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Research article

Neurophysiological method of micro sampling of endogenous substances and their transfer from one structure to another brain structure

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ABSTRACT

A fundamentally new priority method has been developed for the microsampling of endogenous substances from one brain structure for their subsequent introduction into another brain structure. The same universal capillary is first used to extract endogenous substances located in a certain brain structure, and then the same capillary is used to transfer collected substances to another brain structure by increasing the pressure in the capillary when the laser beam heats up its closed cavity located outside.

The method can be used to study the neurochemical mechanisms of the formation of emotional-motivational and pathological conditions, and identify specific endogenous substances involved in these processes.

The developed method can be used in neurophysiological, psychophysiological, pharmacological, biochemical studies, as well as in molecular biology.

1. Introduction

In modern neurophysiology and neurosurgery, electrophysiological and neurochemical methods are widely used. Among them, there are two separate independent methods of introducing biologically active substances and extracting endogenous substances from the brain.

The first is intended only for the introduction of various biologically active substances into individual brain structures with a further assessment of their physiological effects. Using stereotactic coordinates, a thin cannula is inserted into a specific brain structure filled with biologically active substance, which is brought into the brain structure under hydrostatic pressure. This method of injecting microscopic amounts of solutions into a tissue under pressure using a thin needle or cannula is called microinjection (Poland et al., 2015).

This method is focused only on the injection of substances into the structure of the brain. It does not allow endogenous substances to be taken from the brain structure and introduced into another brain structure of the same or another individual.

The second method, called tissue microdialysis, is intended for the collection of endogenous substances from intercellular fluid in vivo, and subsequent biochemical analysis.

The work of Delgado JM et al. (Delgado et al., 1972), who in 1972 described the use of glass catheters for sampling extracellular fluid, was the basis for modern microdialysis. Then, Urgenstedt U. and Pycock C. (Ungerstedt and Pycock, 1974) published a similar technique, which was further developed and led to the creation of tissue microdialysis in its current form (Lonnroth et al., 1987; Sadykov et al., 2013; Microdialysis; Neuropro).

Thin cannulas are used to extract various endogenous substances from the brain structures, which are introduced by the stereotactic method into the studied brain structure. A micro capillary is inserted inside the cannula, at the end of which there is a semi-impermeable membrane through which from the tissue, due to the concentration gradient, endogenous substances diffuse into the dialysis fluid circulating inside the capillary. Continuous renewal of the solution in the membrane area ensures the transmembrane concentration gradient.

The microdialysis catheter is a two-sheet concentric polyurethane tube with an outer diameter of 1 mm and the end section represented by a semi-impermeable membrane (standard length 10 mm). A special infusion pump with a solution similar in electrolyte composition to tissue fluid is connected to the catheter.

Membrane permeability for most substances is determined by its pore size (membrane cut-off size). Typical catheters have pores of size 20 kDa

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(catheter CMA70, for the identification of small molecules, ions, amino acids) and 100 kDa (catheter SMA71, allows additional extraction of small proteins and cytokines). A solution containing the extracted substances accumulates in a 200-μl micro ampoule.

With standard membrane lengths (10 mm) and perfusion rates (0.3 μL/min), relative recovery is 70% of the true concentration (Hutchinson et al., 2002).

In the collected dialysis fluid, the content and concentration of various substances are determined using an analyzer (liquid chromatography, mass spectroscopy, etc.).

The concentration of the substance in the dialysate never corresponds to its concentration in the tissue fluid, and depends on the perfusion rate of the dialysis fluid and the properties of semipermeable membranes, which, in accordance with permeability, limit the release of substances into the dialysis fluid. Therefore, all substances present in brain tissue are not represented in the dialysis fluid. The recoverable amount is part of the true extracellular concentration of a substance in the tissue.

The microdialysis method is focused only on the collection of endogenous substances from the tissue, and does not allow the substance extracted from the tissue to be transferred to another brain structure.

These methods have different purposes and none of them allows:

- extracting endogenous substances from the studied brain structure and transfer them into another brain structure;
- determining the true content of endogenous substances in the structures of the brain under various physiological conditions;

Thus, none of the existing methods makes it possible to extract endogenous substances from brain structures and transfer them to other brain structures of one or more individuals.

The goal is to develop and create of a fundamentally new technology for the extraction of endogenous substances from individual microstructures of the brain and their subsequent transfer into other brain structures under various emotional-motivational and pathological conditions, to determine the physiological role of endogenous substances in the activity of the brain, and their chemical analysis.

2. Description of the method for taking endogenous substances from one brain structure for their subsequent transfer into another brain structure

All procedures were in adherence with the ethical standards of the "Rules for carrying out work with the use of experimental animals", approved at a meeting of the ethical commission of the P.K. Anokhin Research Institute of Normal Physiology (Protocol No. 1 of 3.09.2005), the requirements of the World Society for the Protection of Animals (WSPA) and the European Convention for the Protection of Experimental Animals.

The priority method developed by us (Yumatov, 2017) for sampling endogenous substances from individual micro structures of the brain and their subsequent transfer into other micro structures of the brain is based on the fact that the (single) one and the same universal capillary is first used to extract endogenous substances located in a certain structure of the brain, and then the same capillary is used to transfer the substances taken from it into another structure of the brain, by increasing the pressure in the capillary when the laser beam heats its closed cavity located outside.

A device for implementing the method (Figures 1 and 2) consists of:

- a thin guide cannula, with a diameter of not more than 1 mm, along the length corresponding to its immersion depth in the brain made of a biologically inert polymer or metal;
- a mandrel corresponding to the cannula in length and inner diameter, and placed in each cannula with the possibility of longitudinal movement in it, made of a biologically inert polymer or metal wire;
- a single universal capillary for micro-sampling and transfer of substances, equal in diameter to the mandrel and replacing it in a cannula g > made of a biologically inert polymer, which has openings at both ends for communication with the atmosphere during the intake of substances, and a closed external hole when transferring substances;
- a laser with a power of 500–3000 mW, for remote heating within 1 m of the cavity in the outer part of the capillary.

Initially, the sampling of endogenous substances is performed from a specific brain structure into a microcapillary. The entry of fluid with endogenous substances into the microcapillary occurs due to capillary forces of surface tension.

The height of the capillary rise is determined by the Jurin's law:

$$H = \frac{2A}{rgd} MM,$$

where H is the height of the rise of water, mm;

A is the surface tension coefficient,

r is the radius of the capillary, mm;

g - acceleration due to gravity, m/s;

d - is the density of water at a given temperature g/^{cm3}.

It follows from the formula that the liquid rises in the capillaries the higher, the greater the surface tension, the smaller the diameter of the capillary and the lower the density.

It is important to note that the concentration of all substances in the microcapillary is equivalent to their content in the studied brain structure.

Then the microcapillary with its contents is removed from the cannula and inserted to transfer substances into another cannula located in the brain structure of the same individual or another individual. To remove substances from the capillary into the brain structure, the hole in the upper, outward, part of the capillary is pre-sealed (sealed). The transfer of substances into the structure of the brain from the microcapillary occurs due to the thermal expansion of air in the sealed upper cavity of the capillary when it is heated to 40–50° using remote laser action.

Knowing the volume of a microcapillary, it is possible to calculate and see with a microscope the volume of liquid located in the microcapillary before and after and introducing it into the selected brain structure.

To conduct the study, two identical cannulas are made in advance, the corresponding mandrels and capillaries of the required length and diameter, for immersion in certain brain structures, one of which is selected for sampling of substances, the other for their transferring.

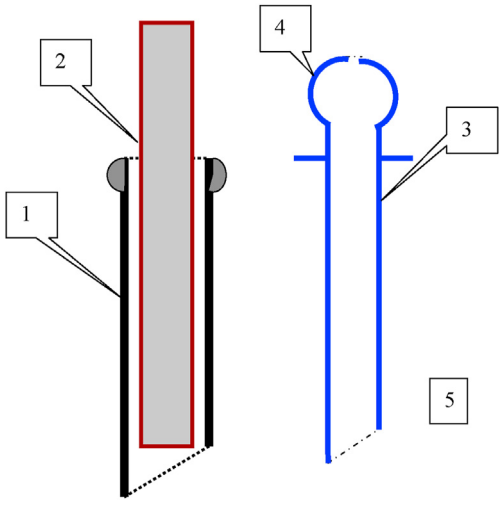

Figure 1. The layout of the cannula and mandrel, which are in the brain structure, before the endogenous substances are taken into the capillary from the brain, and the prepared capillary is inserted into the cannula. Designations: 1 - cannula, 2 - mandrel, 3 - capillary, 4 - the outer part of the capillary with an open hole, 5 - laser.

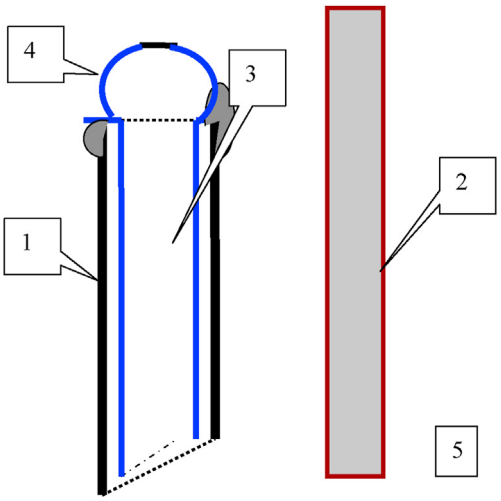

Figure 2. The layout of the cannula and capillary located in the structure of the brain during the sampling into the capillary of endogenous substances from the brain, and the mandrel removed from the cannula. Designations: 1 - cannula, 2 - mandrel, 3 - capillary, 4 - the outer part of the capillary with an open hole, 5 - laser.

Cannulas, mandrels and capillaries can be made of glass or a biologically inert polymer by stretching to the desired diameter when heated. The outer end of the capillary has an extension ending in a hole. The inner end of the capillary, the open tip facing the brain, may be stretched to the required micron size in diameter.

Previously, using stereotactic anesthesia, the prepared cannulas with mandrel are implanted into the brain structures, from which substances will be extracted and into which these substances will be transferred.

During the experiment, mandrel is removed from the cannula and replaced with a capillary, into which endogenous substances from the brain will proceed. Then, the capillary filled with substances is transferred to another cannula located in the structure of the brain into which the collected endogenous substances must be transferred. The capillaries free of the cannula are closed with mandrels and these cannulas are kept closed for repeated examinations.

During the sampling of substances and after a micro dose of endogenous substances is transferred into the selected brain structure, the researcher observes and records various manifestations of the individual's behavioral, vegetative, and other reactions.

Depending on the purpose of the study, cannulas are implanted into different brain structures, some for sampling substances, others for transferring substances into the brain of one or more individuals. The transfer of substances can be performed from one brain structure to another brain structure of the same individual, or from the brain structure of one individual to the brain structure of another individual.

In each particular study, depending on the purpose and objectives, cannulas and capillaries of different diameters are used. By changing the diameter of the tip of the capillary located inside the brain tissue, more or less large endogenous substances can be selectively taken from the structure of the brain.

Having collected the required amount of endogenous substances contained in microcapillaries, it is possible to carry out chemical analysis using liquid chromatography, mass spectroscopy, etc., since this is carried out during microdialysis.

3. Conclusion

The developed method of micro sampling and transfer of endogenous substances from one structure to another brain structure has significant advantages over existing neurochemical methods. The method makes it possible to:

- extract endogenous substances from one brain structure and transfer them to another brain structure;
- to study the role of endogenous substances in individual brain structures in the formation of emotionally motivational states, memory, and other forms of brain activity;
- to study endogenous substances in individual brain structures under conditions of unrestrained animal behavior;
- to determine the true content of endogenous substances using modern analytical methods in the structures of the brain under various physiological conditions of animals.

The developed method for microexchange of endogenous substances between individual brain structures can be used to study the role of different brain structures and to identify the participation of endogenous substances in the formation of various conditions, in particular, hunger and satiety, emotional reactions, sexual, narcotic drives, memory, epileptic, motor disorders, etc.

The method can be widely used in neurophysiological, psychophysiological, pharmacological, biochemical studies, in molecular biology to determine the chemical structure of substances that cause various psycho-emotional states.

Data associated with this study has been presented in the patent description: Yumatov E.A. A method and a device for micro-sampling of endogenous substances from one brain structure and their subsequent transfer into another brain structure of unrestrained animal. RF patent, 2017, No. 2017101458/14 (002472) dated 01/17/2017, as well as included in the article and in the references.

Declarations

Author contribution statement

Evgeny Yumatov: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Data availability statement

Data associated with this study has been presented in the patent description: Yumatov E.A. A method and a device for micro-sampling of endogenous substances from one brain structure and their subsequent transfer into another brain structure of unrestrained animal. RF patent, 2017, No. 2017101458/14 (002472) dated 01/17/2017, as well as included in the article and in the references.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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