HANDBOOK OF CHEMICAL NEUROANATOMY

Volume 12

L.W. Swanson

INTEGRATED SYSTEMS OF THE CNS
PART III

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HANDBOOK OF CHEMICAL NEUROANATOMY

Series Editors: A. Björklund and T. Hökfelt

Volume 12

INTEGRATED SYSTEMS OF THE CNS, PART III
Cerebellum, Basal Ganglia, Olfactory System

Editors:

L.W. SWANSON
Department of Biological Sciences, University of Southern California, Los Angeles, CA, U.S.A.

A. BJÖRKLUND
Department of Medical Cell Research, University of Lund, Lund, Sweden

T. HÖKFELT
Department of Neuroscience, Histology, Karolinska Institute, Stockholm, Sweden

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Dedicated to
János Szentágothai
and
Walle J.H. Nauta

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List of contributors

MATTHEW ENNIS
Department of Anatomy
The University of Maryland School of Medicine
Baltimore, MD 21201
U.S.A.

CHARLES R. GERFEN
Laboratory of Systems Neuroscience
National Institute of Mental Health
Bldg 36 Room 2D-10
Bethesda, MD 20892
U.S.A.

D. JAARSMA
Department of Anatomy
Erasmus University Medical Center
P.O. Box 1738
3000 DR Rotterdam
The Netherlands

E. MARANI
Department of Physiology
Leiden University
Rijnsburgerweg 10
2300 RC Leiden
The Netherlands

JOHN H. MCLEAN
Division of Basic Medical Sciences
Memorial University of Newfoundland
St. John's, Newfoundland
Canada A1B 3V6

MICHAEL T. SHIPLEY
Department of Anatomy
The University of Maryland School of Medicine
Baltimore, MD 21201
U.S.A.

J. VOOGD
Department of Anatomy
Erasmus University Medical Center
P.O. Box 1738
3000 DR Rotterdam
The Netherlands

CHARLES J. WILSON
Department of Anatomy and Neurobiology
University of Tennessee School of Medicine
Memphis, TN
U.S.A.

LEE A. ZIMMER
Department of Anatomy
The University of Maryland School of Medicine
Baltimore, MD 21201
U.S.A.

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Preface

It is with a mixture of pleasure and sadness that we dedicate this third volume of the *Integrated Systems* series of the *Handbook of Chemical Neuroanatomy* to the memory of two outstanding structural neuroscientists, János Szentágothai and Walle J.H. Nauta, who are widely regarded as having led the Romantic School of neuroanatomy through the Twentieth Century. Szentágothai was born on October 31, 1912, in Budapest, and passed away on September 8, 1994 in his native city. He was a student of Cajal's friend von Lenhossék, and like Cajal made enduring contributions to our understanding of many components of the nervous system, including (roughly in chronological order) the autonomic system, spinal cord, vestibulo-ocular and stretch reflex circuitry, neuroendocrine system, cerebellum, thalamus, and cerebral cortex. What sets his work apart from many of his contemporaries was the ability to generalize sensibly. This led, for example, to the concepts of synaptic glomeruli and neuronal modules, and to the synthesis for which he will always be remembered, *The Cerebellum as a Neuronal Machine,* published in 1967 with his collaborators John Eccles and Masao Ito.

Nauta was born on June 8, 1916 in Medan, Indonesia; received the M.D. and Ph.D. degrees at the University of Utrecht; served the last 30 years of his career at the Massachusetts Institute of Technology; and died on March 24, 1994. He perhaps will be remembered longest for the 'Nauta method', the first selective silver impregnation technique for degenerating axons. It was introduced in 1950 and variants were the method of choice for tracing axonal connections for about 25 years, until the use of more sensitive intraaxonal transport techniques became widespread. However, Nauta was a brilliant writer and an inspiring lecturer; and he published very influential experimental analyses of many forebrain systems in a variety of mammals. The limbic system and basal ganglia were his specialties, and indeed his work with Mehler on the lentiform nucleus of the cat and monkey was the first paper published in *Brain Research* (*1*:3-42, 1966) and is a classic with regard to both style and content.

We are profoundly grateful to the authors who have committed so much time and thoughtfulness to the chapters in the third part of the *Integrated Systems* component of the *Handbook*. When planning began in 1983, we had hoped to review each of the major sensory and motor systems, along with parts of the broader system that controls motivated and emotional behavior. Furthermore, each chapter was to be written from a dual perspective – a classical functional neuroanatomical overview, combined with what has been learned more recently about neurotransmitters and receptors within the circuitry. For the usual reasons familiar to editors, all of the planned chapters were not written, and it proved impossible to devote single volumes to an internally consistent theme. Nevertheless, the series as a whole does survey the major sensory systems (*retina* by Ehinger and Dowling, part I; *central visual pathways* by Parnavelas, Dinopoulos, and Davies, part II; *auditory system* by Aitkin, part II; *somatosensory system* by Rustioni and Weinberg, part II; *gustatory and related chemosensory systems* by Kruger and Mantyh, part II; and *olfactory system* by Shipley, McLean, Zimmer, and Ennis, part III); two important parts of the motor system (*cerebellum* by Voogd, Jaarsma, and Marani, part III; *basal ganglia* by Gerfen and Wilson, part III); and three key parts of

the limbic system (*hypothalamus* by Swanson, part I; *amygdala* by Price, Russchen, and Amaral, part I; *hippocampus* by Swanson, Köhler, and Björklund, part I). The literature in the field as a whole continues to explode. Keeping pace is a challenge that we hope will be facilitated by the imminent revolutions in electronic publishing, database management, and computer graphics.

Los Angeles, Lund and Stockholm in June 1995

LARRY W. SWANSON ANDERS BJÖRKLUND TOMAS HÖKFELT

Contents

CHAPTER I

The cerebellum: chemoarchitecture and anatomy

J. VOOGD, D. JAARSMA AND E. MARANI

> ..... but the Spirits inhabiting the Cerebel perform unperceivedly and silently their Work of Nature without our Knowledge or Care.
>
> Thomas Willis. Of the Anatomy of the Brain. Englished by Samual Pordage, Esquire, London. Printed for Dring, Harper, Leigh and Martyn, 1681. Facsimile Edition, McGill University Press, Montreal, 1965. p. 111.

1. INTRODUCTION

During the last 150 years the morphology of the cerebellum attracted numerous histologists. Its relatively simple structure, with its three-layered cortex and clearly defined afferent and efferent connections made it one of the favourite sites in the brain to test out new hypotheses on the connectivity, the development and chemical interaction in nervous tissue. We have attempted to review present knowledge about the external and internal morphology of the cerebellum and to relate the 'classical' topography of the cerebellum to the more recently discovered chemical specificity of its neurons and afferent and efferent pathways. Not all what is new in the histochemistry of the cerebellum is relevant to a better understanding of its chemoarchitecture. This review, therefore, does not pretend to be complete. It is focussed on afferent and intrinsic connections of the cerebellum. The efferent connections of the cerebellum to the brain stem and the spinal cord have not been systematically covered.

2. CYTOLOGY OF THE CEREBELLAR CORTEX

A complete description of the histology of the cerebellar cortex was given by Ramon y Cajal (1911) (Figs 1 and 4). More recently the anatomy of the cortex including its ultrastructure was reviewed by Braitenberg and Atwood (1958), Eccles et al. (1967), Fox et al. (1967), Mugnaini (1972), and Palay and Chan-Palay (1974). Three layers are distinguished in the cortex (Fig. 3). The granular layer borders on the central white matter of the cerebellum. The Purkinje cell layer contains the cell bodies of the Purkinje cells, that are arranged in a single row. The perikarya of the Bergmann glia (the Golgi epithelial cells) are intercallated between the larger Purkinje cells (Fig. 9A). The molecular layer has a low cell content. It contains the dendritic arbors of the Purkinje cells and the Bergmann glial fibers, which run up to the pial surface where they constitute the external glial limiting membrane. The morphology of the cerebellar cortex can be characterized as a lattice: '... it can only be represented in two planes perpendicular to each other and having definite relations to the longitudinal and transversal axes of the

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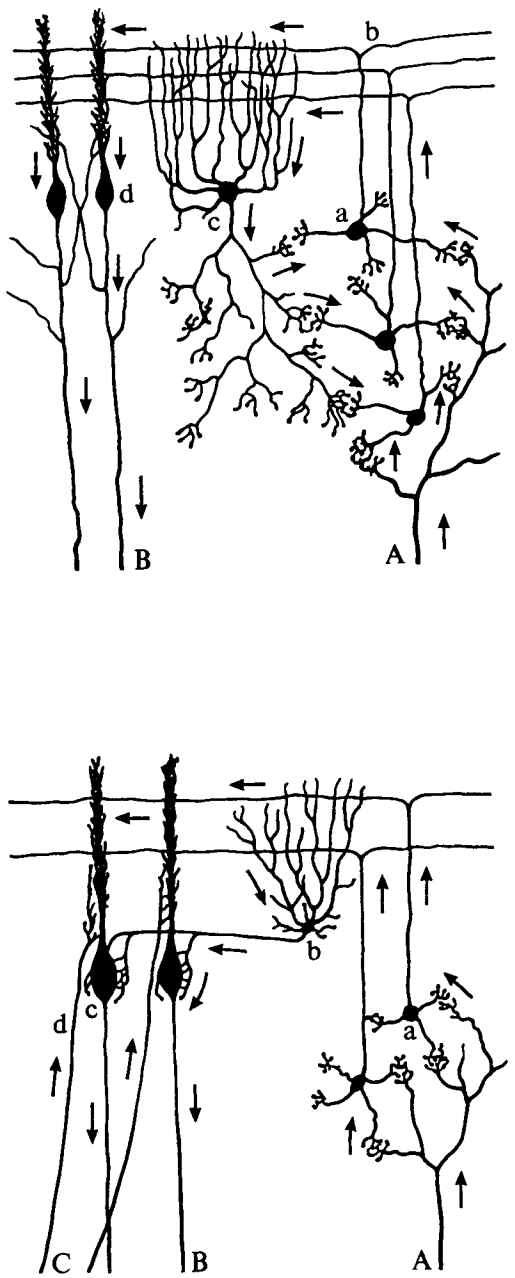


Fig. 1. Cerebellar cortical circuits. Top. Diagram showing the main mossy fiber-granule cell-Purkinje cell circuit and the innervation of the granule cells by the axonal plexus of the Golgi cell. A: mossy fiber; a: granule cell; B: Purkinje cell axon; b: parallel fiber; c: Golgi cell; d: Purkinje cell. Bottom. Similar diagram showing the main cortical circuit and the connection of the basket cell with the Purkinje cell somata. A: mossy fiber; a: granule cell; B: Purkinje cell axon; b: basket cell; C: climbing fiber; c: Purkinje cell soma. Redrawn from Ramon y Cajal (1911).

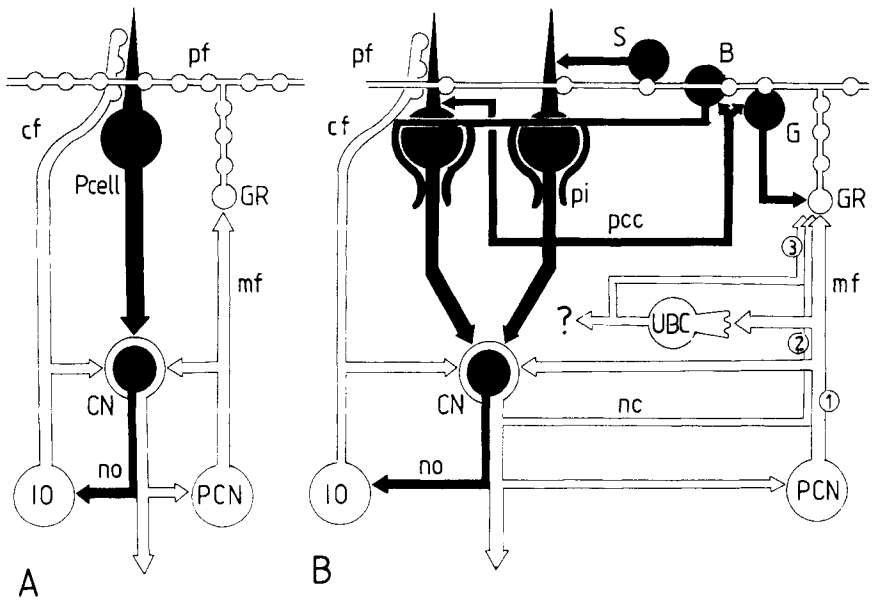


Fig. 2. Diagrams of the cerebellar circuit. Inhibitory neurons are indicated in black. A. Main circuit. B. Cortical interneurons and recurrent pathways. Abbreviations: B = basket cell; cf = climbing fiber; G = Golgi cell; GR = granule cell; IO = inferior olive; mf = mossy fiber; nc = nucleocortical axons; no = nucleo-olivary axons; pcc = recurrent Purkinje cell axon collaterals; P cell = Purkinje cell; PCN = precerebellar nuclei; pf = parallel fiber; pi = pinceau of basket cell axons; S = stellate cell; UBC = unipolar brush cell; 1 = extracerebellar mossy fiber; 2 = nucleo-cortical mossy fiber; 3 = mossy fiber collateral of uni-polar brush cell.

animal. The whole three dimensional structure, therefore, cannot be obtained by rotation but by translation in two directions, thus producing a lattice' (Braitenberg and Atwood, 1958, p.1).

The elements of the main cerebellar circuit were discovered by Ramon y Cajal (1888, 1911). The electrophysiological properties of the circuit were established by Eccles et al. (1967). The main circuit (Figs 1 and 2) consists of the mossy fiber afferent system, that terminates on the granule cells; the granule cell axons that ascend to the molecular layer and bifurcate into parallel fibers, that run in the long axis of the folium and terminate on the Purkinje cells and the projection of the Purkinje cells to the cerebellar or vestibular nuclei. Each Purkinje cell is innervated by a single climbing fiber (Ramon y Cajal, 1911; Eccles et al., 1966a) that takes its origin from the contralateral inferior olive. The synaptic connections of mossy fibers, parallel fibers and climbing fibers are excitatory. The Purkinje cells are inhibitory and use gamma aminobutyric acid (GABA) as a transmitter (Ito and Yoshida, 1964). Small interneurons of the cerebellar cortex (stellate, basket and Golgi cells) receive a parallel fiber input and constitute inhibitory feed back and feed forward loops terminating on the granule cells and the Purkinje cells (Figs 1, 2 and 4). The main determinant of the firing rate of Purkinje cells is the mossy fiber-parallel fiber system. Excitatory coupling between climbing fibers and Purkinje cells is very strong, but the frequency of the complex spikes evoked in Purkinje cells by the climbing fiber is too low to contribute significantly to its firing rate. The function of the climbing fibers, therefore, is one of the main problems in cerebellar neurobiology. Purkinje cells project to the cerebellar or the vestibular nuclei, where their axons terminate with inhibitory synapses. The cerebellar nuclei receive their excitatory drive from collaterals of the mossy and the climbing fibers.

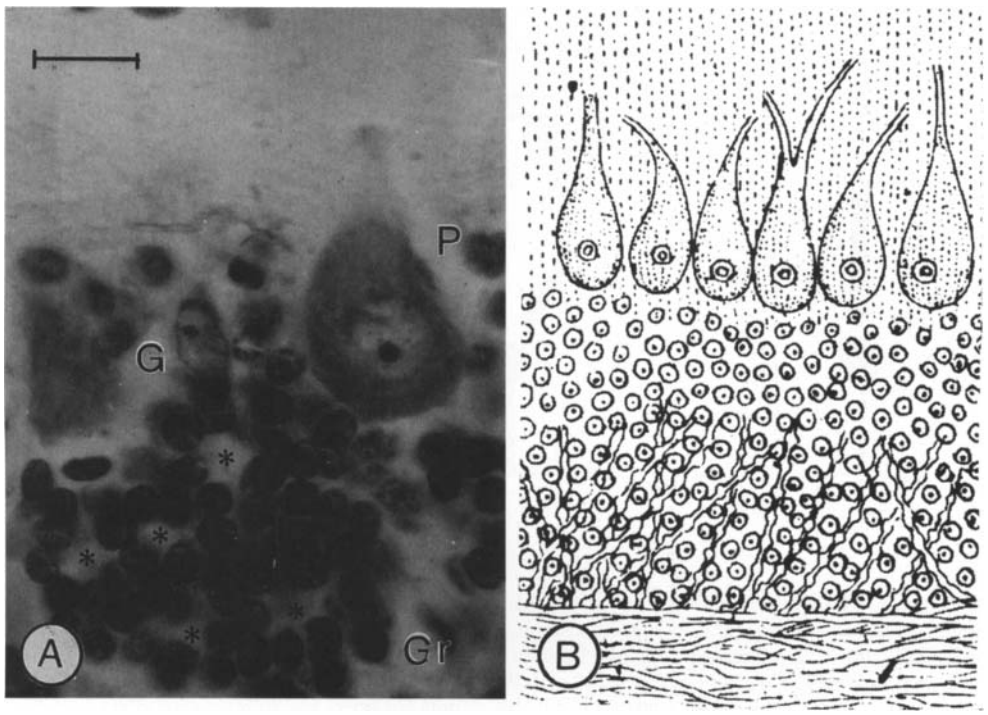


Fig. 3. A. Nissl-stained section of the cerebellar cortex of the cat. G = Golgi cell; Gr = granule cells; P = Purkinje cell, asterisks: protoplasmatic islands of Held. Bar = 20 μm. B. diagram of the cerebellar cortex of Purkinje (1837).

Granule cells are small neurons located in cell nests in the granular layer. Cell-free spaces in the granular layer, that are known as the protoplasmatic islands of Held, contain the terminals of the mossy fibers (Fig. 3A, asterisks). Mossy fibers originate from many different sites in the spinal cord and the brain stem and constitute the main afferent system of the cerebellar cortex. Mossy fibers are myelinated fibers that branch extensively within the cerebellar white matter and the granular layer. They terminate with large irregular swellings (the mossy fiber rosettes, Figs 1, 5 and 6) that are located along or at the end of the axon. Each rosette forms the center of a complex synapse (cerebellar glomerulus) between the mossy fiber rosette, the dendrites of several granule cells and the terminals of one type of short axon (Golgi) cell of the cerebellar cortex. More than one mossy fiber rosette may be present within a protoplasmatic island.

Granule cells possess 3–4 short dendrites, terminating in claw-like excrescenses (Fig.7). The thin, unmyelinated axon ascends towards the molecular layer, where it bifurcates in the form of a T. The two branches, that are known as the parallel fiber, pursue a straight course in the long axis of the folia, parallel to the thousands of other parallel fibers that constitute the bulk of the molecular layer.

Parallel fibers synapse with dendrites of Purkinje cells and short axon cells in the molecular layer. Both the ascending portion of the granule cell axon and the parallel fiber are beaded. These varicosities probably correspond to the synaptic sites (Fig. 7D-E). Parallel fibers are very long. In monkeys their length varied between 0.8 and 5 mm. (Fox and Barnard, 1957). Maximal lengths of parallel fibers of 4.6–5.0 mm were reported for the rat (Brand et al., 1976; Schild, 1980; Mugnaini, 1983). The mean length

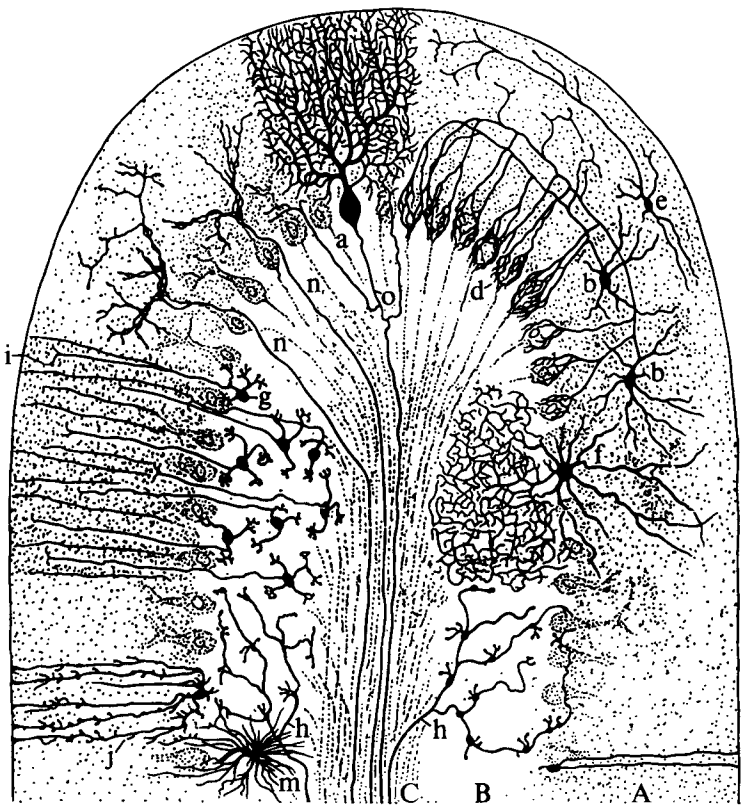


Fig. 4. Semidiagrammatic parasagittal section through a folium of the mammalian cerebellum, based on data from Golgi-stained material. A: molecular layer; B: granular layer; C: white matter; a: Purkinje cell; b: basket cells of the lower molecular layer; d: terminal basket formation of the basket cell axon; e: superficial stellate cells; f: Golgi cell; g: granule cells with their ascending axons; h: mossy fibers; i: the bifurcation of the granule cell axons; j: epithelial glial cell; m: astrocyte of the granular layer; n: climbing fiber; o: branching point of Purkinje cell recurrent axon collaterals. Redrawn from Ramon y Cajal (1911).

of parallel fibers of 4.4 mm, measured after microinjections of biocytin in the granular layer in the rat (Pichitpornchai et al., 1994) is close to the mean length of these fibers of 5 mm, estimated with stereological techniques by Harvey and Napper (1988). The two branches of the parallel fiber are of equal length (Pichitpornchai et al., 1994). Shorter parallel fibers are located at the base of the molecular layer (mean branch length 2.08 mm), they become progressively longer as they approach the pial surface (mean branch length 2.35 mm: Pichitpornchai et al., 1994). Parallel fibers in the superficial molecular layer are of a smaller calibre than deep parallel fibers (Fox and Barnard, 1957, monkey). A similar increase in size of the parallel fibers from superficial to deep laminae of the molecular layer was noticed by Pichitpornchai et al. (1994) in the rat. They also observed proximo-distal tapering of parallel fibers. Van der Want et al. (1985a,b) observed corresponding differences in synaptic size in superficial and deep layers of the molecular layer in the cat. The size and the spacing of the varicosities along the parallal fibers was found to be correlated with their caliber. The mean interval between two varicosities was 5.2 μm for the parallel fibers, 4.02 μm for the ascending axon of the granule cell (Pichitpornchai et al., 1994). The lamination in the molecular layer may be the expression of a deep to superficial gradient in the development of the parallel fibers

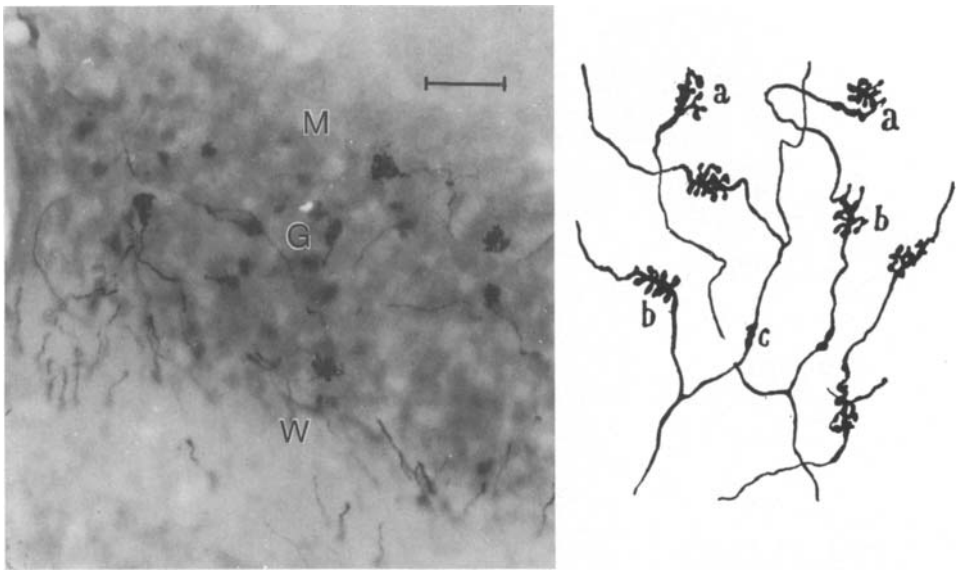


Fig. 5. Mossy fiber rosettes in the granular layer. Left. Mossy fiber rosettes from neurons of the lateral reticular nucleus, labelled with antegrade transport of *Phasaeolus vulgaris* lectin. Bar = 25 μm. Right: Mossy fibers, Golgi impregnation. Cajal (1911). Abbreviations: a = large, terminal rosettes; b = rosettes 'en passage'; c = small rosette 'en passage'; G = granular layer; M = molecular layer; W = white matter. Courtesy of Dr. T.J.H. Ruigrok.

(Pellegrino and Altman, 1979). A population of thick, short parallel fibers was noticed by Pitchitpornchai et al. (1994) in the deep parts of the molecular layer. Deep lying parallel fibers may be myelinated and are one of the constituents of the supraganglionic plexus located above the Purkinje cells (Mugnaini, 1972).

The mossy fiber-parallel fiber-Purkinje cell pathway is characterized by a large divergence. Each mossy fiber terminates on a great number of granule cells and each granule cell contacts hundreds of Purkinje cells along its parallel fiber. An average parallel fiber with a length of 6 mm forms approximately 1100 boutons (Brand et al., 1976). A portion of the molecular layer 6 mm wide contains approximately 750 Purkinje cell dendritic sheets (Brand and Mugnaini, 1976). This number is somewhat lower than the number of available boutons, when a parallel fiber would synapse once with each Purkinje cell it meets on its way (Brand et al., 1976). It is higher than the estimate of Napper and Harvey (1988b) in the rat that 15% of the boutons on parallel fibers synapse with non-Purkinje cells and that the rest synapses once with half of the Purkinje cell dendritic sheets it meets on its way. The granule cell/Purkinje cell ratio was estimated at 274/1 by Harvey and Napper (1988) and at 350-500/1 for different lobules of rat vermis by Drüge et al. (1986). Napper and Harvey (1988) concluded that there are some 175.000 parallel fiber synapses on a single Purkinje cell of the rat. Fox et al. (1967) arrived at a number of 120.000 in monkeys.

The actual strength of the convergence of individual mossy fibers to Purkinje cells depends on the distribution of their mossy fiber rosettes. Electrophysiological studies of Bower and Woolston (1983) in the rat demonstrated that Purkinje cells are most responsive to mossy fiber input that reaches the granule cells located immediately below them. Llinas (1982) explained this strong radial connectivity by the greater number of

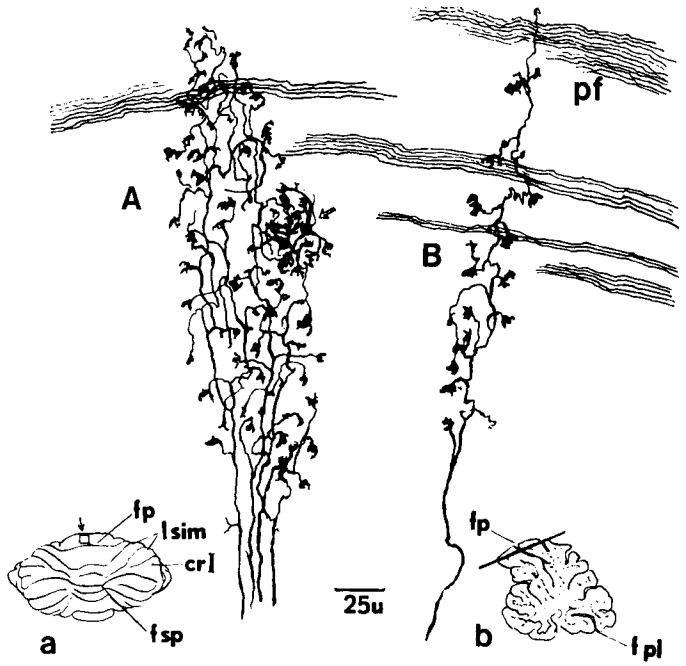
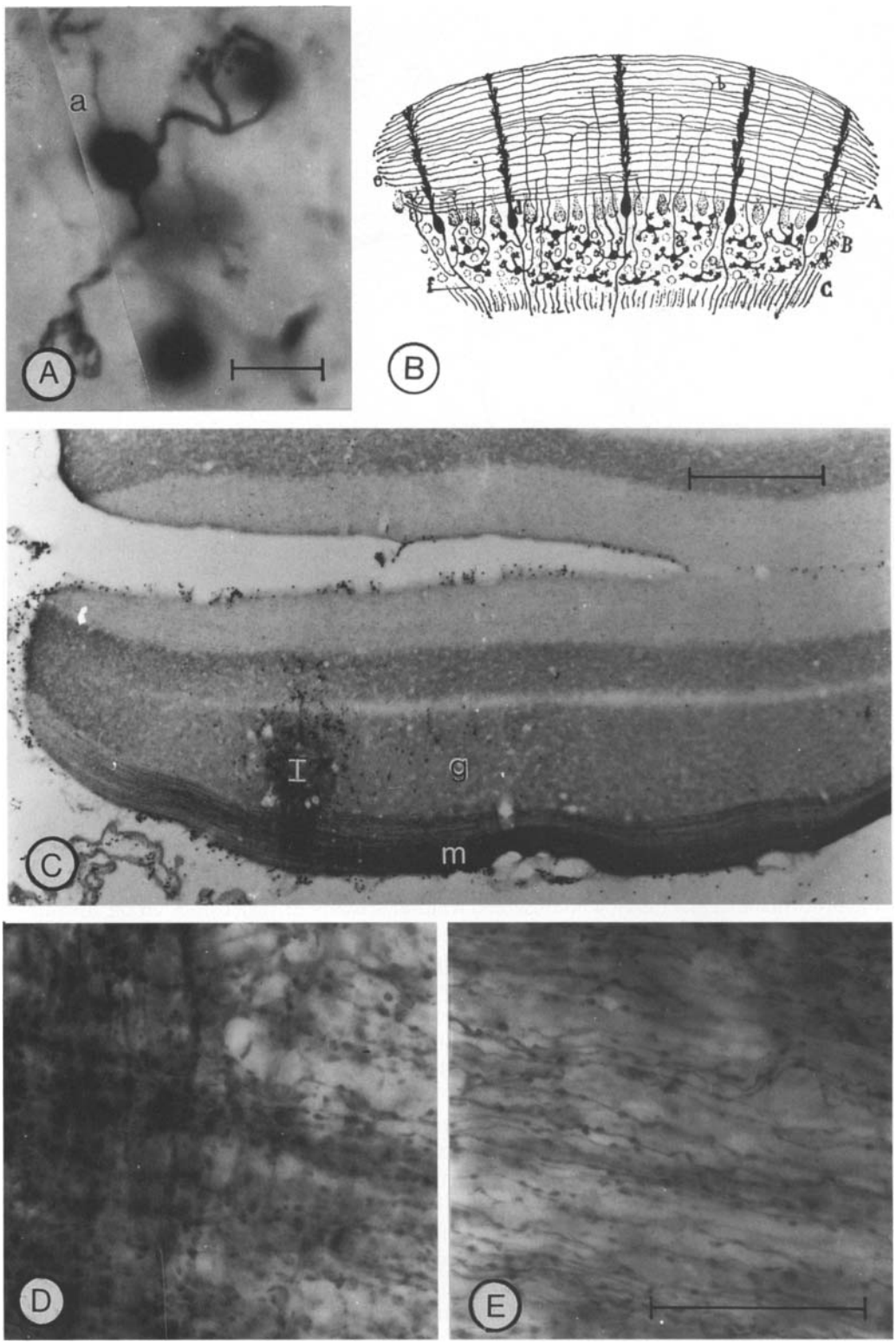


Fig. 6. Drawing of horizontal section through rat cerebellum showing orientation of mossy fibers. A. Elliptical segment or stripe of mossy fiber terminals in the medial portion of the anterior lobe showing the strong caudal-rostral organization of the terminal neuropil. Note the small cluster of granule cell bodies at the open arrow. B. Single mossy fiber from the next adjacent section showing the almost linear caudal-rostral pattern of the related terminals and small groups of parallel fibers (pf). a: View of rat cerebellum from the above showing approximate position of the field illustrated (note square and arrow). b: Medial sagittal section through cerebellum showing approximate location and plane of section. Abbreviations: fp = fissura prima; Isim = lobulus simplex; crI = crus I; fsp = fissura superior posterior; fpl = fissura posterolateralis; pf = parallel fiber. Golgi modification; 21-day-old rat. Scheibel (1977).

synapses with Purkinje cells on the ascending portion of the parallel fiber. However, according to Napper and Harvey (1988) the synapses on ascending portions of parallel fibers would account for only 3% of the total number of synapses of these fibers. Pichitpornchai et al. (1994), who observed a closer spacing of varicosities on the ascending axon and the proximal branches of the parallel fibers than on their distal branches, concluded that parallel fibers will exert a graded synaptic influence on their target Purkinje cells, with the most powerful influence occurring on cells located around the proximal regions of the fibers where they bifurcate. Mossy fiber terminal branches in the granular layer are oriented longitudinally, in the same plane as the Purkinje cells (Scheibel, 1977), (Fig. 6) (see also Section 6.4.2.). Mossy fibers, therefore, preferentially activate longitudinally oriented patches of Purkinje cells.

Different types of mossy fiber rosettes were described by Brodal and Drabløs (1963) with the Glees and Rheumont-Lhermitte silver impregnations and the Golgi method in rat and cat. Highly branching mossy fibers terminating in small, relatively simple rosettes, located along or at the end of the fiber, occur in all parts of the cerebellum. Large rosettes, consisting of aggregations of larger and smaller argyrophilic particles, interconnected by fiber fragments occur exclusively in nodulus and adjoining uvula, lingula and flocculus.

The dendritic tree of the Purkinje cell is flattened in a plane perpendicular to the long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