.7 cm. To avoid the reflection of ultrasound by the skull, the transducer was positioned directly against the dura mater through a 1 cm-diameter hole in the skull over the temporal cortex on the side of the head opposite from the tested ear. The intensity was 30 W/cm^2 and the pulse duration was 20-70 μs. Authors consider that the cats apparently respond to the radiation pressure accompanying the absorption of ultrasound in the brain tissue. They suggest also that the mechanism of ultrasound stimulation is related in that case with the bone conduction.

Because the ultrasound dozes necessary to stimulate neural structures of labyrinth in animals were in several orders lower than ones that cause structural changes in the tissues (see Chapter 1), the studies were continued in volunteers.

3.2. Studies in Humans

The data on the safety of ultrasonic stimulation of the auditory organ obtained in animal experiments became a motivation to carry out similar studies in humans (during long time they were participants of the work). In the very first experiments it was shown (Gavrilov *et al*. 1975 b) that irradiation of the labyrinth of human volunteers with 2 MHz focused ultrasound (SPTP intensities 50-200 W/cm^2, pulse duration 1 ms) evoked click type auditory sensations.

During this research, a method of ultrasonic input of hearing information to humans was proposed (Gavrilov *et al*. 1977b, 1980; Gavrilov, Tsirulnikov 1980; Vartanyan *et al*. 1985). The essence of the proposed 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 0.5–5 MHz), 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 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. With 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 subjects with normal hearing (Gavrilov *et al*. 1977b, 1980). A coordination system used for studying hearing sensations induced by amplitude-modulated focused ultrasound is shown in Figure 20 (Vartanyan *et al*. 1985). The limits of a controlled movement of a transducer relative to the object were the following: 100 mm in a horizontal plane, 1000 mm in a vertical plane (coarse adjustment), and 50 mm in a vertical plane (fine adjustment). The error of determination of the coordinates for each of three mutually perpendicular directions was no more than 0.1 mm. Part of the experimental setup is shown in Figure 21 (Gavrilov 1984). A patient 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.

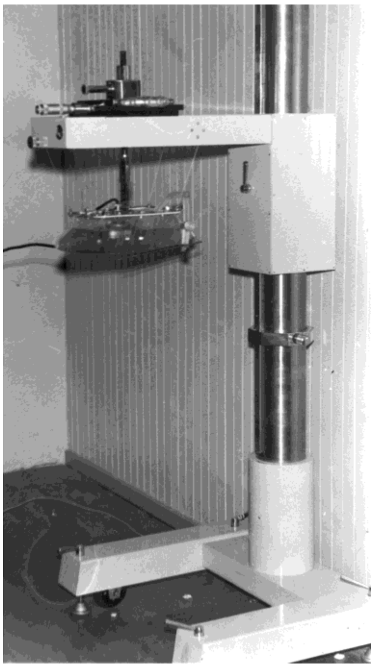


Figure 20. A coordination system used for studying hearing sensations induced by amplitude-modulated focused ultrasound (Vartanyan *et al*. 1985).

Figure 21. Experiment on stimulation of human’s auditory system (Gavrilov 1984).

A bag with a focusing transducer was filled by 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 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 a presumed site of irradiation.

The frequency of focused ultrasound was ranged from 0.67 to 3.7 MHz. 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 1 ms at a repetition frequency from 5 to 1000 Hz. In the mode of amplitude-modulated oscillations, the modulation frequency ranged from 20 to 20000 Hz, and the modulation index ranged from 0 to 1. In addition, the signals from the microphone, tape recorder, radio, etc. were used as the 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 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 W/cm^2 and at a frequency of 0.67 MHz. 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 presented.

With the use of focused ultrasound modulated in amplitude by sinusoidal oscillations with frequencies ranging from 50 to 15000 Hz, 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 as is customary in audiometric studies. The frequency-threshold curves for a subject with normal hearing are shown in Figure 22 (Gavrilov, Tsirulnikov 1980). 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 1 MHz. It is evident that the character of the curves, especially at modulation frequencies of 1–2 kHz, is similar to the curves of the perception thresholds of sound signals by humans (Zwicker, Feldtkeller 1998). 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.

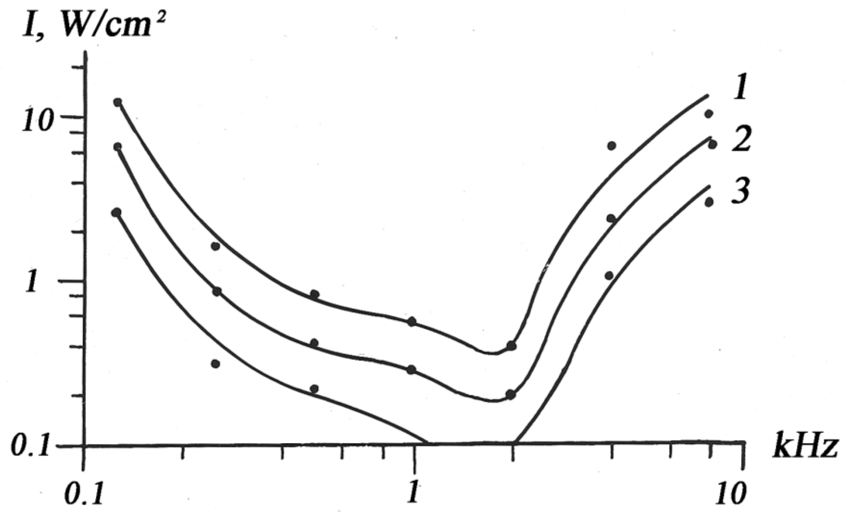


Figure 22. Typical dependences of threshold intensities of ultrasound in the focal region corresponding to the appearance of auditory sensations from the modulation frequency and modulation index *m; 1 – m=0.25; 2 – m =0.5; 3 – m =1* (Gavrilov, Tsirulnikov 1980).

The dependence of the threshold intensity of ultrasound from the carrier frequency when it changes from 0.67 to 3.7 MHz is shown in Figure 23 (Gavrilov, Tsirulnikov 1980). In all cases, the value of the modulation index is equal to 1. It is evident that an increase in the carrier frequency leads to a significant increase in the value of the threshold intensity.

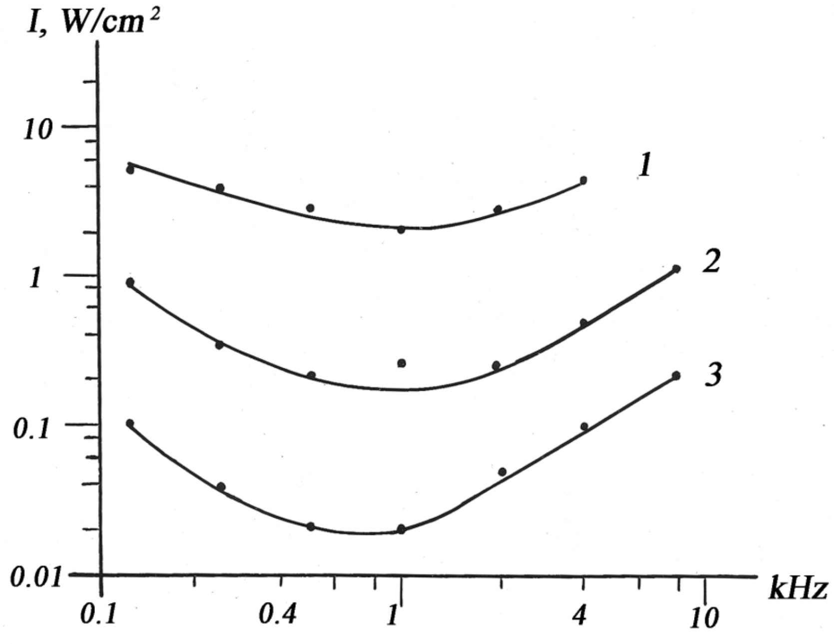


Figure 23. Frequency-threshold curves for one subject with a modulation index m = 1 and different ultrasonic frequencies: curve *1* for f = 3.7 MHz; *2* for f = 2.47 MHz, and *3* for f = 0.67 MHz (Gavrilov, Tsirulnikov 1980).

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 transmitted auditory information.

It is interesting to compare these results with data on the auditory perception of ultrasound studied previously (Pumfrey 1950; Sagalovich, Pokrivalova 1964). It is well known that human beings can hear sounds with frequencies much higher than 16–20 kHz (the normal limit for hearing in the air) if the radiator is in direct contact with the tissues of the body. This induces a sensation of a high-pitched tone. The pitch does not change when the ultrasonic frequency increases up to 225 kHz (the highest frequency at which these sensations have been registered). The radiator can be applied to various sites of the head, neck, and trunk, which results only in changes in the sensations strength, but not in their tone. In contrast with this method, the impact on the ear labyrinth of humans using amplitude-modulated focused ultrasound allowed the experimenters to input complicated auditory information (speech, music, etc.) with high-quality perception.

3.3. Mechanisms of Ultrasound-Induced Auditory Sensations

The mechanism of stimulation effects of amplitude-modulated ultrasound at the neural structures of the labyrinth was also studied (Gavrilov *et al*. 1977b; Gavrilov, Tsirulnikov 1980; Gavrilov 1984; Vartanyan *et al*. 1985). First, it is worth to keep in the mind that the human organ of hearing 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 $S = ½\rho\omega^2A^2$, 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 $aCos\Omega t$, where $\Omega = 2\pi F$ (F is the frequency of modulation), it is necessary to replace A by $A+cos\Omega t$. Then (Altenberg, Kästner 1952):

$$S = ½\,\rho\omega^2\,(A+ aCos\Omega t\,)^2 = ½\,\rho\omega^2A^2(1+m^2/2+ 2mCos\Omega t + ½\,m^2cos2\Omega t), \qquad (22)$$

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 frequency modulation. 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. $P_F = 2mCos\Omega t$ and its second harmonic $P_{2F} = ½\,m^2cos2\Omega t$, 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 going 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 in Chapter 1 (see Table 3).

Also, there is an evidence that ultrasound has a direct stimulation effect on the fibers of the auditory nerve (Vartanyan *et al.* 1982, 1985). In particular, the possibility of stimulating the fibers of the nerve auditory system by means of amplitude-modulated focused ultrasound was confirmed by experiments on a grass frog with a beforehand destroyed receptor apparatus of the ear labyrinth (Vartanyan *et al*. 1982) (see in the next Section). Bioelectric responses to ultrasound stimulation similar in configuration to the responses to sound in the normally functioning receptor apparatus but with higher thresholds were registered in the auditory center of the midbrain. In addition, it was shown using special histochemical methods that, when the ear labyrinth was pre-destroyed, just the nerve fibers were activated (Vartanyan *et al*. 1982).

Finally, there were clinical observations at the Leningrad Institute of Ear, Nose, Throat, and Speech that 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 (Gavrilov, Tsirulnikov 1980).

3.4. 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*) (Vartanyan *et al*. 1982). The frequency of ultrasound was 2.34 MHz, duration of pulses 1 ms, the maximal intensity used in experiments achieved 700 W/cm^2. Action potentials evoked by focused ultrasound were similar to those evoked by activation of the intact contralateral labyrinth but with higher thresholds (Vartanyan *et al*. 1982). The threshold intensity without destruction of labyrinth was 3 W/cm^2, but after destruction it became 80 W/cm^2. In the case of the intact labyrinth, the amplitude of the responses was increased until 350 W/cm^2 and then decreased. For the destroyed labyrinth, the amplitude increased until the maximal intensity (700 W/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 auditory fibers of the VIIIth nerve even without participation of the receptor apparatus (Vartanyan *et al*. 1982).

Clinical studies also support the demodulation mechanism. It is known that the conductive apparatus is important for perception of ultrasound, and various impairments in the conductive apparatus specifically affect the transmission (and hence perception) of ultrasound with different modulation frequencies, including a complete disappearance of sensitivity to some modulation frequencies in otosclerosis (see Section 5.6 and Tsirulnikov *et al*. 1988). At the same time, some clinical data, especially those of patients with pathology of the cerebellopontine angle, suggest a direct effect of ultrasound on the acoustic nerve fibers. 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 (Gavrilov, Tsirulnikov 1980; Tsirulnikov *et al*. 1988).

3.5. Temporal Summation and Aural Ability to Determine Time Intervals

It was of interest to study a phenomenon of the temporal summation, or in other words, to compare the dependence of the thresholds of aural sensations on the duration of a stimulus for different locations of a focal region relatively the subject's head (Gersuni *et al*. 1981; Gavrilov *et al*. 1980). Three various directions of a focal region were investigated: a) the focal region is directed to an ear labyrinth; b) focal region is located into a forehead bone; c) focal region is located outside a head, i.e. in water without any acoustical contact with the head (Figure 24). In the last case, hearing did not related with any effect of ultrasound and determined only by sound signals.

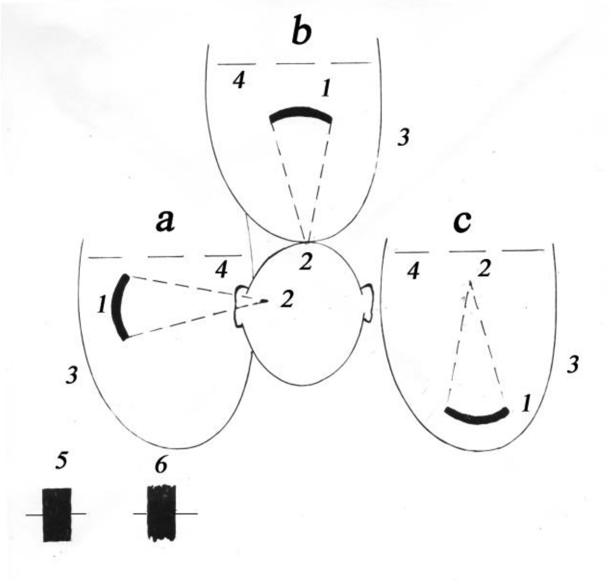
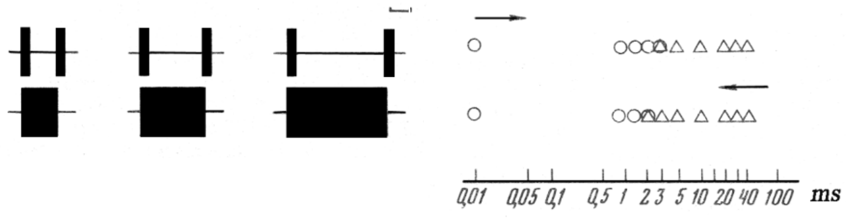


Figure 24. Three different locations of the focal region relative the head of a tested person. a- a focal region is directed to an ear labyrinth; the center of the focal region is located in a forehead bone; c – the focal region is located outside a head, in water. 1- focused radiator; 2 – center of the focal region; 3 – polyethylene bag; 4- level of water in the bag, 5 - ultrasound signal amplitude-modulated by a rectangular pulse; 6 - ultrasound signal amplitude-modulated by a white noise (Gersuni *et al*. 1981)

Focused ultrasound with the frequency of 2.47 MHz was modulated by rectangular pulses with a different duration (from 0.05 to 100 ms) or by white noise with a frequency band of 20-20000 Hz. It was obtained that the time duration after which the value of the threshold does not change (it is called as the critical duration of stimulus or the critical time of summation) varied in all these situations in the range of several orders. When the focal region is directed to the ear labyrinth it is equal to 0.5-1 ms, when the focal region is located in a forehead bone it is about 0.05 ms, and at last, for the case of usual sound stimuli it is larger than 100 ms, which corresponds to the literature data (Arapova *et al*. 1972; Pedersen, Elberling 1972). The difference in more than two orders between the time of summation for sound and ultrasound stimulations of labyrinth, suggests that the simple explanation of mechanisms of ultrasound-induced auditory sensations by the action of the radiation pressure only, presented in section 3.3 is not exhaustive. That means that the studies of those mechanisms should be continued.

In the work of Vartanyan, Tsirulnikov (1985) the curves of the temporal summation obtained at the action on the ear of a person with normal hearing by short stimuli of electric current, pulses of focused ultrasound with the frequency of 2.47 MHz and sound were compared. These studies showed that the critical time of summation for electric current was about 0.05-0.1 ms, for focused ultrasound was about 0.5 ms, and for sound about 200 ms. That means that the critical time of summation for ultrasound is two orders of magnitude smaller than one for sound signals (Arapova *et al*. 1972) and comparable with the critical time for stimulation by electric current (Volohov *et al*. 1934).

Essential advantage of ultrasound method of stimulation of hearing organ is a possibility to generate stimuli with the duration of less than 1 ms which is extremely difficult with sound stimuli (Gersuni *et al*. 1982). Such a possibility was used for the research into the aural ability to determine time intervals between two stimuli and estimate the duration of a single stimulus with the use of proposed ultrasound method. The parameters of ultrasound were the same as in the previous article (Gersuni *et al*. 1981). First of all, it was obtained that if the duration of a stimulus was larger than a few milliseconds, two sequential clicks are perceived. The longer stimulus elicits a correspondingly greater increase in the time interval between clicks. As an example, Figure 25 (Gersuni *et al*. 1982) presented examples of stimuli induced the sensations of two clicks.



(a) **(b)**

Figure 25. a) Short and long ultrasound stimuli of induce similar sensations of two clicks. Calibration at the right above is 5 ms; b) Character of perception when durations of stimuli are varied. Designations: circle is the perception of a single click; triangle is two clicks. Arrows indicate the order of presenting of stimuli: the left is from lesser duration to greater; the right is from greater duration to lesser (Gersuni *et al*. 1982)

It is seen that for the duration of stimuli equal to 1-2 ms one click was elicited and after duration of about 5 ms two separate clicks were perceived. An interval 2-5 ms is a zone of uncertainty.

When one click was perceived, the subject could distinguish stimuli of different durations one from another without error even with a difference of 10 μs. Thus, hearing can evaluate the duration of a single stimulus significantly shorter than 2 ms (Gersuni *et al*. 1982).

3.6. Possibilities for Focused Ultrasound Prosthesis in Hearing

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 (Gavrilov, Tsirulnikov 1980). In order to help such patients, an attempt to use ultrasonic technique for audio prosthetics of the deaf people was undertaken. 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.

3.7. Common and Different Features Between the Effects of Ultrasonic and Sonic Stimulation

A similarity of the threshold audiometric curves obtained with the use of sound and ultrasound modulated by audiometric frequencies served as the basis for comparative studies of the identity of perception of frequencies of sound and amplitude modulation of ultrasound. To that end, to one ear of a tested person a pure tone or a series of pulses of a certain frequency were presented, and to another ear the stimuli of focused ultrasound were delivered. The aim of the tested person was to determine the frequency of a sinusoidal or pulse modulation of ultrasound which created the sensation with a pitch and loudness corresponding to the sensation induced by sound. It was obtained that the frequency of ultrasound modulation in all the cases was assessed as equal to a corresponding frequency of sound. If not a sinusoidal but a pulse modulation was used, the tested person defined the repetition frequency of pulses as equal or close to the frequency of the reference sound signal.

At the same time, some differences between the effects of ultrasonic and sonic stimulation have been found. For example, the shortest stimulus duration above which the hearing threshold does not decrease is about 1 ms for ultrasonic stimuli (Gavrilov, Tsirulnikov 1980) as compared to over 100 ms, or two orders of magnitude higher, for sonic stimuli (Arapova *et al*. 1972; Pedersen, Elberling 1972). The specificity of ultrasonic stimulation seems to be due to the following reasons. First of all, ultrasonic signals from the focused radiator should reach the sensory structures by different path as compared to sonic signals. Secondly, it was proposed on the basis of physiological, morphofunctional and psycho-physical data that not only the receptor structures but also the auditory nerve fibers took part in the perception of focused ultrasound (Gavrilov, Tsirulnikov 1980; Vartanyan *et al*. 1982; Vartanyan *et al*. 1984; Vartanyan, Tsirulnikov 1985). Therefore, an investigation of patients with hearing disorders by ultrasound should help obtain new information on the location and degree of the impairment, primarily on the functioning of the receptors and the auditory nerve, which is important for the diagnostics of hearing diseases.

The second difference is related with a fact that ultrasound stimulus, contrary to sound one, might in some circumstances include, in addition to the sound component (radiation pressure), the effects of sign-variable ultrasound oscillations, i.e. thermal effects and mechanical actions of high-intensive ultrasound discussed in Chapter 1 (see Table 3).

Chapter 4

FOCUSED ULTRASOUND AND SOME OTHER NEURAL STRUCTURES

The content of this chapter is related with the use of focused ultrasound for stimulation of Pacinian corpuscles, central nervous structures of invertebrates, electroreceptor system of skates, human acupuncture points, to induce taste sensations in humans, and also for the study of the effects of focused ultrasound on animal single fibers and on sensory and motor structures in the brain of animals.

4.1. Effects on the Pacinian Corpuscles

As is well known, a Pacinian corpuscle is a type of touch receptor located in the subcutaneous tissue of the skin. It is classed as a mechanoreceptor, meaning it is a part of the group of sensory receptors that respond to touch and pressure. Therefore single Pacinian corpuscles isolated from cat mesentery were used as one of convenient objects for the study of possibility of stimulation of mechanoreceptors by stimuli of focused ultrasound (Gavrilov *et al*. 1976a; 1977b). The operating frequency was 0.48 MHz and the length and diameter of the focal region were in this case, correspondingly, 34 mm and 6.4 mm, which was essentially larger than the sizes of Pacinian corpuscle. Ultrasonic stimuli of a rectangular shape had the duration from 0.1 ms to hundreds ms.

An isolated receptor along with the attached nerve fiber was placed in the vaseline oil, over the ultrasonic irradiator, in the center of the focal region. Cotton threads suspending the receptor were moistened with a physiological solution and served as detecting electrodes. Ultrasonic irradiation of Pacinian corpuscles, with appropriate parameters of stimulation, produced receptor and peak potentials of the usual type. The receptor potentials appeared first of all, its amplitude gradually increased with the rise of the intensity of ultrasound stimuli. When the receptor potential reached a critical level, an action potential was generated in an all-or-none manner. Examples of the responses of isolated Pacinian corpuscles to pulses of focused ultrasound are presented in Figure 26 (Gavrilov *et al.* 1976a, 1977b) for two different receptors. Stimulation with long (several ms and longer) stimuli gave both on- and off-responses. The stimulation level for action potentials varied between of 0.4 and 2.5 W/cm^2.

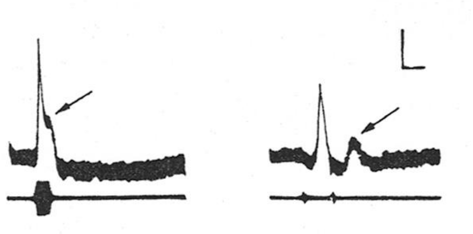


Figure 26. Responses of isolated Pacinian corpuscles to stimuli of focused ultrasound. Top tracing are the oscillogram of responses (action and receptor potentials); bottom tracing are the stimuli. The results for two different receptors are presented. At the left: response to a single stimulus of 1 ms duration; to the right: responses to double stimuli with the duration of 0.1 ms and interval 3 ms between them. Arrows show the receptor potentials. Calibration: 50 μV**,** 2.5 ms (Gavrilov *et al*. 1976a; 1977b)

The data that the receptor and peak potential appear in response to the action of focused ultrasound on the Pacinian corpuscle indicates that this physical agent can be used for stimulation of mechanoreceptors. This fact also explains the capability of the focused ultrasound to evoke the tactile sensations.

It has been shown that thresholds for the tactile sensations in the human finger skin induced by focused ultrasound of the same frequency (0.48 MHz) for stimulus duration of 1 ms is equal to 8 W/cm^2 (see Table 7 in Chapter 2). Comparison of these values with thresholds for excitation of Pacinian corpuscles shows that just these receptors can be responsible for threshold tactile sensations during ultrasonic stimulation.

4.2. Stimulation of Central Nervous System Structures of Invertebrates

Analysis of the works related with attempts to stimulate neural structures in the brain of mammals, including human beings, by ultrasound stimuli, shows (see below) that there are no unambiguous and reliable data confirming such a possibility. Therefore, 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 two publications (Gavrilov *et al*. 1978; Tsirulnikov, Kovalev 1983).

In the first work (Gavrilov *et al*. 1978) edible snails without shells were used as an object of investigation. This object has the following advantages: accessibility for microscopic observation of the irritated 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. Focusing radiator with the resonant frequency of 2.25 MHz was used. The focal distance of the radiator was 70 mm*;* the diameter and length of the focal region were 1.6 mm and 10 mm correspondingly. The maximal intensity of focused ultrasound in the center of the focal region was 2000 W/cm^2. The impact on the neural structures was carried out by single pulses of ultrasound with the duration of 1 ms.

A subpharyngeal ganglion of the snail containing nervous cells in diameter of 100-150 μm has been chosen from central nervous structures for stimulation by focused ultrasound. Another object of research was also statocyst which is a balance organ in some invertebrates. It represents a formation with the diameter of 150 μm and contains receptor cells. The conditions of the experiments permit to expose simultaneously both ganglion and statocyst and to compare functional activity of cells.

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 (Figure 27a). 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 W/cm^2. If the intensity of stimuli exceeded 1000 W/cm^2, the evoked impulse activity of cells of ganglion was appeared (Figure 27b). With the subsequent increase of the ultrasound intensity, the activity increased and at achievements of the maximal value of the intensity equal to 2000 W/cm^2 did not show the tendency to reduction.

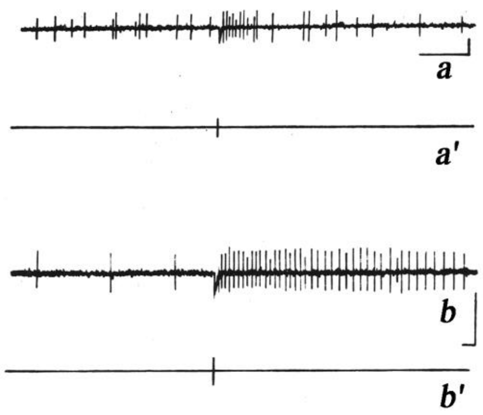


Figure 27. Oscillograms of an impulse cellular activity of a statocyst (a) and ganglion (b) of snails. On the right - calibration: horizontal – 200 ms, vertical – 250 μV; a' and b' - oscillograms of ultrasound pulses with the duration of 1 ms, frequency 2.25 MHz, intensity of 460 W/cm^2 (a') and 1100 W/cm^2 (b') (Gavrilov *et al*. 1978).

Thus, in this study a principle possibility to induce a stimulation of some central nervous structures of invertebrates has been demonstrated.

These investigations were continued in another work (Tsirulnikov, Kovalev 1983). In electrophysiological experiments, the reactions of suboesophageal ganglion neurons of the gastropod mollusks *Helix vulgaris* and *Lymnaea stagnalis* to focused ultrasound stimulation have been studied. The data obtained indicate the existence of neurons which respond to ultrasound presumably directly, neurons which conduct excitation of neighbors ones and those which do not respond to ultrasonic stimulation. These data explain the fact that in the previous study (Gavrilov *et al*. 1978) the stimulation effect of ultrasound was registered not in all the cases.

4.3. Effects on Electroreceptor System of Skates

One of the most convenient objects for the study of stimulation action of focused ultrasound are various receptor formations, i.e. specialized structures of the organism most sensitive to the action of mechanical, thermal, physical and chemical factors (Gavrilov *et al*. 1976a)**.** 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 10-15 cm 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 the Black Sea skates (*Raja clavata*, *Dasyatis pastinaca*) (Broun *et al*. 1981). An experimental setup is schematically presented in Figure 28. An anesthetized fish was placed in an experimental bath on a grid from plastic strings. The ultrasound focusing radiator was located in water under a grid and could move relatively an animal with the accuracy of 0.1 mm. The radiator with the operating frequency of 1.95 MHz was used. It has the diameter of 70 mm, the radius of curvature of 70 mm and the angle of convergence of 31^o. The length and diameter of the focal region were 7.6 mm and 1.4 mm. Both pulsed and continuous modes of an irradiation were used. The duration of pulses varied from 1 up to 1000 ms, the total exposition of ultrasound varied from 1 ms up to several minutes. The maximal intensity of ultrasound, averaged over the area of the focal region was 600 W/cm^2. The bioelectrical activity of single fibers of the anterior lateral line nerve going from ampullae of Lorenzini was registered.

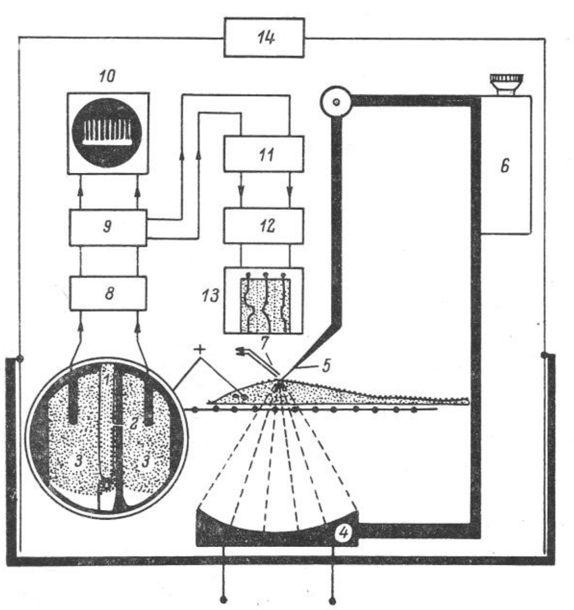


Figure 28. The scheme of an experimental setup. *1* - trunk of a lateral line nerve; 2 - single nervous fiber; *3* - space filled by a cerebrospinal fluid; *4* - focusing radiator; *5* - pointer of the focus; *6* - positioner; *7* – thermistor; *8* - cathode follower; *9* - amplifier; *10* - oscilloscope; *11* - device for generation of standard impulses; *12* - integrator; *13* - recorder; *14*- generator of rectangular impulses (Broun *et al*. 1981).

Because the majority of afferent fibers of an anterior lateral line nerve, even at absence of stimulation of receptors, possesses a background impulse activity, it was possible, studying its change, to estimate both stimulating and inhibitory effects. The responses of single fibers of anterior lateral line nerve were different depending on the place of ultrasonic stimulation and parameters of the stimuli. At overlapping the focal region with a pore of the ampulla, there was, as a rule, an increase of an impulse activity (Figure 29, Broun *et al*. 1981). Such reactions appeared in response to the action of ultrasonic impulses with the duration of 1 ms at the intensity of ultrasound beginning from 12 W/cm^2 and became more expressed with the increasing of the intensity and duration of the ultrasonic action (see oscillograms 1-3, Figure 29). With the increase of the duration of a stimulus up to 1000 ms, the pulse reaction became biphasic: an increase in the beginning of action of stimulus was replaced by a full inhibition of the activity (see oscillogram 4, Figure 29). The latter is, apparently, a consequence of the thermal action of ultrasound on the neural structure.

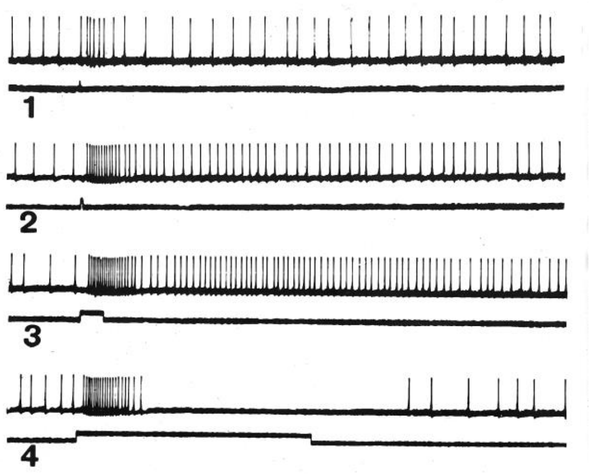


Figure 29. The responses of ampulla of Lorenzini at the action of stimuli of focused ultrasound on the area of the pore. The top beam is a bioelectrical response; the bottom is a mark of stimulation by ultrasound. Duration of action: *1* – 1 ms , *2* – 10 ms, *3* – 100 ms, *4* – 1000 ms. Intensity of ultrasound is 40 Вт/см^2 (Broun *et al*. 1981)

For the study of the mechanism of ultrasound stimulation at the effect on the pore of the ampulla it was important that pulse bioelectrical reactions arose only in the case when the pore was above the surface of water, and did not arise when the pore was in water. At the same time the similar response was also generated at pressing on the area of a pore by a glass stick irrespectively on the position of the pore relative water or air. The fact that reaction of electroreceptors to ultrasonic action appears only in the case when the pore of the ampulla was above a surface of water allows to believe that the effective factor of ultrasonic stimulation is a mechanical one, namely the radiation pressure which is revealed usually at the borders of media with different acoustic properties, i.e. in this case at the border of air and tissue of a fish. It is indicative that the reaction to ultrasound was absent if the media were more acoustically homogeneous (water and tissue of the animal in the cases when the pore was in water), but appears in response to a touch by a glass stick. The least value of the radiation force on the pore area, corresponding to the origin of the bioelectrical reactions of an electroreceptor, was 6 mg at the duration of ultrasound stimulation of 1 ms. This value is well agree with the data of the literature on a mechanical stimulation of a pore (Murray 1960). Results of stimulation of a pore by a glass stick and ultrasound (in the case when a pore was located above water) allow to assume, that there is some mechanism of mechanoelectric transformation at the level of a pore.

Action of single ultrasonic impulses with the duration up to 50 ms on ampullae of Lorenzini, i.e. on the area of location of receptor cells, did not induce bioelectric responses in corresponding nervous fibers even at the maximal intensity of ultrasound equal to 600 W/cm^2. At the action of similar ultrasonic impulses on a trunk of a lateral line nerve and on its separate fibers, the responses of mechano- and electrosensitive fibers also were absent.

Thus, it was shown in this work 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.

4.4. Does Ultrasound Have a Taste?

If to direct the focal region of the focused transducer to a tip of a tongue, the tested person will feel a rather singular and rather unpleasant taste. It reminds a taste which can be perceived after connecting electrodes of a low-voltage battery to a tip of a tongue (Gavrilov, Tsirulnikov 1980; Gavrilov *et al*. 1996).

4.5. EEG Responses of the Human Brain to Stimulation of Different Receptor Structures by Focused Ultrasound

Tsirulnikov *et al*. (2007) studied electroencephalographic responses of the brain to stimuli of focused ultrasound inducing tactile and hearing sensations and skin pain, as well as to sound stimuli that cause hearing sensations. Single stimuli of focused ultrasound with the frequency of 2.5 MHz and the duration of 1 and 5 ms were used in experiments in 17 healthy persons. In experiments related with inducing pain sensations, series of pulses with the repetition frequency of 50 Hz and with different durations were applied. The objects of stimulation were fingers, hand, and skin cheek. The intensity of single stimuli was from 5 dB lower up to 15 dB higher of the tactile threshold. Responses that were characteristic for all kind of sensations were recorded. In particular, it was shown that the simultaneous effect of sound and ultrasonic stimuli that caused, respectively, auditory and tactile sensations increased the amplitude of the response compared with the response to any one of these stimuli without changing its latency.

Experiments with focused ultrasound and the following experiments with adequate stimuli showed for the first time that sensations of different modalities (tactile, thermal, hearing, etc.) were accompanied by nystagmoid eye movements (Tsirulnikov *et al*. 2007). These movements were more noticeable with the use of ultrasonic stimulation rather than with adequate stimuli. The latency and amplitude of eye movements depended on the strength and duration of stimulation by focused ultrasound. In comparison with the evoked potentials of a brain at tactile, temperature, hearing, etc. sensations, the nystagmoid eye movements include the same characteristics: the latent period, amplitude and duration of movements. Thus, there is an opportunity to characterize and compare various sensations induced by focused ultrasound studying the movements of eyeballs.

4.6. Effect of Ultrasound on Sensory and Motor Structures in the Brain of Animals

Research of the effect of ultrasound on sensory and motor structures in the brain of animals (grass frog) was fulfilled (Vartanyan *et al.* 1980, 1985). The ultrasound frequency was 2.34 MHz. The irradiation was performed in continuous (up to 30 s) and pulsed modes (pulse duration of 1 and 10 ms, repetition frequency from 0.5 to 50 Hz). The intensity averaged over the area of the focal region varied from 3 to 580 W/cm^2. A number of pulses with the intensity in this range changed from 1 to 50, the duration of series did not exceed 1 s. The targets were various brain structures of the grass frog. At irregular time intervals after the exposure by ultrasound, microphone potentials in the inner ear, potentials of the auditory center of the midbrain caused by sound, displacement of the eye-ball, changes in the diameter of a pupil, the slope of the head and trunk in motionless frogs, the direction of motion while jumping, and changes in the pattern of the vocal reaction were registered. Morphological control of the irradiated area was carried out.

There were observed the suppression of the microphonic response to sound, a decrease in the amplitude of the potentials in the midbrain caused by sound, the displacement of the eyeball from its normal position, a long-term dilation or contraction of the pupil and changes in its reaction to light, movement of the animal in a circle with an inclined head, and changes in the temporal pattern of its vocal reaction. The degree of manifestation and reversibility of the above reactions depended on the parameters of ultrasound exposure and, primarily, on the mode of irradiation. In a pulsed mode of irradiation, compared with a continuous mode, the reactions were more pronounced and more complete and the recovery time was smaller. It was found that, despite the clear changes in functional performance, there were no morphological changes in brain tissues during irradiation in a pulsed mode.

For illustration, Figure 30 (Vartanyan *et al.* 1980, 1985) represents the recordings of the artificially induced voice reactions of the frog before the ultrasound irradiation and through the different time after the action. The intensity of ultrasound was 470 W/cm^2, the total ultrasound exposition was 22 s at the duration of stimuli of 1 ms and the repetition frequency of 300 Hz.

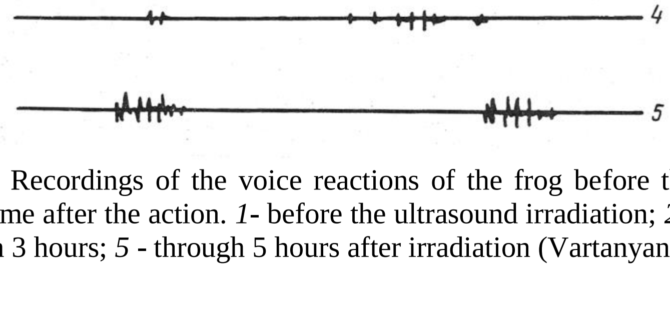


Figure 30. Recordings of the voice reactions of the frog before the ultrasound irradiation and through different time after the action. *1*- before the ultrasound irradiation; *2*- through 1 hour; *3* - through 2 hours; *4* - through 3 hours; *5* - through 5 hours after irradiation (Vartanyan *et al.* 1980, 1985).

It is seen that after irradiation, the temporal structure of the induced bioacoustics signals is disturbed, the signs of its regeneration are noticed through 3 hours after the effect and full restoration of the structure is observed through 5 hours.

4.7. Impulse Activity of Single Fibers of Animals

It has been found from electrophysiological studies in animals (Enin *et al*. 1989, 1992; Tsirulnikov *et al.* 1993) that stimuli which in human are associated with the initiation of warmth and cold sensations evoke in animals impulse activity of single nerve fibers which differs from the activity evoked by other stimuli.

From the results of the studies all receptor units (single afferent nerve fibers with the corresponding receptive fields) of animals may be divided into three groups. In the first and third groups are the low- and high-threshold units respectively. Stimuli which were threshold for these units evoked in humans tactile sensations and pain respectively. The second group consisted of middle-threshold receptor units. Presentation of ultrasound stimuli which are threshold for this group evoked tactile and thermal sensations in humans (Enin *et al*. 1989).

In acute experiments on rats studies have been carried out on impulse activity of single fibers of the sciatic nerve evoked by stimulation of the receptive fields of the sole of the rat paw by pulses of focused ultrasound. Mechanical effects were produced by rectangular ultrasonic stimuli and the thermal one by trapezoid ones (Enin *et al*. 1992). Such the stimulus allows to decrease the mechanical action of ultrasound at the neural structure and therefore to avoid the inducing of the tactile sensations. Being applied to the skin of humans, these stimuli evoked the sensation of warmth and with the increase of the intensity induced the nonspecific pain. Their application to animals gave rise to impulse activity in single afferent fibers.

For illustration, Figure 31 (Enin *et al*. 1992) presents the impulse activity of the middle-threshold receptive unit registered in response of stimulation of the sole of the rat paw by ultrasound stimulus. Focused ultrasound with the frequency of 2.6 MHz in a form of a single trapezoidal pulse with the duration of 400 ms was applied. The duration of increase and recession of the pulse was 100 ms, therefore the duration of the plateau was 200 ms. Such the stimulus elicited in humans the sensation of warmth changing by the burn pain.

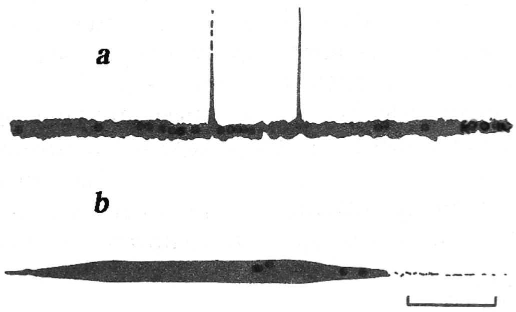


Figure 31. The response of the middle-threshold receptor units at the stimulation of the sole of the rat paw by ultrasound stimulus of a trapezoidal form; a - impulse activity recorded from a single nerve fiber; b - mark of a stimulus; calibration is 100 ms (Enin *et al*. 1992)

4.8. 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 (Tsirulnikov *et al.* 1986). Activation of these points was carried out by single rectangular pulses of focused ultrasound (frequency of 2.67 MHz) with the duration of 1 ms for the study of tactile sensitivity and 30 ms for investigation of pain sensations. Since corporal acupuncture points are located at various subcutaneous distances, for comparison also were 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 (Tsirulnikov *et al.* 1986, 1993). 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. The regularity of increasing of thresholds in proximal direction was confirmed also for acupuncture points. 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. An increase in the duration of the ultrasound stimulus decreased the threshold of the sensations evoked by them (Vartanyan *et al.* 1990).

4.9. Possibility to Activate Unmyelinated Nerve Fibers

It has been shown in Section 3.4 that when the ear labyrinth of animal was destroyed by special means, just the nerve fibers could be activated (Vartanyan *et al*. 1982). Similar results of ultrasonic activation of the nerve fibers were obtained by recording the impulse activity in single afferents fibers of the limbs in rats under the action of focused ultrasound on the peripheral endings of these fibers in receptive fields of the soles (Enin *et al*. 1992; Tsirulnikov *et al*. 1993).

Chapter 5

FROM SCIENTIFIC INVESTIGATIONS TO PRACTICE

This Chapter 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 prosthesis, on estimation of the bone tissues regeneration after fractures, and evaluation of the efficiency of anesthetic and analgesic drugs, on tactile sensitivity in children with inborn and postamputational stumps of the forearm, and on the possible use of phased arrays for stimulation of neural structures.

5.1. Ultrasound 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 may prove the diagnosis, evaluate the extent of pathological processes, monitor the results of treatment and so forth (Godovanik *et al*. 1978; Gavrilov, Tsirnlnikov 1980; Gavrilov 1984).

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 (Godovanik *et al*. 1978). 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 using 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 32 (Godovanik *et al*. 1978; Gavrilov 1984) presents the results of an examination of the tactile sensitivity in one of the patients with syringomyelia. The frequency of focused ultrasound was 1.95 MHz, the maximum intensity averaged over the area of the focal region was 1400 W/cm^2 and the duration of single stimuli varied from 0.1 to 100 ms. 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 0.1 ms the tactile sensitivity on the right (affected) hand was absent even at the maximum intensities (1400 W/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.

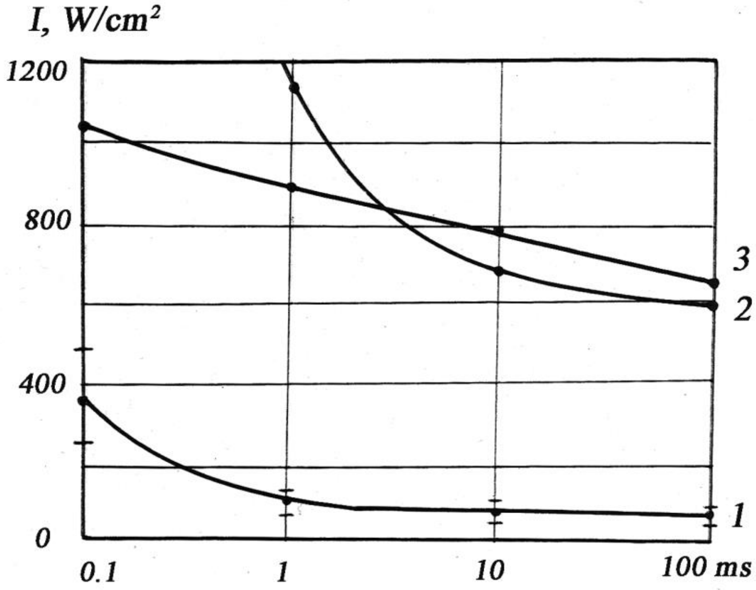


Figure 32. Thresholds of tactile sensitivity in a patient with syringomyelia: *1* - thresholds in healthy persons (control group); *2* - thresholds in the right (affected) hand; *3* - thresholds in the left hand, where the standard neurological examination showed no sensitivity disorders (Godovanik *et al*. 1978; Gavrilov 1984).

Figure 33 presents the data of similar measurements in the patient with polyneuritis. It is seen that thresholds of tactile sensitivity in both hands of a patient were much higher than in the control group. There are other examples of applications of ultrasound method for diagnostics of neurological diseases (Godovanik *et al*. 1978).

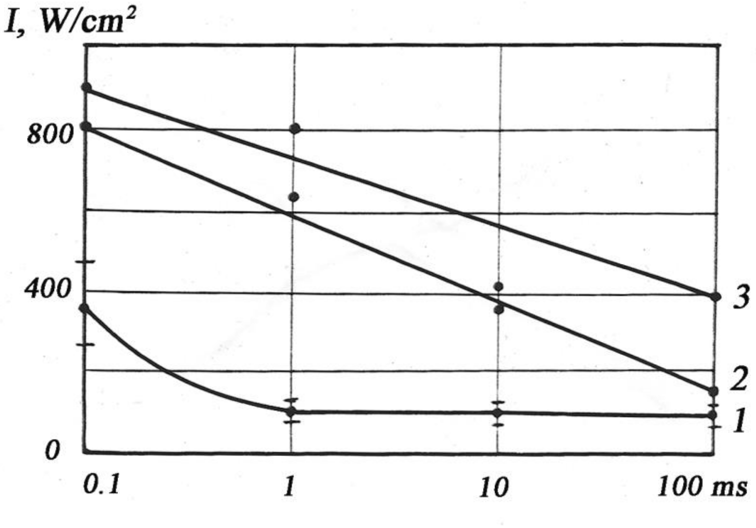


Figure 33. Thresholds of tactile sensitivity in the patient with polyneuritis: *1* - thresholds in healthy persons (control group); *2* - thresholds in the left hand; *3* - thresholds in the right hand (Godovanik *et al*. 1978).

In some patients, pain in bones appeared under the effect of focused ultrasound, whereas in healthy people a stimulus of the same intensity did not cause any pain. It follows that, during some diseases, along with a reduction in tactile sensitivity (increased thresholds), the thresholds of other sensations, such as pain, may decrease.

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 (Ashkinazi *et al*. 1992). 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.

5.2. Ultrasound 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 1.7 MHz) with the duration of 1 ms has been used (Tsirulnikov *et al*. 1988). If the sensation was absent, the duration was increased up to 6 ms and sometimes up to 12 ms. 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 (Tsirulnikov *et al*. 1988). 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 1 ms did not appear, in these cases the duration was increased up to 6 and 12 ms. 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 12 ms. 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 pathological processes in skin of various genesis.

5.3. Applications in Orthopedics for Estimating the Regeneration of the 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 has 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.

5.4. Tactile Sensitivity in Children with Inborn and Postamputational Stumps of the Forearm

In research into healthy children as well as into children with inborn and postamputation stumps of the forearm, the relationship between the level of tactile sensitivity in the skin of the forearm and the level of its muscular motor activity has been studied (Tsirulnikov *et al*. 1990). These studies were performed using focused ultrasound stimulation in children of different ages (7; 10 and 14 years). It was found that, with the increase of motor activity of the forearm, irrespectively of the age, tactile thresholds decrease. The success of prosthetics depends on the ratio between the skin tactile sensitivity and the motor activity of the forearm. The lowest thresholds were found in a zone innervated by the median skin nerve of the forearm.

The results of this study led to principally new data about the relationship of a tactile sensitivity with the innervation zones of different nerves. It appears that the tactile sensitivity related with some nerves might be higher than a sensitivity related with other nerves. There were not found such data in the literature, probably because earlier there was no possibility to measure the thresholds with the accuracy comparable with measurements fulfilled by ultrasound method.

5.5. 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 administrations seems self-evident (Gavrilov 1984; Gavrilov *et al*. 1996).

One of examples of application of this approach has been demonstrated in a number of works (Gavrilov *et al*. 1996; Davies *et al*. 1996). 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 3.5 h following the administration of the drug showed a significant correlation with the corresponding visual analogue scale score (Wright *et al*. 1993). 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 (Wright *et al*. 1993). Restoration of the amplitude of the evoked potential to its original, predrug value by increasing one or more of the 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 (Gavrilov *et al*. 1996, Davies *et al*. 1996).

5.6. Ultrasound Diagnostics of Hearing Diseases

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, computer 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 diseases. 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 (Gavrilov *et al*. 1983; Vartanyan *et al*. 1985; Tsirulnikov *et al*. 1988). 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 Department of Hearing Physiology and Pathology headed by Prof. A. S. Rosenblyum in the Leningrad Institute of the Ear, Throat, Nose, and Speech Disorders. Another active participant of these works was Dr. E.M. Tsirulnikov. In addition to fundamental problems of hearing physiology, were elaborated some original, new methods of diagnostics, treatment and prognosis of the pathological process for different forms of hearing and vestibular function disorders.

In this study a single element focused radiators were used. The focal distance was 70 mm, and the convergence angle was 30°. The operation frequency of different transducers was varied from 0.4 to 3.7 MHz, although most of the studies, including those described below, employed

2.5 MHz 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 2.5 MHz was 1.5 mm, the length of the focal region was 9 mm and the area of the focal spot was 1.75 mm^2.

Precise location and controllable acoustical matching of the focusing radiator to the ear labyrinth was ensured with the use of a coordination system described in Section 3.2 (see Figs. 3.4 and 3.5). It provided the movement of the radiator in three mutually perpendicular directions with an accuracy of 0.1 mm. The radiator was placed in a sound-transparent polyethylene bag filled with distilled water heated to 30-35°C. A removable focus pointer located outside the bag was mounted to the radiator. 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 external auditory meatus was blocked with a piece of cotton. 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 30-35 mm 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. It was found that the threshold for hearing sensations induced by ultrasound increased by 2-3 times when the radiator acoustic axis was displaced by more than 5 mm towards the external acoustic meatus and by more than 10 mm in other directions. In view of these findings, displacement of the focus pointer at the end of each experiment was checked. The results were not taken into account if the displacement value exceeded the above values.

The use of the described setup was found rather labor-consuming, and a handier device including an ultrasonic generator and focusing radiator was developed (Figure 34). The radiator was mounted on a headband. The acoustic axis of the radiator should be placed perpendicularly to the sagittal plane of the head. As a rule, the radiator was not displaced noticeably during the trial.

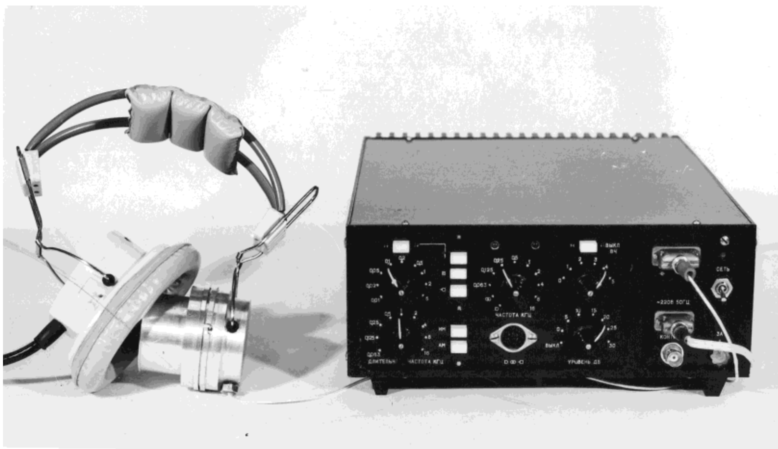


Figure 34. Ultrasonic generator and a radiator with a headband for diagnostics of hearing diseases (Tsirulnikov *et al*. 1988)

Ultrasound was amplitude-modulated by a sinusoidal sound signals at standard audiometric octave frequencies in the range of 125-8000 Hz, as well as by 50-100 μs pulses with the same repetition frequencies. In preliminary investigations, hearing thresholds were expressed in ultrasound intensity values averaged over the focal spot area according to measurements made in water. These measurements were carried out using the well-known radiation force balance method (Robinson 1984). The actual intensity in the head tissues is difficult to estimate due to significant defocusing of the beam by the cranial bones and to the high attenuation of ultrasound in the bone. It is clear, however, that the intensity values are much (perhaps a few orders of magnitude) lower in the labyrinth than in water.

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 35 (Tsirulnikov *et al*. 1988).

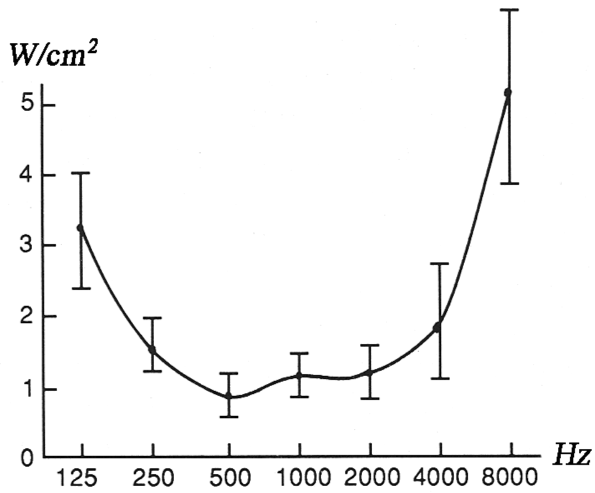


Figure 35. Averaged ultrasonic frequency-threshold curve for normal hearing persons. The horizontal axis shows frequency of amplitude modulations in Hz, the vertical axis is the ultrasound intensity averaged over the focal spot area in W/cm^2 (according to measurements in water). Vertical lines correspond to mean square deviation of the thresholds (Tsirulnikov *et al*. 1988).

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 3.3 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 (see Chapter 1). 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 35.

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 essence 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 the paper of Tsirulnikov *et al*. (1988). 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 (Tsirulnikov *et al*. 1988). 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.

As an example, the method of diagnostics of otosclerosis is desribed below. First of all, it was shown that ultrasonic audiograms of patients with this disease were different from their tonal audiograms (Gavrilov *et al*. 1983; Tsirulnikov *et al*. 1988). Thresholds in response to amplitude-modulated ultrasound were recorded when the air conduction was reduced by 45-50 dB. 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 36). 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 successful surgical intervention the sensitivity to modulated ultrasound was restored and the ultrasonic audiogram became more similar to the tonal audiogram.

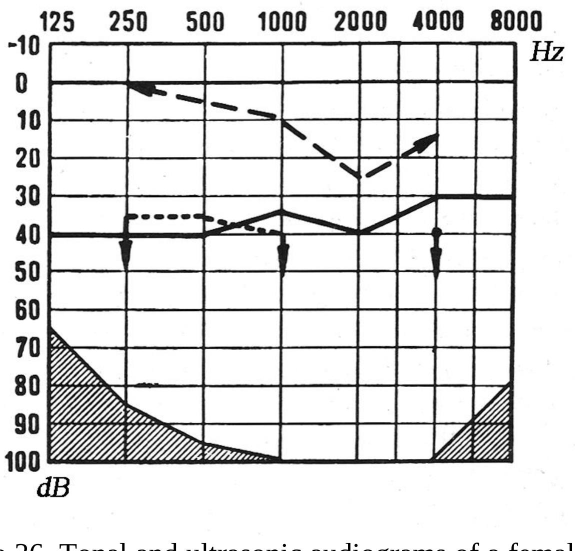


Figure 36. Tonal and ultrasonic audiograms of a female patient aged 45 with otosclerosis. Solid line is an air conduction audiogram; dashed line is a bone conduction audiogram, and dotted line is an ultrasonic audiogram. Horizontal axis is a frequency of tone or of ultrasound modulation in Hz; vertical 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 8000 Hz are absent (Tsirulnikov *et al*. 1988).

Our examinations of otosclerosis patients have also contributed to a better understanding of the routes by which ultrasound reaches perceptive structures. The fact that surgical treatment restores sensitivity in the whole range of frequencies suggests that sensory receptor structures within the labyrinth are activated. Since such operations are based on restoring the mobility of the oval window, it appears that the latter plays a special role in the transfer of stimulating signals into the labyrinth.

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 considerably 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.

Safety of ultrasound diagnostics

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 radiator were not higher than 0.1-0.2 W. Corresponding values of the intensity in the region of the projection point on the patient's skin were not higher than 0.02-0.05 W/cm^2 (Tsirulnikov *et al*. 1988) The possible values of an ultrasonic intensity in deeper tissues couldn't 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 20-30 dB/cm at 2.5 MHz). 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 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 (Vartanyan *et al*. 1980, 1985) 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.

Data on the long-term ultrasound irradiation of the hearing organ were obtained in the study of auditory perception of amplitude-modulated focused ultrasound by a person with normal hearing (one of the leading participants of these studies). In this case, the calculated ultrasonic intensity in the focal region was from units to tens of W/cm^2 (data for water, i.e. without defocusing), and the duration of exposure reached several minutes at each session. No changes in the hearing sensitivity were observed after several years of permanent participation of this person in the experiments. Moreover, none of the natural changes in auditory sensitivity sometimes occurring in people of over 50 years of age were observed in this person.

To the end of 1980s, when these works were terminated due to the reasons independent on participants of these studies, 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 cocnleaneural 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.

In spite of the fact that some details of the mechanisms underlying the perception of focused amplitude-modulated ultrasound remain unanswered, ultrasonic diagnostics is clearly a valuable tool for the complex diagnostics of many hearing diseases.

5.7. Is it Worth to Develop Ultrasound Devices for Diagnostics of Hearing Diseases and for Hearing Prosthesis?

As it has been shown above, there are two large fields of possible applications of amplitude-modulate focused ultrasound in otology: diagnostics of hearing diseases and hearing prosthesis. As for the first possibility, the reply on the question in the title of this Section is uniquely and unconditionally positive. It was shown above that ultrasound methods are useful for diagnostics of hearing diseases and the trustworthiness of the diagnosis is very high; i.e. the diagnosis was highly reliable. But the method of application of this procedure is evidently more complicated than one in the usual tonal audiometry. That is why there is no need to use ultrasound method and technique when the existing diagnostic methods give unequivocal diagnosis. It is advisable to use ultrasound method in the cases with complex pathology when unambiguous diagnosis with traditional approaches has not been defined and the ultrasound diagnostic method can become a “method of choice”.

A situation with ultrasound devices for hearing prosthesis is much more ambiguous for several reasons. As in the previous case, a number of cases when such methods and technique could be useful is relatively small in comparison with the total amount of people having hearing loss or deafness. As it was mentioned, ultrasonic audio prosthetics may be effective in cases of deafness with a complete loss of receptor elements, in condition that the auditory nerve fibers, by which the auditory information is usually transmitted from hair cells to the brain, remain intact. In some of such cases, e.g. for the mentioned above bilateral sudden deafness, standard hearing aids cannot help. It is easy to imagine how important is for such people to have a possibility at least a relatively short time to communicate with relatives, etc. It is especially important because without any training the hearing nerve of such people died off rather soon.

We had tried to develop ultrasound devices for the research into feasibility to help such people. The photographs of devices for diagnostics of diseases of hearing and audio prosthetics developed in 1980s in the Acoustic Institute and V.P. Vologdin Research Institute of High-Frequency Currents (St. Petersburg) in cooperation with the mentioned above medical organizations are shown in Figure 37 (Gavrilov, Tsirulnikov 2012). The “Sensophon” was the first of these devices (Figure 37a). It is an ultrasound analogue of the tonal and speech audiometers. It has a relatively small size and weight and can be used in clinics. The device “Ultraphon” was designed for inputting auditory information to the deaf and hearing impaired people (Figure 37b). It is a simplified ultrasonic diagnostic device with a minimal number of external adjustments.

(a) **(b)**

Figure 37. Devices "Sensophon" (a) and "Ultraphon" (b) for diagnostics of hearing diseases and studying the possibilities of inputting auditory information to the deaf people (Gavrilov, Tsirulnikov 2012).

These investigations were continued in our Institutes until the beginning of 1990s when due to a common economical situation in Russia financing of works related with the research and development of new medical technique were terminated, and then these works were carried out in an informal way, by the efforts of a few enthusiasts.

Obviously, the systems developed in the 1980s for research are cumbersome and are not comparable in size with the individual hearing aids used in conventional sound-amplifying audio prosthetics. It is evident that at the present there is no sense to reproduce such the technique because the modern electronics became qualitatively different.

In addition, it is still unknown whether a daily and long, sometimes lasting for many hours, action of focused ultrasound on ear labyrinth 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, the studies described have not been implemented.

5.8. Possible Use of Phased Arrays for Stimulation of Neural Structures

An idea to use multi-elements two-dimensional phased arrays to stimulate neural structures is extremely valuable and perspective. As it was shown in Section 1.4, the use of such 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 (Hertzberg *et al*. 2010; Naor *et al.* 2012). This idea is in agree with the state-of-the-art approach to the development of ultrasound tactile displays (Section 1.4). Therefore it is worth to stay in brief on the construction and characteristics of modern two-dimensional arrays.

A key advantage of two-dimensional phased arrays is their ability to simultaneously generate and steer in space several foci (Ebbini, Cain 1989; Daum, Hynynen 1998, 1999). It has been shown that this ability is realized most effectively and with the least level of grating lobes by means of the use of so called random arrays, i.e. arrays with elements distributed randomly at its surface (Goss *et al*. 1996; Gavrilov, Hand 2000; Hand, Gavrilov 2000; Gavrilov *et al*. 2000; Gavrilov 2003). However, if a large number of foci is generated simultaneously (for instance, 16 or 25), secondary intensity maxima ("hot spots") are inevitably originated in the acoustic field along the path of the ultrasound beam to the focal plane, which in some practical situations may appear unsafe. In such a case, it is expedient to use a more safe approach (Ebbini, Cain 1989; Daum, Hynynen 1998, 1999). Its idea is that, instead of generation a static field with a rigidly fixed set of secondary maxima of the intensity, it is suggested to use the fields with several configurations (patterns) consisting of a lesser number of foci, which are switched electronically at a frequency of 10–20 Hz. It is desirable to have the same number of foci in each pattern. The design and characteristics of the modern random phased arrays are given in Pernot *et al*. (2003), Hand *et al*. (2010). The detailed analysis of methods of calculations of two-dimensional phased array is presented in a number of papers (Ebbini, Cain 1989; 1991; Goss *et al*. 1996; Gavrilov, Hand 2000; Gavrilov *et al*. 2000; Gavrilov 2003).

The main goal of the work of Gavrilov (2008) was to propose and study in model numerical experiments an alternative way for the development of tactile displays based on the application of a two-dimensional array with elements randomly distributed on its surface. The calculation of the spatial distributions of acoustic fields was performed for the array with the surfaces in the shape of a part of a spherical shell with a curvature radius of 60 mm. The array diameter was 65 mm and the ultrasonic frequency was 3.0 MHz. The arrays consisted of 256 flat elements in the form of discs with a diameter of 2.5 mm (i.e., 5λ at this ultrasound frequency). The distance between the element centers varied and was ≥ 3.0 mm. The total active area of the array elements was 12.5 cm^2, which provides an opportunity to reach the required values of acoustic power (up to 20–30 W) at appropriate values of intensity on the element surfaces. Figure 38 (Gavrilov 2008) shows the positioning of elements for a random array.

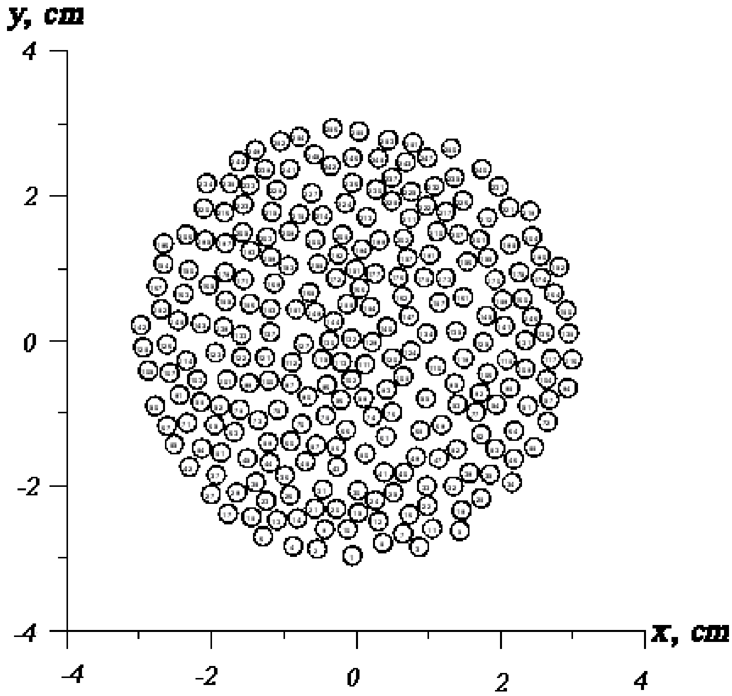


Figure 38. Positioning of elements at the surface of a random array (Gavrilov 2008).

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 39 Gavrilov (2008). To create these symbols, 24 and 25 foci, respectively, were used. The sizes of the fields where the simulations were carried out were 4 x 4 (a) and 1 x 1 cm (b). The absence of significant secondary maxima within the field of interest indicates an appropriate quality of the intensity distributions.

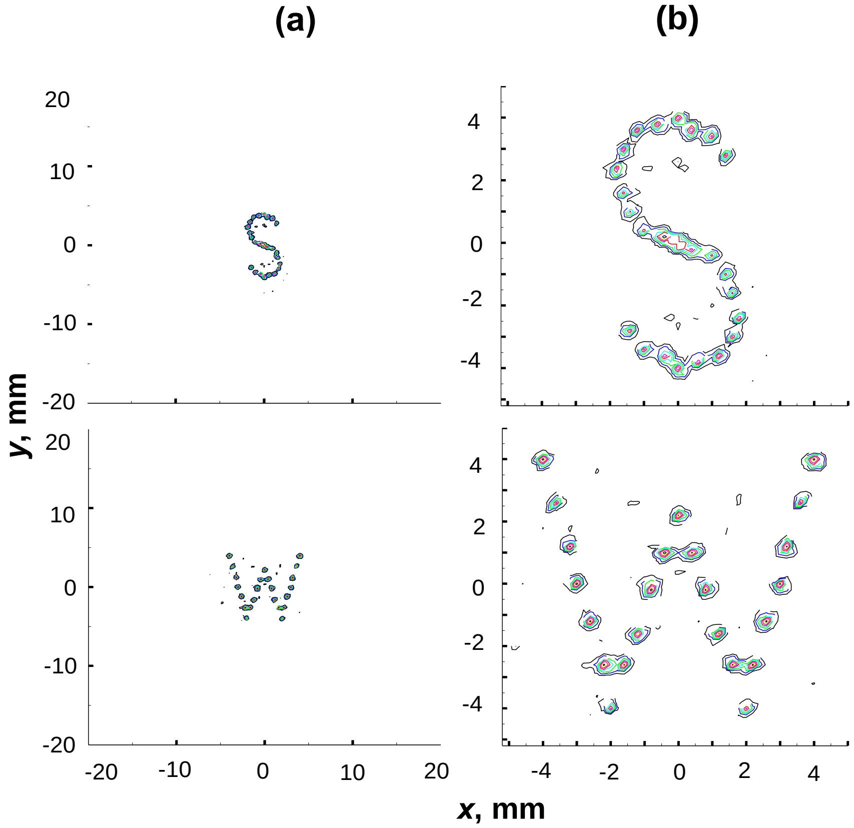


Figure 39. Synthesis of complex symbols in the form of some Latin letters, by a random array. The dimensions of the field under investigation are (a) 4 x 4 cm and (b) 1 x 1 cm (Gavrilov 2008).

In connection with the images presented in Figure 39, it is worthwhile to discuss one of the possible designs of ultrasound tactile display (see Section 1.4). There are mechanical tactile displays allowing blind and even deaf-and-blind persons to perceive textual information displayed by a relief-dot font due to the effect of the radiation pressure. 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. Ultrasonic tactile displays have potential advantages: they are noiseless, contactless, 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. 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 (Gavrilov 2008).

As it was shown in Section 1.4, all the last prototypes of airborne ultrasound tactile displays include as a main stimulating unit just a two-dimensional phased array. 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.

Conclusion

The subtitle of the Conclusion could be "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 (Broun *et al*. 1981) 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 Sections 2.7 and 3.3) 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.

For example, for inducing hearing sensations, still remains non clear the fact that so called critical time of temporal summation (see section 3.5) for sound and ultrasound stimulations of labyrinth differs in more than two orders. According to literature data (Arapova *et al*. 1972; Pedersen, Elberling 1972), this time is equal to 200-300 ms for sound stimulations, whereas our numerous measurements of this time for ultrasound stimulation led to the values of 0.5-1 ms. These data were obtained with the use of focused ultrasound not only in persons with normal hearing, but also in patients with different pathology of hearing (see Section 5.6). Moreover, such small value of the critical time of summation became one of important indicator for clinical diagnostics of a number of hearing diseases. So, the difference in more than two orders between the time of summation for sound and ultrasound stimulations of labyrinth, suggests that the simple explanation of mechanisms of ultrasound-induced auditory sensations by the action of the radiation pressure only, presented in section 3.3, is not exhaustive.

Another motive to reflections gave our measurements of aural ability to determine time intervals between two ultrasound stimuli of rectangular shape (Section 3.5). It was shown that for the duration of stimuli equal to 1-2 ms one click was perceived by a subject, and after the duration of about 5 ms and more two separate clicks in the beginning and in the end of the ultrasound stimulus were perceived. For rather long ultrasound stimuli with the duration of, e.g., 100 ms and longer, the character of perception did not change: a subject perceived only the beginning and the end of ultrasound stimulus but not its filling by unmodulated oscillations of ultrasound frequency. It was not observed any effects of the action of carrier frequency of ultrasound on functional state of the hearing organ (as effects of suppression, etc.), probably because the ultrasound intensities used in these experiments were too low.

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 (Gavrilov, Tsirulnikov 1980) (see Section 3.3). 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 (Vartanyan *et al*. 1982) (see Section 3.4). Usual sound signals, even of high intensity, are nor 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 2.7). 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 (Gavrilov *et al*. 1976; Gavrilov, Tsirulnikov 1980; Gavrilov 1984). 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 2.4). For example, estimations fulfilled with the use of Eq. 11 and data from Table 7 shows that for the duration of ultrasound stimuli of 1 ms in the frequency range of 0.48-2.67 MHz, the temperature rises corresponding to induce in a person warmth and cold sensation are equal to 0.003-0.39 ^{o}C and 0.007-0.39 ^{o}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 2.6), or, in other words, with deformation of tissues under the action of the gradient of the mechanical force.

Meanwhile, the effects of oscillations of ultrasound carrier frequency on neural structures are worth a special attention. As it was mentioned, these effects were not noticed in all our experiments, but it does not mean that they do nor reveal, for example, at the studies of the action of ultrasound on the structures of the central nervous system. Literature data indicate effects of neuromodulation of the brain activity but also describe various effects of suppression or inhibition of sensory functions of the brain (see Sections 1.3-1.5). There were not obtained reliable and unambiguous data regarding the possibility of ultrasound stimulation of neurons in the central nervous system. From this point of view, the term “neurostimulation”, i.e. an occurrence of functional electric activity of brain neurons due to the effect of a stimulus, is hardly applicable to the impact of ultrasound stimuli on the brain structures.

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 (Gavrilov, Tsirulnikov 2012).

- Ultrasound can activate not only peripheral receptor structures, but also nerve fibers, and it can have a functional effect on brain structures.
- 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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