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ELECTROPHYSIOLOGY STUDY USING PATCH CLAMP TECHNIQUE IN SENSORY NEURONS

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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

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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 neurossomas.

Figure 01 shows sensory neurossome of the dorsal root ganglion (GRD) of rats from the bioterium of the State University of Ceará (CEUA process number 10339956-9). The process of isolating neurossomas 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 neurossomas were plated on coverslips previously treated with poly-D-lysine maintained in supplemented DMEM and incubated at 37 °C and 5% CO_2 [3].

The figure shows a neurossoma 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 micro-hematocrit 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 neurossomas.

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