MODULATION OF THE FUNCTIONAL STATE OF THE BRAIN WITH THE AID OF FOCUSED ULTRASONIC ACTION

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We investigated the possibility of modifying the functional state of the brain with the aid of focused ultrasound and studied various regimes of its action. A specific pattern in the effect of focused ultrasonic action was discovered with regard to its intensity: the effect is absent at low (less than 0.1 mW/cm^2) intensities; activation of bioelectrical activity in the brain takes place at intensities from 1 to 100 mW/cm^2; and suppression of the ECoG takes place at intensities from 1 to 100 W/cm^2. On the basis of our own data and the data in the literature, we suggest that the mechanism of ultrasound action is based upon changes in the permeability of neuronal membranes leading, after a chain of intracellular molecular reactions, to a subsequent general de- or hyperpolarization of the membranes of neuronal populations and to a change in the bioelectrical activity of the brain.

KEY WORDS: focused ultrasonic action; cerebral cortex; ECoG; evoked potentials.

The action of high-intensity focused ultrasound on the central nervous system has been investigated in appreciable detail and is described in a number of reviews [7, 13]. Special attention has been devoted to long-term injury and thermal effects associated with ultrasound, effects which indicate that high-intensity focused ultrasound can destroy brain structures of certain shapes and dimensions [12, 16].

However, the use of focused ultrasound to obtain reversible changes in the bioelectrical activity of structures in the central nervous system has been insufficiently studied, although it is precisely the reversible changes which are of interest for studying the structuro-functional connections in the central nervous system and which are promising for the use of ultrasound in clinical practice. Also, little attention has been given to the action of low-intensity ultrasound on the central nervous system, although such data are becoming increasingly important due to the growing using of ultrasound in medical diagnostic equipment.

Investigations of nerves in frogs and cats [19] have shown that focused ultrasound can reversibly block axonal conduction; a stimulating action of focused ultrasound upon auditory fibers has also been observed [5]. It has been shown in a report [17] that there is a heightening of synaptic reflexes with a subsequent depression when the intensity of ultrasound is increased. Low-intensity but unfocused ultrasonic action on the brains of monkeys leads to reversible suppression of the EEG [15].

The majority of effects of focused ultrasonic action upon the functioning of the central nervous system described in the literature are associated with the suppression or blocking of individual brain functions during high-intensity ultrasound. We know of no data on the activation of brain functions of higher vertebrates during exposure to focused ultrasound accompanied by a study of the bioelectrical activity of the brain.

In our present research, therefore, we set out to clarify the possibility of modulating the functional state of the brain with the aid of focused ultrasonic action and to study various regimes of its action.

METHODS

The investigation was carried out in acute experiments on 17 cats (2.5-4 kg in weight) and in chronic experiments on rabbits (2.5-3.5 in weight). In the acute experiments, the

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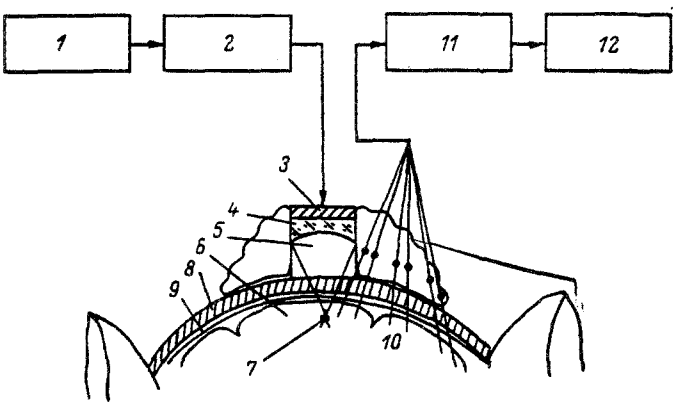

Fig. 1. Block diagram of the set-up for focused ultrasonic action upon the brain of an animal. 1) High-frequency generator, 2) modulation block, 3) piezoceramic emitter, ultrasound generator, 4) focusing lens, 5) liquid ultrasound guide prepared from degassed agar gel, 6) brain of animal, 7) focus of ultrasound, 8) skull bone, 9) dura mater, 10) recording electrodes, 11) electroencephalograph, 12) analyzer.

head of an animal placed under light nembutal narcosis (30-40 mg/kg, given intraperitoneally) was fixed to a stereotaxis apparatus in a screened chamber. Ultrasonic action on brain sites was implemented through a trepanated opening in the animal's skull. In this process, an ultrasound emitter was fixed to the micromanipulator of the stereotaxic apparatus; this allowed us to focus the ultrasound with a fair degree of precision upon the brain site studied. The ECoG from the brain sites irradiated were recorded in bipolar and monopolar fashion using electrodes made from tungsten wire in fluoroplastic insulation. Each electrode was 0.1 mm in diameter and had an unsharpened tip (uninsulated) 0.5 mm in length. Brain sites located in the temporal, sensorimotor, and parietal cortex were subjected to ultrasonic action. The indifferent electrode-screw was fixed to the bone of the frontal sinus. The recording of the ECoG was carried out on a 16-channel "Orion" electroencephalograph.

In the chronic investigations, ultrasonic action was carried out through intact skull bone with the aid of a setup described in the block diagram shown in Fig. 1. The piezoceramic emitter (3), creating the ultrasonic oscillations, and the focusing lens (4) were focused on the skull of an awake animal with the aid of a rapidly hardening plastic so that the focus of ultrasonic oscillations was located at a depth of 1.5-2,0 mm from the surface of the dura mater at the selected site in the neocortex.

Various regimes of focused ultrasonic action were studied: individual pulses with durations ranging from 0.1 to 100 msec and packets of pulses with frequencies of 1-20 pulses/sec. The calculated ultrasound intensity at the focus varied from 1 $\mu W/cm^2$ to 1400 W/cm^2. Various physiological indices were investigated: ECoG, thresholds to direct electrostimulation, evoked potentials to light flashes, and reactions associated with the imposition of rhythms of light stimulation.

Determination of thresholds for motor activity to direct electrostimulation of the motor cortex was performed with the aid of a ESL-2 electrostimulator. Recording and accumulation of evoked potentials was carried out on a NTA-512 analyzer (200 msec epoch of analysis, the number of accumulations was 40 per 2-4 channels simultaneously).

The indices investigated were analyzed in control experiments (without ultrasound) prior to and during the process of focused ultrasonic action and during the post-action period. The reliability of the differences in these indices during various phases of the experiments were evaluated according to Student's criterion.

RESULTS

In the first series, peculiarities associated with changes in the bioelectrical activity of the neocortex during various regimes of focused ultrasonic action administered through a trepanated opening were investigated in acute experiments.

The character of the changes in bioelectrical activity is determined by the duration of the focused ultrasonic action and the intensity of the action. Short pulse action with

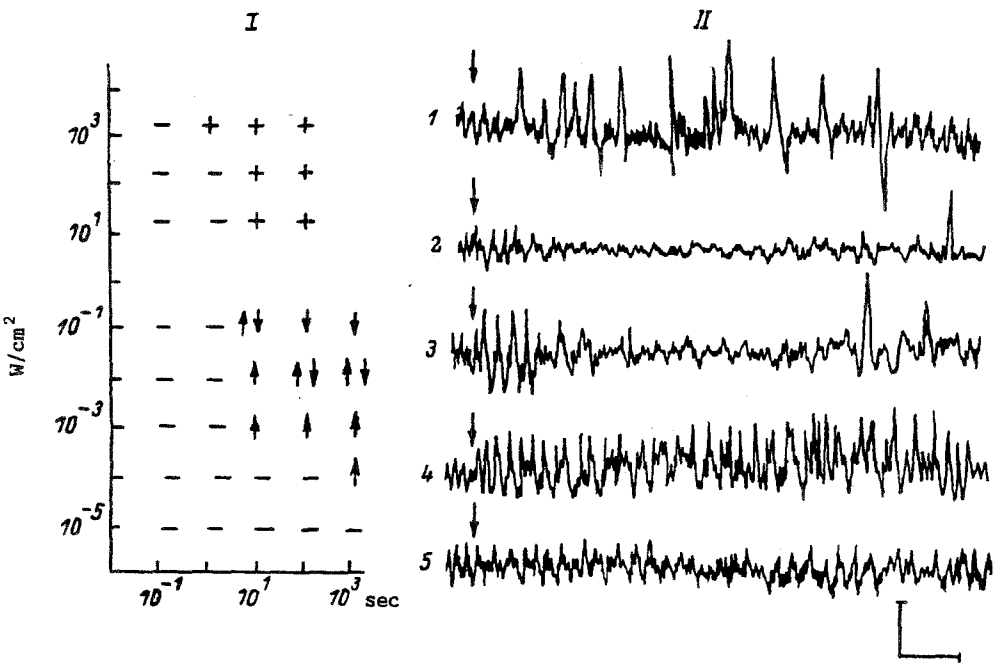

Fig. 2. Changes in the ECoG for various parameters of focused ultrasonic action. I) Character of reorganizations at different intensities and durations of focused ultrasonic action: crosses) manifestation of "peak" activity; downward arrows) suppression of ECoG amplitude; paired arrows) two-phase reaction dependent upon the frequency and duration of ultrasonic action; upward arrows) increase in amplitude of the ECoG. II) Typical patterns of ECoG at different intensities of focused ultrasound; I) 10 W/cm², frequency of modulation 10 Hz; 2) 100 mW/cm², frequency of modulation 16 Hz; 3) 10 mW/cm², frequency 6 Hz; 4) 1 mW/cm², frequency 6 Hz; 5) 0.01 mW/cm², frequency of modulation 8 Hz. Arrows) initiation of ultrasonic action. Calibration bar) 50 μV, 1 sec.

ultrasound intensities ranging from 1 mW/cm² to 1400 W/cm² did not alter the bioelectrical activity; this was determined by the absence of repetition in the time and amplitude characteristics of the extreme and transitional points on the summated ECoG patterns. Evoked potentials to direct focused ultrasonic action were not observed.

Increases in the duration of the ultrasonic action up to 1 or more seconds led to tonic alterations in the ECoG. In these cases, reorganizations of the ECoG can be seen most clearly at durations of the ultrasound action exceeding 10 sec. The character of the changes in the ECoG in these cases depended upon the intensity of the focused ultrasound. Ultrasound with intensities ranging from 1 to 100 W/cm² resulted in a pronounced suppression of bioelectrical activity along with an appearance of high-amplitude peaks. The frequency of the peaks did not coincide with the frequency of ultrasound modulation. In a number of experiments, such activity showed features of typical paroxysmal "peak-wave" activity (Fig. 2, II). Similar changes in the ECoG were noted not only in the focal region of ultrasonic action but also in regions neighboring it. In 30% of the cases, high-amplitude peaks arose only in distant regions of the neocortex; only suppression of activity took place in the focal region. In 10% of the cases, "peak-wave" activity appeared in mirror-image fashion within the opposite hemisphere.

Ultrasound of 10-100 mW/cm² intensity was accompanied by two differently directed reorganizations in the ECoG; the character of these reorganizations depended strongly upon the frequency of modulation and the duration of ultrasonic action. During the action of focused ultrasound over 5-10 sec and during modulation of ultrasonic action by frequencies of up to 6 Hz, an effect involving an increase in the amplitude of the ECoG took place along with a shift in the spectral rhythms to the side of low frequencies (Fig. 2, II, 3). The synchronization developed in the ECoG continued for 30 sec following ultrasonic action. An increase in the duration of the action up to 60 sec and modulation of the ultrasound by frequencies

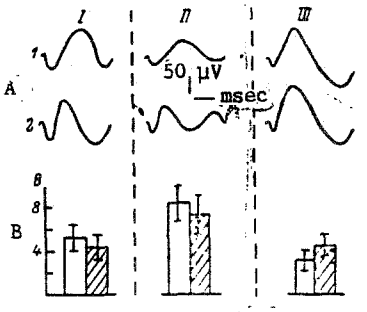

Fig. 3. Changes in the functional state of the brain during focused ultrasonic action. I) Control (prior to ultrasonic action); II) ultrasonic action with an intensity of 10 mW/cm^2, III) with an intensity of 1 mW/cm^2. A) Average evoked potential to light flashes; 1) left motor cortex, zone where ultrasound focus was positioned; 2) visual cortex. Calibration bars: 50 μV, 50 msec. B) Threshold for direct electrostimulation of the brain, C: white columns) left motor cortex, zone where ultrasound focus was positioned, hatched columns) right motor cortex.

over 8 Hz led to a readily reproducible effect of desynchronization of bioelectrical activity. After 30-40 sec of ultrasonic action, a decrease in the amplitudes of all ECoG rhythms by 30-40% from the average background level occurred. A further increase in the duration of focused ultrasound action intensified this effect, and desynchronization of the brain's activity could develop over the course of 5-20 sec following the cessation of ultrasonic action.

Focused ultrasonic action with an intensity of 1-10 mW/cm^2 led to a stable increase in ECoG amplitude (Fig. 2, II, 4) over the entire time of ultrasonic action (5-10 min). The integral amplitude increased during the action by 30-50% in comparison with the background and gradually decreased after cessation of the ultrasonic action, returning to normal after 20-30 sec.

All of the actions applied caused unitypical changes in bioelectrical activity in the temporal, sensorimotor, and parietal cortex; the reorganizations of the ECoG took place in diffuse fashion and were noticed both in the local region of ultrasonic action and at some distance from it. Ultrasonic action at an intensity less than 0.1 mW/cm^2 caused practically no changes in bioelectrical activity.

In the second experimental series, reorganizations of ECoG were noted in chronic experiments on rabbits during which focused ultrasound was administered to the brain of the animal through intact skull bone. The functional state of the neocortex was investigated during ultrasonic action in a range of intensities causing a consistent change in the amplitude of the ECoG (1-100 mW/cm^2). The ECoG reorganization was slightly sensitive to the presence of a barrier to ultrasound transmission in the form of undamaged skull bone. During action through the undamaged bone, the intensity of the ultrasound needed for one or another type of ECoG alteration exceeded the intensity of ultrasound evoking such reorganizations through a trepanated opening by a factor of only 1.5-2. In this case, the threshold values for the intensity of ultrasonic action needed to achieve a specific effect did not exceed the range of individual variability.

In awake rabbits, focused ultrasound with an intensity of 1-100 mW/cm^2 and a frequency of modulation equal to 6-10 Hz results in a stable and pronounced increase in the amplitude of the ECoG along with a shift to low frequencies for durations of the action up to 20 min. The change in the ECoG can be observed not only in the focal region of the ultrasound but also in distant structures of the neocortex and sometimes in the opposite hemisphere. The changes in the ECoG are accompanied by an increase in the range of assimilation of rhythms consisting of light stimuli up to 45-50 Hz. An analysis of the functional state of the neocortex reveals two of its levels. During action of ultrasound with an intensity of 10-100 mW/cm^2, a lowering of the excitability of the neocortex takes place; this is manifest in a decrease in the amplitude of evoked potentials to light stimuli and in an increase in the

thresholds for direct electrostimulation of the motor cortex (Fig. 3, II). During the action of focused ultrasound with an intensity of 10 mW/cm^2, an increase in the amplitude of evoked potentials takes place both at the focus of the action and in remote structures. A decrease in the thresholds for direct electrostimulation also occurs (Fig. 3, III), with the decrease of thresholds within the zone of the focus consistently exceeding background values ($p < 0.05$) but, at the same time, not exceeding random deviations in the opposite hemisphere. Thus the action of focused ultrasound with an intensity of 1-10 mW/cm^2 results in an increase in the excitability of the neocortex.

DISCUSSION

The data obtained indicate that it is possible to distinctly alter the bioelectrical activity of the brain and its excitability under certain conditions with the aid of partially focused ultrasound. The character of the changes in the activity of the cerebrum are determined by the intensity of the ultrasound, the duration of its action, and the character of its modulation (duration, pulse, and on-off time ratio).

Short, solitary pulses of ultrasound (1-0 msec) of various intensities do not evoke stimulation effects even in the case of multiple actions (up to 100 pulses); this may be connected with a theoretical impossibility involved in obtaining evoked potentials with the aid of isolated ultrasound action [10].

During extended action of ultrasound with an intensity ranging from 1 to 100 W/cm^2 against the background of a decrease in the ECoG amplitude, pathological forms of activity ("peak-wave") occur; these are intensified when the excitation time is lengthened.

The lowering of the intensity of focused ultrasound to 10-100 mW/cm^2 clarifies the role of the duration of the action upon the character of reorganization of the functional state of the neocortex. Short-term action (5-10 sec) is accompanied by an increase in the amplitude of the ECoG, changing to suppression during more prolonged ultrasonic action. Short-term action is also accompanied by the appearance of pathological forms of activity. The role of ultrasound modulation in the character of the reorganization of brain activity is also most distinct in this range of intensities. During low-frequency modulation (4-6 Hz), a prolonged increase in the amplitude of the ECoG occurs, then, as at a modulation frequency of more than 8 Hz, ultrasound of the same intensity results in suppression of the ECoG amplitude and the appearance of "peak-wave" activity.

Ultrasonic action with an intensity of 1-10 mW/cm^2 allows formation of a state of the neocortex accompanied by synchronization of the ECoG and shifts of rhythms to a region of low frequencies and allows the state to be retained for a long time (for 10-20 min). Such a change in the functional state is reversible. The change in the character of the ECoG observed in this case is similar to reorganizations described for intercerebral micropolarizations [3], when the formation of an "optimized" functional state [4] takes place.

One feature of the functional state formed during ultrasonic action at an intensity of 1-100 mW/cm^2 is the presence of two levels identified according to the change in the excitability of the brain. The observed increase in the range of assimilation of light stimuli indicates an increase in the lability of the brain, however, its excitability during focused ultrasonic action at an intensity of more than 10 mW/cm^2 remains somewhat lowered and an increase in the excitability of the brain takes place only during an action of 1-10 mW/cm^2.

By analyzing the results obtained, it is possible to see a certain general biological pattern of focused ultrasonic action upon the brain of the animal, a pattern which involves a specific dependence of the effect of ultrasonic action upon its intensity. At low, subthreshold (less than 0.1 mW/cm^2) doses, the effect is absent; during increases in the intensity of the ultrasound (1-100 mW/cm^2), activation of the bioelectrical activity of the brain takes place: a power of 1-100 W/cm^2 causes suppression of the ECoG and a focused ultrasound with an intensity of more than 1000 W/cm^2 results in destruction of brain structures. In a specific interval of intensities (1 mW/cm^2-1 W/cm^2), the effect of focused ultrasonic action upon the bioelectrical activity of the brain is reversible.

There is no clarification at present as to the mechanisms involved in specific responses of the nervous system to ultrasonic action of low and intermediate (nonthermal) intensities. Judging from existing evidence that the amplitude of the displacement of the medium in the focal region of the emitter is a parameter unequivocally associated with the process of exciting the nerve structure [7], however, one can suggest the following hypothesis concerning

the mechanism of focused ultrasonic action upon the bioelectrical activity of the brain.

Elastic deformation forces which deform (dilate) the membrane of the nerve cells arise in the focus of the ultrasonic wave; this results in a change in the passive permeability of the membranes. Simultaneously, conformational alterations in the glycoprotein receptor structures of the membrane enzymes (the Na-K-ATPase type) take place under the influence of deformation forces; this leads to a change in the active permeability of the membranes for Na and K ions. A change in permeability of the membranes can also occur due to a sharp increase in the intensity of diffuse currents near a plastic membrane under ultrasonic action [2].

Such changes in the conformation of the molecule-receptor and the permeability of the membranes leads to the appearance of a signal which triggers the synthesis of cyclic nucleotides; after a subsequent chain of molecular reactions, this results in the a change in the activity of the nerve cells, to an overall depolarization or hyperpolarization of the membranes of the neuronal populations, and to a change in the bioelectrical activity of the brain.

The degree and direction of the change in the permeability of the membranes of the nerve cells (de- or hyperpolarization) and, subsequent to this, changes in the electrographic indices of the brain, depend upon the intensity and duration of the focused ultrasonic action and its modulation. This results in various phenomenal effects. This process is reversible and normalizes ten minutes after the cessation of ultrasonic action if the latter is not too intense and does not cause morphological disturbance to the brain structures.

The suggested hypothesis concerning the mechanism of ultrasonic action upon the nerve cells of the brain is in agreement with a variety of experimental data. Research has demonstrated [1, 9, 14] a change in the permeability of membranes during the action of low-intensity ultrasound (50-600 mW/cm^2). It has also been observed that a mechanical deformation of the membranes of the nerve cells leads, in the end, to a change in the membrane potential and a change in the frequency of action potentials.

By analyzing similar changes in the EEG during the action of various physiological factors (micropolarization, ultrasound, optical radiation [6, 11], one can surmise that a common molecular-structural mechanism involved in the primary reaction of nerve cells is at work in all of these cases; this is the change in the permeability of membranes. This mechanism is apparently the primary, general, and universal reaction of the nerve cell to any physiological influence.

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INTRASPECIES BEHAVIOR OF ANIMALS BASED ON A MODEL OF EXPERIMENTAL BRAIN ISCHEMIA

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The features of the intraspecies behavior of rats after ligation of the right, left, or both common carotid arteries were studied. It was shown that certain characteristics of behavior change differently following ligation of the right or left common carotid artery. The approach used may be utilized for the assessment of the severity of ischemic brain damage.

KEY WORDS: intraspecies behavior of rats; ischemic brain damage.

Changes in behavior in human vascular diseases stand at the initial stages of study. The creation of models of experimental ischemic insult offers the possibility of studying the changes in the behavior of animals, and the mechanisms underlying pathological behavioral reactions, which will promote the development of methods of pharmacological correction of the pathological forms of behavior.

The purpose of the present study was the elucidation of the features of behavior as a function of the individual characteristics entering into the makeup of a group of individuals, and the development of a model and criteria for the assessment of the behavior of rats with experimental ischemia of the brain.

METHODS

The experiments were performed on 84 mixed breed male rats weighing 150-200 g. To study the character and qualitative accounting of the group behavior of the rats a communicational apparatus was used [1]. It is a closed system formed of three intercommunicating

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