METHOD OF LOCAL APPLICATION OF FOCUSED ULTRASOUND TO DEEP BRAIN STRUCTURES OF AN UNRESTRAINED UNANESTHETIZED ANIMAL

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To study structural–functional relations in the CNS a widely used method is that based on blocking brain structures by exposure to various factors: electric, temperature, chemical, and so on. Changes in the animal's responses after blocking of brain structures are then studied. However, the use of all known blocking procedures is accompanied by unavoidable damage to brain structures located above the blocked formation, and in addition, the operations are complicated by secondary phenomena in neighboring structures as a result of disturbance of the circulation in part of the vascular system. The method based on application of focused ultrasound (FUS) to block deep brain structures is known to be free from the above drawbacks [1, 2].

However, with the technique described previously it is impossible to induce such a block in an unrestrained animal, because the experimental procedure of application of FUS to deep brain structures requires fixation of the animal in a stereotaxic apparatus, to which the ultrasonic generator is rigidly connected. The animal must therefore be immobilized or anesthetized.

The aim of this investigation was to devise a method of studying functional changes after blocking of CNS structures through application of FUS in the course of performance of a behavioral act by the animal. The result of ultrasonic application was assessed by studying changes in electrical activity (evoked potential — EP) of the optic tract.

EXPERIMENTAL METHOD

In the method developed, the fixing device with the ultrasonic generator is oriented in accordance with the coordinates of the structure to be blocked and is implanted on the animal's skull, and ultrasound is applied during performance of a behavioral act by the animal. This device has a body consisting of a hollow cylinder, whose internal diameter corresponds to the external diameter of the ultrasonic radiator, and is filled with sound-conducting fluid, and a miniature piezoceramic ultrasonic radiator, shaped like the segment of a sphere 1.8 cm in diameter with focal distance of 2.5 cm, and a working frequency of 0.975 MHz (Fig. 1). To implant the apparatus, the head of the previously anesthetized animal was fixed in the halter of a stereotaxic apparatus. A midline skin incision was made, the muscles dissected, and the part of the cranial bone above the structure to be irradiated was exposed. Using the indicator of the micromanipulator of the stereotaxic instrument, a point on the cranial bone located above the center of the area to be blocked was determined. A circle with its center as its point, and with a diameter equal to that of the radiator, was marked out on the animal's skull. A burr-hole of this diameter was then drilled. The body of the instrument was placed above the burr-hole and fixed to the skull with screws. The airtightness of fitting of the apparatus was ensured by the use of self-hardening plastic of the "Stirakril" type.

The effect of blocking and the course of adaptation were monitored by recording electrophysiological parameters through implanted electrodes: cortical (a silver wire 0.3 mm in diameter insulated with varnish) and subcortical, bipolar (nichrome wire 0.1 mm in diameter insulated with varnish).

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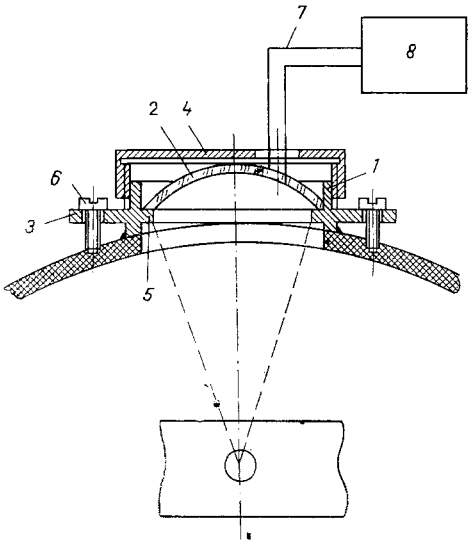

Fig. 1

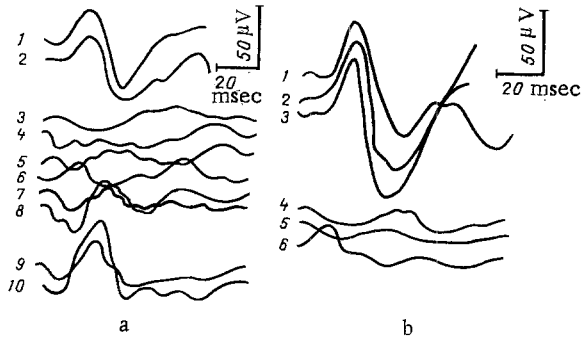

Fig. 2

Fig. 1. Diagram of apparatus for local application of FUS to brain structures of an unrestrained animal. 1) Body, 2) focusing radiator, 3) device for fixing body to animal's skull, 4) tight-fitting lid, 5) flange of body, 6) screws, 7) flexible cable, 8) ultrasonic generator.

Fig. 2. Reversible (a) and irreversible (b) changes in evoked potentials of optic tract before and after application of FUS (continuous irradiation). Explanation in text.

The period of adaptation during which the normal brain activity of the animal was restored to its preoperative level (the amplitude of the EEG reached 20-30 μV, a desynchronization reaction was observed during 5-10% of the recording time, and the level of correct conditioned-reflex responses was 85%) as a rule lasted 3 days. The animal was then placed in an experimental chamber, the tight-fitting lid was removed, and the body filled with degassed physiological saline, warmed to 37°C. The focusing radiator, connected by a flexible cable to the ultrasonic generator, was placed inside the body (Fig. 1). The electrodes were connected through a plug and socket with a flexible cable to the input of a biopotential amplifier or electroencephalograph.

EXPERIMENTAL RESULTS

The possibility of reversible blocking of the optic tract by the use of continuous application of ultrasound was studied. With an intensity of ultrasound of 63 W/cm^2 and a duration of application of 30 sec, a reversible blocking effect was obtained, consisting of initial depression and subsequent restoration of EP. The presence of this effect is illustrated by Fig. 2a, in which traces of biopotentials 1 and 2 were obtained before application of ultrasound, and traces 3-8 at various times after application. Traces 9 and 10 were obtained 30 min after ultrasonic irradiation. Depression of all phases of EP will be noted. The shape of the EP was gradually restored 15 min after the end of irradiation, and in the initial stage of recovery the amplitude of the first stage of EP was higher than normal, whereas the amplitude of the second phase remained unchanged, although its latent period was appreciably lengthened. Recovery of all phases of EP was observed in this case after a few tens of minutes.

If the duration of ultrasonic irradiation was increased to 60 sec, irreversible blocking of conduction of excitation, accompanied by irreversible damage to brain tissue was observed. Disappearance of all phases of EP after irradiation can be seen in Fig. 2b: Traces 1-3 were obtained before application of ultrasound, traces 4-6 a few hours after ultrasonic irradiation.

The method described above can thus be used to study the dynamics of the phases of behavior during the first minutes of blocking of particular brain structures while the animal is performing a behavioral act; in this way additional reliable information can be obtained on the character of functioning of brain structures and on the localization of cerebral functions.

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DETERMINATION OF NUCLEOTIDE AND ISOPLITH COMPOSITION OF DNA BY THIN-LAYER CHROMATOGRAM SCANNING IN UV LIGHT

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The results of previous investigations [3, 7, 9] showed that during scanning of thin-layer chromatograms in reflected light dependence of the areas of the peaks of the densitograms for different substances on the quantity of material in the chromatographic stains is determined both by the quantity and character of the material. Over a certain range of quantity, this dependence may be a virtually linear function [6, 9]. However, despite all the evident advantages of scanning of thin-layer plates compared with the laborious spectrophotometry of eluates for quantitative analysis of DNA derivatives, this approach has not yet been used because of the lack of any suitable coefficients for converting areas of densitographic peaks into corresponding quantities.

The aim of this investigation was the qualitative and quantitative analysis of spectra of UV light reflection by DNA hydrolysis products [2, 10] in thin layers of cellulose after chromatographic separation and determination of the nucleotide and isoplith compositions of DNA.

EXPERIMENTAL METHOD

Nitrogenous bases and pyrimidine isopliths of DNA were fractionated by methods described previously [1, 5]. The quantity of material applied at the start, calculated as DNA, was 5 μg. Scanning was carried out by means of an "Opton" chromatogram spectrophotometer (West Germany) in reflected UV light with a slit measuring 12 × 0.2 mm; the velocity of movement of the beam relative to the plate was 10 mm/min and the tape winding speed of the automatic writer was 30 mm/min. Spectra were recorded in the middle of the zone occupied by a separate component, using the same width of slit as for scanning, relative to a control region having the same R_f value.

EXPERIMENTAL RESULTS

Spectral data for DNA bases separated in an alkaline solvent: N-butanol 60, methanol 20, water 20, 25% NH_4OH 1 (v/v) [8], and an acid solvent: methanol 70, concentrated HCl 20, water 10 [4], are given in Figs. 1 and 2, respectively. The marked differences between the spectral characteristics of the bases in the systems used reflect differences in the degree of protonation of the bases in each system. If dry plates retaining bound water were kept under ordinary conditions, the spectral characteristics of the bases were unchanged. Quantitative analysis showed that coefficients of molar extinction of the substances analyzed on thin-layer chromatograms in reflected light vary depending on the properties of the layer (different batches of cellulose); however, the ratio between the coefficients remained constant in this case. Coefficients which can play the role of coefficients of molar extinction within the

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