

**CONFOCAL MICROSCOPY VISUALISATION OF CHOLINERGIC NEURONS
IN LIVE TISSUES
WITH A NOVEL PROBE, FLUORESCINEINATED PHYSOSTYGMINE (Ph-F)**

J.W.Dobrucki¹, J.Galas², B.W.Lee³, G.L.Johnson³, Z.Darzynkiewicz⁴

¹Jagiellonian University, Dept. of Biophysics, Kraków, Poland

²Jagiellonian University, Institute of Zoology, Kraków, Poland

³Immunochemistry Technologies, Bloomington, MN, USA

⁴Brander Cancer Research Institute, New York Medical College, Valhalla, N.Y., USA

E-mail: dobrucki@mol.uj.edu.pl

KEY WORDS: live cholinergic neurons, acetylcholinesterase, fluorescent physostigmine

Acetylcholinesterase (AChE, EC3.1.1.7) is involved in signal transduction in synapses in various tissues, including skeletal muscle, diaphragm, heart and brain. Several neurological disorders are associated with improper expression and activity of AChE. The number of cholinergic nerves and activity of AChE is known to fall during the progress of Alzheimer disease.

A construct combining physostigmine (Ph), the high-affinity ligand of AChE, and fluorescein was developed (Ph-F) and shown to specifically label neurons in a diaphragm, a femoral nerve, skeletal and heart muscle and a brain of a mouse and a bank vole. A study of neurons in a diaphragm demonstrated a complex architecture of nerve cells, including neuromuscular junctions and neurons, with characteristic features like terminal boutons and dendritic spines. The mechanism of accumulation of Ph-F in nerve cells, its specificity for cholinergic nerves and potential applications to studies of the architecture and function of nervous system and the effects of drugs on cholinergic synapses is currently under investigation.

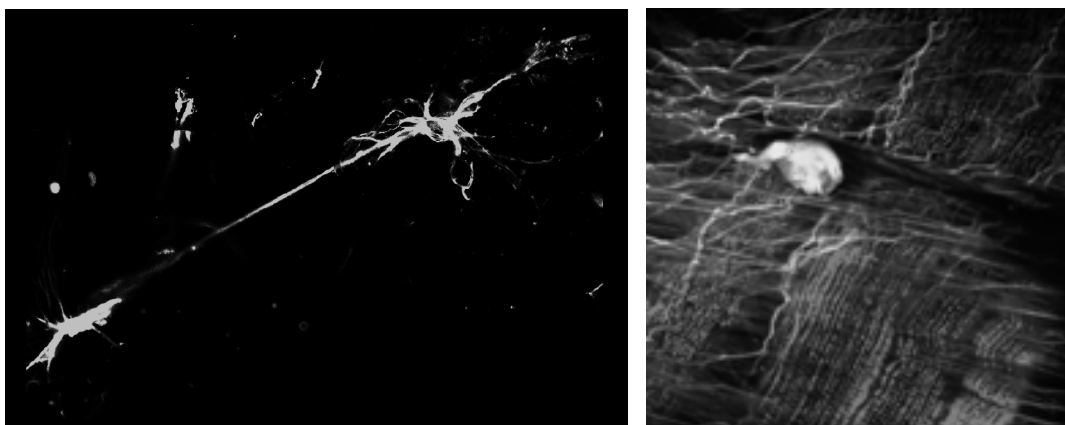


Fig.1. Confocal images demonstrating Ph-F-labeled live neurons in diaphragm of a mouse; the right panel shows the neurons against striated muscles and cell nuclei counterstained with other fluorescent probes.