Cerebellar Classic

Exploring Subcellular Cerebellar Fractions with the Electron Microscope

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Abstract

Differential ultracentrifugation and subcellular fractionation historically helped to study the components of the cell, to discover new cellular organelles, and to decipher their morphological and molecular properties. In neuroscience, the technique has yielded important results on neuron biochemistry and the mechanisms of synaptic transmission. This *Cerebellar Classic* is devoted to the pioneering work of Manuel del Cerro, Ray S. Snider and Mary Lou Oster-Granite, who isolated purified fractions after successive centrifugations of the rat cerebellum from birth to adulthood, and studied them under the electron microscope.

Keywords

Cell organelles, Cerebellar development, Differential ultracentrifugation, Subcellular fractionation, Center for Brain Research, University of Rochester

Cell fractionation by differential centrifugation combined with electron microscopy was pioneered by Albert Claude (1899–1983) of Rockefeller University in 1946 [1], and heralded the beginning of decades of unprecedented breakthroughs, including the description of mitochondria and endoplasmic reticulum by Claude [1], ribosomes and protein synthesis by George Palade (1912–2008) [2], and lysosomes and peroxisomes by Christian de Duve (1917–2013) [3].

The technique aimed to separate cellular components while preserving their functions, by combining the steps of homogenization of tissues, filtration, and density gradient centrifugation. Ultrastructure merged into biochemistry, and the biochemistry of mass-isolated subcellular components became the best way to get at the function of newly discovered structures of the cell [2]. Actually, de Duve discovered a new cellular organelle without using a microscope [3]. Having differential centrifugation as a tool, he thought that, in order for the other components of the cell not to be affected and for the cell in its entirety not to be destroyed by the action of acid phosphatase, such enzymes should be enveloped in some membranous encasing, and suggested the existence of an independent organelle, the 'lysosome' (named so because its role was digestive, or 'lytic'). In 1955, he sent his subcellular fraction to the cytologist Alex B. Novikoff (1913–1987) at the Albert Einstein College of Medicine, who examined the specimen under the electron microscope, and saw that it contained the mysterious structures that for years morphologists had called 'microbodies' [4]. This avalanche of discoveries set the stage for the growth of modern Cell Biology as a new scientific discipline [5, 6].

Subcellular fractionation was applied to nervous tissue by biochemist Victor P. Whittaker (1919–2016) in Cambridge and by cell biologist Eduardo De Robertis (1913–1988) in Buenos Aires. Beginning in 1958, by combining differential and density gradient centrifugation, Whittaker and his coworkers isolated detached presynaptic nerve terminals, which they termed 'synaptosomes' [7]. They demonstrated that synaptic vesicles store acetylcholine. Cell fractionation has been widely used to study neuron biochemistry and the molecular mechanisms of synaptic neurotransmission in particular [8]. This has led to major discoveries regarding the identity of neurotransmitter systems and their uptake mechanisms [9].

In the present *Cerebellar Classic* [10], the authors, using successive centrifugations, isolated purified subcellular particles from the cerebella of Sprague-Dawley rats, and studied

them under the electron microscope.

Having trained under De Robertis in his native Argentina, Manuel del Cerro (now retired) joined the faculty of the University of Rochester Medical Center. In 1963, Ray S. Snider (1911–1991) had become director of the Center for Brain Research, the first USA interdisciplinary group of its kind [11], succeeding its founder, Erwin R. John (1924–2009). The cerebellum was at the center of del Cerro's research. He documented early synaptogenesis [12] and the progressive reduction of extracellular space [13], the fine structure of the Purkinje cell cilium [14], and the growth cones [15, 16] that Ramón y Cajal had discovered in 1890 [17, 18].

Snider had been studying the cerebellum at a time when that part of the CNS was considered 'a silent area of the brain, a region of subtle and tantalizing mystery'; he foresaw that the major function of integration that the cerebellum subserved might extend into the mental realm [19]. While working at Johns Hopkins University with the neurosurgeon Averill Stowell in the 1940s, Snider proposed that the cerebellum, besides proprioception and equilibrium, was also involved in the coordination of tactile, auditory, and visual processes in a somatotopic organization typified by cerebellar 'homunculi' or projection areas showing localization of function [20, 21].

The discussed classic was assisted by Mary Lou Oster-Granite, then an undergraduate student at the University of Rochester majoring in Biology and Chemistry. After earning her Ph.D. from Johns Hopkins University, she went on to serve on the faculties of the University of Maryland, Johns Hopkins, and University of California–Riverside, and as the health sciences administrator at the National Institutes of Health.

The various obtained fractions were highly homogeneous and comprised nuclei, mitochondria, endoplasmic reticulum ('microscomes'), synaptosomes, ribosomes, and myelin. By studying animals at ages ranging from newborn to adult, the authors documented increases of both synaptic vesicles and myelin fragments over time. The novelty of the study was that it focused on the cerebellum at a time when it was common among neurochemists, who tacitly considered the brain 'a homogeneous population' — when in fact it is 'a pool of regions known to have important histochemical and physiological differences' [10] — to either study the brain as a whole or keep only the cerebrum. With a few exceptions [22], the cerebellum at

that time was generally dissected away and discarded. The isolation of subcellular fractions from developing cerebella [10] further extended the possibility of analyzing the biochemical underpinnings of cerebellar histogenesis.

Subcellular fractionation was later used to study the cerebellar glomeruli [23], the differential incorporation of glutamate and GABA in molecular and granular layer homogenates [24], biochemical changes in the cerebellum of mutant mice [25], and to isolate and sequence new cerebellar peptides, the cerebellins [26], among others. In more recent years, age-related changes in energy metabolism in the rat cerebellar cortex were characterized *in vitro* by means of functional proteomics after combined differential/gradient centrifugation; specific and independent metabolic features were documented for mossy and parallel fiber synaptosomes regarding maximum rate (V_{max}) of selected enzymes of glycolysis, the Krebs cycle, glutamate and amino acid metabolism, and acetylcholine catabolism [27]. Overall, the study of synaptosomes led to a confirmation that the brain was composed of highly specialized areas at a histological, biochemical and neurochemical level, with numerous stereospecific regions [28].

Current studies dissecting neuron-glia interactions and aiming to understand how elemental synaptic defects impact on complex brain functions such as cognitive operations are in the direct line of these earlier experiments [29]. The history of cell fractionation has contributed to our understanding of the structural and functional properties of the cerebellum, showing a high degree of heterogeneity of fractions obtained by centrifugation in discontinuous and continuous sucrose gradients [30]. In all, the technique of cellular fractionation has played a major role in advancements in neuroscience.

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Declarations

Ethics committee approval Not applicable.

Conflict of interest The authors declare no conflict of interest.

References

1. Claude A. The coming of age of the cell. Science. 1975;189:433-5.
2. Palade G. Intracellular aspects of the process of protein synthesis. Science. 1975;189:347-58.
3. de Duve C. Exploring cells with a centrifuge. Science. 1975;189:186-94.
4. de Duve C. Alex Novikoff reminiscences. J Histochem Cytochem. 1987;35:931-4.
5. Tashiro Y. Accomplishments of Drs. Albert Claude and George E. Palade and the birth of cell biology. Tanpakushitsu Kakusan Koso (Tokyo). 1975;20:74-6.
6. de Duve C, Palade GE. Albert Claude, 1899–1983. Nature. 1983;304:588.
7. Whittaker VP. Thirty years of synaptosome research. J Neurocytol. 1993;22:735-42.
8. De Robertis E. Ultrastructure and cytochemistry of the synaptic region. Science. 1967;156:907-14.
9. Evans GJO. The synaptosome as a model system for studying synaptic physiology. Cold Spring Harb Protoc. 2015;2015(5):421-4.
10. del Cerro MP, Snider RS, Oster ML. Subcellular fractions of adult and developing rat cerebellum. Exp Brain Res. 1969;8:311-20.
11. Snider RS. The neurosciences preserved: truth, friendship and self-control. Integr Psychol Behav Sci. 1975;10:63-5.
12. del Cerro MP, Snider RS. Axo-somatic and axo-dendritic synapses in the cerebellum of the newborn rat. Brain Res. 1972;43:581-6.
13. del Cerro MP, Snider RS, Oster M. Evolution of the extracellular space in immature nervous tissue. Experientia (Basel). 1968;24:929-30.
14. del Cerro MP, Snider RS. The Purkinje cell cilium. Anat Rec. 1969;165:127-30.
15. del Cerro MP, Snider RS. Studies on the developing cerebellum: ultrastructure of the growth cones. J Comp Neurol. 1968;133:341-62.
16. del Cerro M. Uptake of tracer proteins in the developing cerebellum, particularly by the growth cones and blood vessels. J Comp Neurol. 1974;157:245-79.
17. García-Marín V, García-López P, Freire M. The growth cone as seen through Cajal's original histological preparations and publications. J Hist Neurosci. 2009;18:197-210.
18. Tamariz E, Varela-Echavarría A. The discovery of the growth cone and its influence on the study of axon guidance. Front Neuroanat. 2015;9:51.
19. Snider RS. The cerebellum. Sci Am. 1958;199(2):84-90.
20. Snider RS, Stowell A. Receiving areas of the tactile, auditory and visual systems in the cerebellum. J Neurophysiol. 1944;7:331-57.

21. Manni E, Petrosini L. A century of cerebellar somatotopy: a debated representation. Nat Rev Neurosci. 2004;5:241-9.
22. Gaunt GL, de Duve C. Subcellular distribution of D-amino acid oxidase and catalase in rat brain. J Neurochem. 1976;26:749-59.
23. Balázs R, Hajós F, Johnson AL, Reynierse GL, Tapia R, Wilkin GP. Subcellular fractionation of rat cerebellum: an electron microscopic and biochemical investigation. III. Isolation of large fragments of the cerebellar glomeruli. Brain Res. 1975;86:17-30.
24. Mikoshiba K, Changeux JP. Morphological and biochemical studies on isolated molecular and granular layers from bovine cerebellum. Brain Res. 1978;142:487-504.
25. Mallet J, Huchet M, Pougeois R, Changeux JP. Anatomical, physiological and biochemical studies on the cerebellum from mutant mice. III. Protein differences associated with the weaver, staggerer and nervous mutations. Brain Res. 1976;103:291-312.
26. Slemmon JR, Blacher R, Danho W, Hempstead JL, Morgan JI. Isolation and sequencing of two cerebellum-specific peptides. Proc Natl Acad Sci USA. 1984;81:6866-70.
27. Ferrari F, Gorini A, Villa RF. Energy metabolism of synaptosomes from different neuronal systems of rat cerebellum during aging: a functional proteomic characterization. Neurochem Res. 2015;40:172-85.
28. Coyle JT, Snyder SH. Catecholamine uptake by synaptosomes in homogenates of rat brain: stereospecificity in different areas. J Pharmacol Exp Ther. 1969;170:221-31.
29. Matta SM, Moore Z, Walker FR, Hill-Yardin EL, Crack PJ. An altered glial phenotype in the NL3R451C mouse model of autism. Sci Rep. 2020;10(1):14492.
30. Wilkin GP, Reijnierse GL, Johnson AL, Balázs R. Subcellular fractionation of rat cerebellum: separation of synaptosomal populations and heterogeneity of mitochondria. Brain Res. 1979;164:153-63.