

ABSTRACT

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Título: In vitro studies of the central nervous system of the sea cucumber *Holothuria glaberrima*

Among animal phyla, some of the least studied nervous systems are those of the phylum Echinodermata. Part of the problem lies in that most of their nervous components are embedded in the body wall that has calcareous skeletal components. We have now developed a dissection technique for successful isolation of the radial nerve cords (RNCs) and an in vitro system where the isolated RNCs can be cultured. This in vitro system is thus amenable to experimental manipulations aimed at characterizing the nervous system of the sea cucumber *Holothuria glaberrima* and to extend our studies on its regeneration capabilities. In this procedure, the body wall containing the RNCs is surgically excised and digested with collagenase producing RNCs explants that can be kept in culture for up to 2 weeks. We have performed histological studies to determine changes in the RNC anatomy during culture. We have also performed immunohistochemistry using neuronal and collagen markers to identify specific cellular populations. Labeling with a nuclear dye (DAPI) shows the presence of cells throughout the cultured period with a slight increase in nuclear counts in the medial and lateral regions of the RNC neuropile through days 3 to 7 when compared to *in vivo* RNCs (control). Neuronal markers, RN1 monoclonal antibody and anti-GFSKLYFamide polyclonal antibody, identified nerve fibers and, neuron-like cell bodies along the RNC periphery, as has been documented previously in *in vivo* studies. In addition, anti-collagen marker IBA2 highlighted the demarcation between the ectoneural and hyponeural subdivisions of RNC explants kept for at least 3 days in culture. Our results showed that the central nervous system components of an echinoderm can be kept alive, maintaining its structure and morphology for various length of time. These RNC explant cultures provide a unique tool to study cellular and molecular basis of processes occurring in nervous systems such as neuronal regeneration and degeneration.