This Week's Citation Classic

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De Robertis E, Pellegrino de Iraldi A, Rodríguez de Lores Arnaiz G & Salganicoff L. Cholinergic and non-cholinergic nerve endings in rat brain—I. Isolation and subcellular distribution of acetylcholine and acetylcholinesterase. *J. Neurochemistry* 9:23-35, 1962. [Inst. Anat. General y Embriol., Fac. Ciencias Médicas, Univ. Buenos Aires, Argentina]

This paper gave the first detailed description of the isolated nerve endings, i.e., synaptosomes, from the brain. The methodology introduced allowed the separation of myelin, mitochondria, and two types of cholinergic and noncholinergic synaptosomes. These fractions were characterized by electron microscopy and by several biochemical markers. [The *SCI*® indicates that this paper has been cited in over 610 publications since 1962, making it one of the most cited ever published in this journal. Its continued relevance is reflected in the existence of numerous *ISI/BIOMED*™ research fronts on synaptosomes.]

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"At the end of the 1950s, and after a long exile, I returned to my country to become chairman of the Institute of Cell Biology with the task of starting a research group in the neurosciences. By that time, the electron microscope had revealed the extraordinary complexity in the ultrastructure of the central nervous system. Inside the nerve endings, the synaptic vesicles had been discovered,[1] and many structural details of the synaptic membranes, perikarya, dendrites, and glial cells were described. I thought that to simplify the structural and biochemical analysis of the brain, it was essential to develop new methods of cell fractionation adapted to its complex ultrastructure and fragile nature, and to obtain subcellular fractions as homogeneous as possible.

"This project involved an interdisciplinary approach in which investigators from the biochemical field (G. Rodríguez de Lores Arnaiz and L. Salganicoff) joined efforts with others (A. Pellegrino de Iraldi and myself) from the field of ultrastructure. We made the homogenization milder by increasing the clearance between the tube and the pestle, so as to protect the nerve endings from disruption. We separated the crude mitochondrial fraction by differential centrifugation, and from this we isolated five subfractions on a discontinuous sucrose gradient. A systematic electron microscope investigation revealed that the lighter fraction was myelin; the sediment free mitochondria and the three other fractions contained isolated nerve endings. For these structures the name 'synaptosomes,' suggested in 1964 by V.P. Whittaker *et al.*,[2] prevailed in the literature. We also used several biochemical markers and found that two of these fractions were rich in acetylcholine and acetylcholinesterase and the other poor in these biochemical markers (i.e., cholinergic and noncholinergic synaptosomes).

"The high citation of this paper appears to be justified because the isolation of the synaptosome started a whole new field of research, which was pursued in many laboratories. This work provided the foundation for the isolation of synaptic vesicles which, since our early work, were considered to be the structural units for the storage and release of the neurotransmitter.[3] It also led to the separation of the synaptosomal membranes and the localization of pre- and postsynaptic receptors.[4]

"The synaptosome is a self-contained particle having all the structural and many of the functional characteristics of the synaptic region. Within its exiguous limits it contains, in a miniature form, all the molecular constituents, and the complex structural and biochemical machinery, needed for the transmission of the nerve impulse. In addition, the synaptosome is probably able to execute other less known functions related to neurogenesis, plasticity, memory, and learning. It is good fortune that, after two decades, the work on synaptosomes is still wide open for research."[5]

1. **De Robertis E & Bennett H S.** Some features of submicroscopic morphology of synapses in frog and earthworm. *J. Biophys. Biochem. Cytol.* 9:229-35, 1955.
2. **Whittaker V P, Michaelson I A & Kirkland R J A.** The separation of synaptic vesicles from nerve-ending particles ('synaptosomes'). *Biochemical J.* 90:293-303, 1964.
3. **De Robertis E, Rodríguez de Lores Arnaiz G & Pellegrino de Iraldi A.** Isolation of synaptic vesicles from nerve endings of the rat brain. *Nature* 194:794-5, 1962.
4. **Criado M, Aguilar J S & De Robertis E.** Action of detergents and pre- and postsynaptic localization of ^{3}H-naloxone binding in synaptosomal membranes. A structural approach. *J. Neurobiology* 12:259-67, 1981.
5. **De Robertis E.** *The synaptosome. Two decades of cell fractionation of the brain.* New York: Raven Press. In press, 1982.