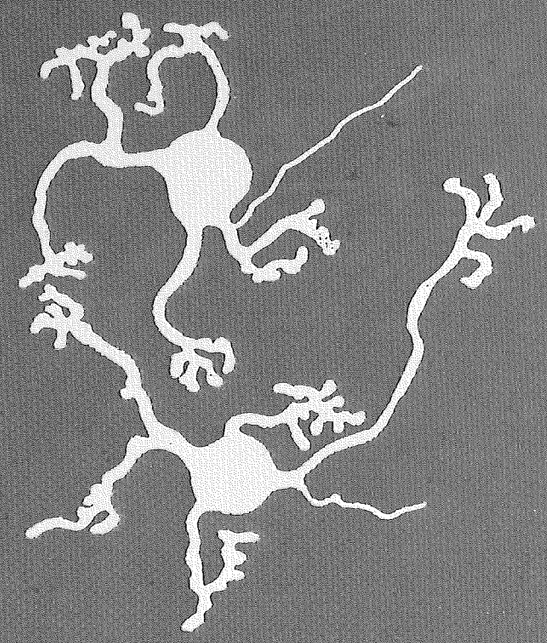
Palay
Chan-Palay

Cerebellar Cortex



Sanford L. Palay · Victoria Chan-Palay

Cerebellar Cortex

Cytology and Organization

With 267 Figures including 203 Plates

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«... on ne peut, ni l'on ne pourra jamais parler du cervelet sans que Cajal ne vienne au devant, et quiconque l'ignorerait serait forcément obligé à coïncider avec lui sur beaucoup de points, soit dans l'interprétation, soit dans les faits qui constituent les fondaments de toutes les constructions scientifiques.»

CLEMENT ESTABLE 1923
Trab. Lab. Invest. Biol. (Madrid)
vol. XXI, p. 187.

«Malheureusement pour moi, d'autres, courant la même carrière, virent plusieurs des choses que j'avois vuës, & s'étant fait un plan moins étendu, m'enlevèrent, en publiant leurs observations, une espèce d'honneur que je croiois avoir également mérité.»

PIERRE LYONET 1762
from the preface to *Traité anatomique de la Chenille, qui ronge le Bois de Saule.*
Pierre Gosse, jr. et Daniel Pinet, La Haye.

Preface

The origins of this book go back to the first electron microscopic studies of the central nervous system. The cerebellar cortex was from the first an object of close study in the electron microscope, repeating in modern cytology and neuroanatomy the role it had in the hands of RAMÓN Y CAJAL at the end of the nineteenth century. The senior author vividly remembers a day early in 1953 when GEORGE PALADE, with whom he was then working, showed him an electron micrograph of a cerebellar glomerulus, saying "That is what the synapse should look like." It is true that the tissue was swollen and the mitochondria were exploded, but all of the essentials of synaptic structure were visible. At that time small fragments of tissue, fixed by immersion in osmium tetroxide and embedded in methacrylate, were laboriously sectioned with glass knives without any predetermined orientation and then examined in the electron microscope. After much searching, favorably preserved areas were studied at the cytological level in order to recognize the parts of neurons and characterize them. Such procedures, dependent upon random sections and uncontrollable selection by a highly erratic technique of preservation, precluded any systematic investigation of the organization of a particular nucleus or region of the central nervous system. It was difficult enough to distinguish neurons from the neuroglia. Even so, much was learned about the fine structure of the nerve cell, especially about the perikaryon, axons, and dendrites, and, most important for the purpose of this book, about the structure of the synapse.

During the past twenty years vast improvements in technique have made it possible to study all parts of the nervous system at the fine structural level. Now we are able to fix the tissue before fragmenting it, thus retaining the orientation of its components. That signal improvement has made it relatively easy to recognize types of cells and their processes with the minute clues found in thin sections, whereas before it was generally impossible. Improved fixing solutions, embedding and staining methods, as well as refinements in the electron microscope itself, have greatly increased the number and subtlety of the discriminations that can be made, so that many details formerly nonexistent in the electron micrographs were now useful in identifying structures. All of this technical advance, to which we have been privileged to contribute, has made it possible to attempt such a work as the present volume.

Concentrated labor on this book began in the autumn of 1969, when the senior author was joined in this endeavor by the junior author. With this collaboration, the pace of the investigation was greatly accelerated. Vast numbers of Golgi preparations and electron micrographs were made, permitting a fruitful interplay between traditional optical microscopy and electron microscopy.

Actually, the book was conceived a long time ago as a comprehensive demonstration presented to the annual meeting of the American Association of Anatomists in April 1964 at Denver (PALAY, 1964a). Some fifty electron micrographs of cells, fibers, and synapses in the cerebellar cortex were exhibited. During the display a well-known elder statesman of the traditional neuroanatomical school came by and, casting a

scornful glance at the micrographs, came out with "Well, what have you learned that we didn't know before?" In vain to tell him about the synapses of parallel fibers on Purkinje cell thorns, or about a new understanding of the glomerulus, or a fresh view of the pericellular basket and the pinceau, or about a thousand other points. It had all been worked out by RAMÓN Y CAJAL almost a century before. Nor were anatomists the only ones who thought that such investigation was futile. In the late 1950's a well-known neurophysiologist chided the senior author for working on the structure of the cerebellum. "Why do anatomists," he asked, "always like to study the cerebellum? Nothing interesting goes on there!" In the intervening years a great many investigators, both morphologists and physiologists, have found new and interesting things in the cerebellar cortex, so much, in fact, that the literature on the subject has burgeoned beyond the ability of anyone to cope with it. Once again the notion is prevalent among neuroanatomists that the subject is exhausted. This book is testimony to the fact that although much has been learned, a great many questions still go unanswered.

In the present volume each of the cell types and afferent fibers in the cerebellar cortex is taken up in turn and described. Both optical and electron microscopy are used and illustrated. A careful study of the cerebellar cortex with these two methods indicates that considerable reliance may be placed on the Golgi technique for the general architecture and the three-dimensional form of the cells and fibers. All of the known synapses are characterized and their function is discussed from the anatomical point of view. It would be presumptuous on our part to undertake a review of the electrophysiology of this cortex, although we have drawn freely upon the results and insights derived from that discipline. All of the drawings are original India ink tracings made with the aid of a camera lucida at high magnifications. They were prepared by the junior author especially for this book or for recent journal articles.

Finally, a word may be in order to explain our use of the laboratory rat for this study instead of the cat, which has long been the favorite of neurophysiologists. Besides believing that the cat is a most peculiar animal, we could claim a dangerous sensitivity to feline dander. But, more pertinently, the cerebellum of the rat has all of the essential machinery of the mammalian cerebellum. It is much more reliably preserved than that of the cat and there is no cytological advantage in studying the larger animal. Furthermore, our work may encourage neurophysiologists to make use of this lowly beast in which a great deal of fundamental biology has been explored.

It remains to acknowledge the support of our laboratory by research grant NS 03659 and training grant NS 05591 from the National Institute of Neurological Diseases and Stroke, Bethesda, Maryland. The composition of the manuscript was made possible by a fellowship from the John Simon Guggenheim Memorial Foundation, granted to the senior author while he was on sabbatical leave from the Harvard Medical School. We wish to express our gratitude to CAROL WILUSZ for her patient assistance in making counts and measurements, tracing fibers in Golgi preparations, and preparing the graphs; to PHOEBE FRANKLIN for her meticulous preparation of the typescript for publication and for her bibliographic searching. Finally, we should like to thank VICTORIA LI MEI PALAY for the use of Fig. 10.

Boston, September 1, 1973 SANFORD L. PALAY and VICTORIA CHAN-PALAY

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Chapter I

Introduction

Probably no other part of the central nervous system has been so thoroughly investigated and is so well known as the cerebellar cortex. For nearly a century all of its cell-types have been recognized, and the course and terminations of their processes have been described countless times by numerous authors. Yet unanimity on many doubtful points has not been reached. Most of our knowledge of the cerebellar cortex derives from the early work of RAMÓN Y CAJAL. It was, in fact, during the period of his first successes with the Golgi method, when RAMÓN Y CAJAL was seized with what he described in his autobiography as a *fièvre de publicité*, that the basic plan for the organization of the cerebellar cortex was worked out. Subsequent research has confirmed many of his intuitions and added only details. For a long time these observations and the plan of the cerebellar cortex that he derived from them were far in advance of the physiological understanding of this organ. The information that this cortex is divisible into three layers, each with its own distinct populations of cells, and that they are interconnected in a few simple neuronal chains was already sufficient to baffle comprehension. No one had any idea of how the cerebellar cortex should work or what operations it should perform with the impulses coming into it from diverse sources. The Sherringtonian concept that the cerebellum was somehow related to the maintenance of muscle tone, muscle coordination, equilibration, and proprioception balanced securely on a knowledge of afferent and efferent pathways that was already too encumbered with detail. The interpretation of coarse experiments with ablations and evoked surface potentials had no need for intracortical circuits (see, for example, FULTON, 1949, and DOW and MORUZZI, 1958).

1. A New Morphology

It is only in the past decade that a more precise knowledge of the anatomy of the cerebellar cortex has been required. Today we still have only glimmerings of how the cerebellar cortex operates and no clear vision of what its function is, but the situation with respect to anatomicophysiological correlations has vastly changed. Physiologists are now probing the activities of individual neurons and eavesdropping on the coordinated interchanges between the members of neuronal assemblies. A much more detailed and precise knowledge of the morphology of the cerebellar cortex is required now than hitherto in order to guide these experiments and to inform their results, as well as to contain the speculations that they induce in physiologists, cyberneticists, and morphologists alike.

This new level of morphology can be achieved by the exercise of three technical procedures—one ancient, the others relatively recent—which provide complementary views of the cerebellar cortex. The old technique is the Golgi method, which figured so strongly in the early advances in our knowledge of the cerebellar cortex, and the centenary of which should be celebrated this year. Although this method is still poorly understood, it has undergone a revival of interest and confidence during the past decade such as it has not enjoyed since the end of the nineteenth century. Its survival through this long period of neglect and antipathy is owing to a handful of adepts, whose painstaking observations kept it alive until the modern era of neurocytology reasserted its value. Even now the method has few practitioners and unnecessarily appears esoteric to the uninitiated. Important data are still to be derived from the careful study of Golgi preparations, especially when they are coordinated with the results of the more modern methods. The second method of present usefulness is the method of experimental degeneration, particularly the Nauta technique and its recent variants, for locating the terminal arborizations of axons within the neuropil. This technique provides essential data about the distribution of nerve fibers connecting one region of the nervous system with another. While these data are necessary for the location of terminals belonging to specific systems, they do not identify the actual sites of synapses. This deficit is filled by electron microscopy.