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Poster Presentation

Evaluating the Function of Motoneuron-Like Cells Differentiated from Rat Adipose Derived Stem Cells through Voltage-Sensitive Dyes (Rh 795) and Investigating the Synaptic Vesicle Recycling

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Abstract

Cell replacement therapy has provided the basis for future clinical applications to treat central nervous system injuries following by car accidents. Induced functional neurons are an option for replacing the lost neurons. The ability to monitor changes in membrane potential is a useful tool for studying neuronal function, but there are only limited options available at present. Here, investigated the potential of voltage-sensitive dyes, RH 795, for imaging the membrane potential of motoneurons like cells (MNLCs) differentiated from adipose derived stem cells (ADSCs) using an epifluorescence-based cell imaging system. In this study described a novel method for the detection of action potential-capable MNLCs differentiated from ADSCs using voltage-sensitive dyes (VSDs). We compared the results of extracellular applied VSDs in a more detailed labeling of cellular processes with calcium indicators. MNLCs were maintained in culture medium and then loaded with the VSDs RH795. For the RH795 loading, cultures were maintained in a artificial cerebrospinal fluid (ACSF) buffer and incubated at 37°C in the dark. The cells were then washed 3 times and incubated for 60 minutes in ACSF buffer in the dark. With RH-795, a fluorescence change was observed in the frame immediately following the stimulation onset, reaching a maximum at 10–20 ms after stimulation onset and then decaying during the subsequent frames. This method allows for a repeatable fast and accurate stimulation of neurons derived from stem cell cultures to assess their differentiation state, which is capable of monitoring large amounts of cells.

Keywords: Voltage-Sensitive Dyes, Central Nervous System Injury, Motoneurons Like Cell.

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