ELECTROPHYSIOLOGY STUDY USING PATCH CLAMP TECHNIQUE IN SENSORY NEURONS

Tiago dos Santos-Nascimento 1*, Kleyane Morais Veras 2, José Ossian Almeida Souza Filho 3, Luiz Moreira-Júnior 4

1 Superior Institute of Biomedical Sciences, State University of Ceará, Ceará, CE, Brazil.
2 Post-Graduation program in Education, State University of Ceará, Ceará, CE, Brazil.
3 Faculty of Vale do Jaguaribe, Ceará, CE, Brazil.
4 Department of Physiology, University of Tennessee, Tennessee, USA.

*Corresponding author: Instituto Superior de Ciências Biomédicas, Universidade Estadual do Ceará, Campus do Itaperi, Av. Silas Munguba, 1700, Itapery, 60714-903, Fortaleza, CE, Brazil. Phone: +55 (85) 9 99665-0627. E-mail: santosnascimento.t@gmail.com

Research Ethics Committee Approval (if necessary): Approved by the Ethics Committee on Animal Research of Ceará State University (CEUA/UECE) (#10339956–9).


Figure 1. Sensory neurossoma of the dorsal root ganglion (GRD) of rats in patch clamp whole cell mode.

The electrophysiological and pharmacological study involving sensory and autonomic neurons enables the development of new effective agents.
in the treatment of neuropathic disorders, since they enable the elucidation of the mechanisms underlying the malfunction of the nervous system. In this context, the patch clamp technique increased the study of cells, providing a high-resolution method at the molecular level for observing the flow of ions through ion channels characteristic of excitable cells [1], such as the neurons.

When using different protocols with combinations of intracellular and extracellular solutions with specific pharmacological agents, this technique allows different unit and/or macroscopic records of active and passive electrical variables of cellular activity [2] that it favored the Nobel Prize in physiology or medicine to Erwin Neher and Bert Sakmann in 1991. Although the whole cell mode is the most used configuration in health-related researches, little is known in health courses. To apply this technique to neurons, it is commonly necessary to dissociate neurosomas.

Figure 01 shows sensory neurosomes of the dorsal root ganglion (GRD) of rats from the biotern of the State University of Ceará (CEUA process number 10339956-9). The process of isolating neurosomas from the intact ganglion consists of two phases: 1) Collagenase (1mg / ml for 75 min) and Trypsin + EDTA (0.25% and 0.025%, respectively, for 12 minutes); 2) Mechanical dispersion with 3 Pasteur glass pipettes with decreasing diameter (2.5 mm, 1 mm and 0.5 mm, respectively). Then, the neurosomas were plated on coverslips previously treated with poly-D-lysine maintained in supplemented DMEM and incubated at 37 °C and 5% CO2 [3].

The figure shows a neurosoma 24h after plating. This cell has approximately 25 μM in diameter, which it plays role nociception function [4]. Furthermore, the nucleus is not centralized, the cell does not have neurites. As for the micropipette, capillaries were used for microhematocrit without heparin (75 mm length, 1 mm inner diameter and 1.5 mm outer diameter) for making with tip resistance range from 1 and 3 MΩ after filling with the solution to compose intracellular medium [5].

In this technique, a microelectrode was micrometrically move toward until it lightly touched the plasma membrane. Then, a continuous negative pressure was applied to increase the contact of the glass with the membrane, stabilizing the seal (interaction between membrane and glass) and increasing it until its resistance reaches the order of 109 ohm (GΩ). Then, more suction was applied to cause the cell surface under the microelectrode to rupture, thus providing access to the interior of the cell, allowing excellent control of the cell membrane potential and, consequently, high-fidelity records of ionic currents that flow through ion channels present
in the plasma membrane of neurosomas.

References


Conflict of interest: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence in the work.

Acknowledgements: The authors would like to thank P. Militão Albuquerque-Neto for technical support and José Henrique Leal-Cardoso for provided help during the research.

Funding: This work was supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Fundação Cearense de Apoio ao Desenvolvimento Científico e Tecnológico (FUNCAP), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Universidade Estadual do Ceará (UECE).

How to cite this article: Santos-Nascimento T, Veras KM, Souza-Filho JOA, Moreira-Junior L. Electrophysiology study using patch clamp technique in sensory neurons. Brazilian Journal of Case Reports. 2021 Jan-Mar;01(1):12-14.