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THE MICROTRANSPLANTATION TECHNIQUE: A SIMPLE AD USEFUL APPROACH TO STUDY RECEPTORS TRANSPLANTED INTO XENOPUS OOCYTES

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Abstract — Neuroreceptors are involved in many neurological diseases and represent the preferential target for the pharmacological treatments. Thus functional studies of their activity, by the use of electrophysiological techniques, are a fundament approach to understand not only the pathological mechanism of many neurological diseases but also the mechanism of action of potential drugs. Unfortunately, this cannot be applied for studying the receptor activity in all the human tissues. The option is the use of animal models, however they often resemble only some of the neurological diseases in human. In addition, adult or old animals are not always suitable for electrophysiological studies of age-related diseases. Here, we propose the microtransplantation technique as a novel and useful method to study receptors in humans and, more in general, in adult animals.

Index Terms — receptors, Xenopus oocytes, microtransplantation, neurological diseases, two-electrode voltage-clamp

1 BACKGROUND

With the microtransplantation technique exogenous cell membranes are injected into the Xenopus oocytes, where receptors and ion channels are functional transplanted into the oolemma. The main advantages of this technique are:

- already-assembled receptors are still surrounded by their native environment maintaining intact their functional properties;
- the membrane preparations are easy to obtain and they can be isolated from animal and human biopsies;
- a simple approach to analyse the biophysical properties of the receptors in human and adult animals.

2 OBJECTIVES

Analyze the functional and pharmacological properties of the receptors involved in neurological disease. This approach is suitable to study receptors isolated from:

- human biopsies [1];
- adult animal models [2].

3 APPROACH & METHODS

General approach

With this approach it is possible to "resuscitate" receptors and ion channels from tissues kept frozen for many years. Cell membrane preparations are isolated as described in [1] and samples storage at - 80° C until used for the experiments.

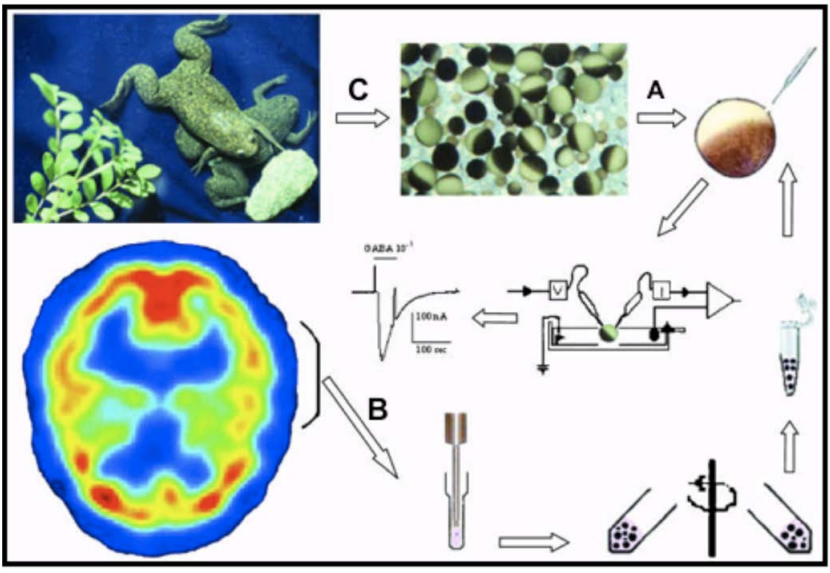

Figure 1: Schematic presentation of the microtransplantation technique.

Methods

The main steps of the technique are summarized in Figure 1. Briefly, Xenopus oocytes, isolated from anesthetized animal (A), are dissected from segments of ovary, defolliculated with collagenase, and maintained at 16 °C in Barth's solution. The next day, each oocyte are injected with membranes (i.e. human cortex) (B). Two electrode voltage clamp recordings are performed to analyzed the functional incorporation of the channels (C).

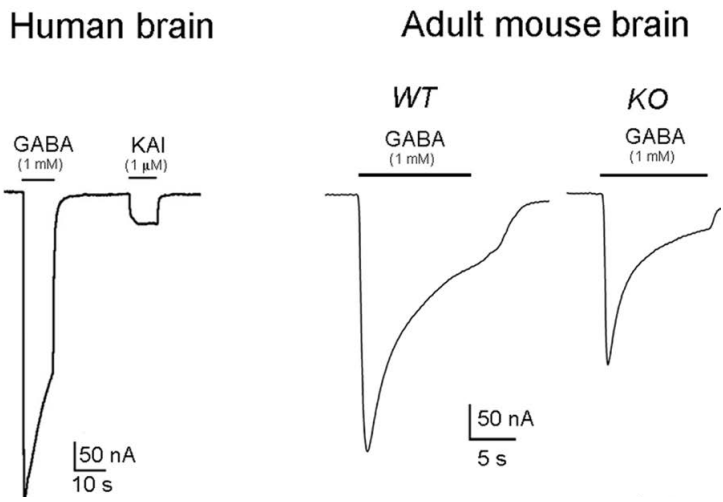

Figure 2: Examples of GABA and KAI-currents recorded in two microtransplanted oocytes.

4 RESULTS

The injection of cell membranes into Xenopus oocytes allows the functional characterization of the receptors. In Figure 2, examples of GABA- and KAI-currents recorded in oocytes injected with cell membranes isolated from human temporal cortex (left) and of GABA-currents recorded in oocytes injected with mouse cortex cell membranes from WT and KO model for Rett Syndrome (right).

5 POTENTIAL NEW PRODUCTS & SERVICES

Product: Functional characterization of receptors from human and animal models tissues and their role in the diseases.
Service: A simple approach for screening the efficiency of new drugs for the treatment of neurological diseases.

6 CURRENT COLLABORATIONS

Prof. Enrico Tongiorgi, Dept. of Life Sciences, University of Trieste, Italy
Prof. Jean-Michel Rigo, BIOMED Research Institute, Hasselt University, Belgium

7 CONTACT OR COLLABORATIONS NEEDED

Source of human biopsies of tissues from animal models. Companies interested in delivery of new drugs for treatment of neurological disease.

8 COMMUNICATION TOOLS

- The high level of expertise quality and performance of the presented methods is disseminated through the high quality scientific publications.
- Dissemination of scientific results using media for the general public.

9 FUNDS NEEDED

9.1 For basic research (investigation of biological mechanisms): € 80.000,00

9.2 For pilot & demonstrator activities (to develop a prototype): € 20.000

10 CONCLUSION

Our model represents a powerful alternative for studying the mechanism of action of potential drugs for neurological disorders on the receptor activity. By using human post mortem tissues, as well animal models, it is now possible to perform the experiments avoiding the limitation of the "classical" electrophysiological approaches. Moreover, it is possible to extend the study in non-neuronal tissues [3].

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REFERENCES

[1] Bernareggi A, Duañes Z, Reyes-Ruiz JM, Ruzzier F, Miledi R (2007). Properties of glutamate receptors of Alzheimer's disease brain transplanted to frog oocytes. Proceedings of the National Academy of Sciences of the United State of America 104: 2956-60.
[2] Bernareggi A, Grilli M, Marchi M, Limatola C, Ruzzier F, Eusebi F (2011). Characterization of GABA (A) receptors expressed in glial cell membranes of adult neocortex using a Xenopus oocyte expression system. Journal of Neuroscience Methods 198: 77-83.
[3] Bernareggi A, Reyes-Ruiz JM, Lorenzon P, Ruzzier F, Miledi R (2011). Microtransplantation of acetylcholine receptors from normal or denervated rat skeletal muscle to frog oocytes. Journal of Physiology 589: 1133-42.