# NEUROHISTOCHEMISTRY
## Modern Methods and Applications

Editors
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Department of Anatomy
University of Helsinki
Helsinki, Finland

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# Contributors

**Luigi F. Agnati,** Department of Human Physiology, University of Modena, 41100 Modena, Italy **[137]**

**Hannu Alho,** Departments of Biomedical Sciences and Public Health, University of Tampere, SF-33101 Tampere, Finland, and Laboratory of Preclinical Pharmacology National Institute of Mental Health, Saint Elizabeth's Hospital, Washington, DC 20032 **[453,569]**

**Daryll M. Baker,** Department of Anatomy, University College, Cardiff CF1 1XL, United Kingdom **[569]**

**Elena Battenberg,** Division of Preclinical Neuroscience and Endocrinology, Scripps Clinic and Research Foundation, La Jolla, CA 92037 **[3]**

**Floyd E. Bloom,** Division of Preclinical Neuroscience and Endocrinology, Scripps Clinic and Research Foundation, La Jolla, CA 92037 **[3]**

**Peter Böck,** Department of Micromorphology and Electron Microscopy, University of Vienna, A-1090 Vienna, Austria **[617]**

**Martha C. Bohn,** Department of Neurobiology and Behavior, State University of New York, Stony Brook, NY 11794 **[245]**

**E. Costa,** Laboratory of Preclinical Pharmacology, National Institute of Mental Health, Saint Elizabeth's Hospital, Washington, DC 20032 **[453]**

**A.C. Cuello,** Departments of Human Anatomy and Pharmacology, and Sir William Dunn School of Pathology, Oxford University, Oxford OX1 3QX, United Kingdom, and Department of Pharmacology and Therapeutics, McGill University, Montreal, Quebec H3G 1Y6, Canada **[49]**

**M. Fujimoto,** Laboratory of Preclinical Pharmacology, National Institute of Mental Health, Saint Elizabeth's Hospital, Washington, DC 20032 **[453]**

**Kjell Fuxe,** Department of Histology, Karolinska Institute, S-10401 Stockholm, Sweden **[137]**

**Menek Goldstein,** Department of Psychiatry, New York University Medical Center, New York, NY 10016 **[137]**

The number in brackets is the opening page of the contributor's article.

**Ann-Charlotte Granholm,** Department of Histology, Karolinska Institute, S-10401 Stockholm, Sweden [273]

**A. Guidotti,** Laboratory of Preclinical Pharmacology, National Institute of Mental Health, Saint Elizabeth's Hospital, Washington, DC 20032 [453]

**Maria Hadjiconstantinou,** Department of Pharmacology, Ohio State University, Columbus, OH 43210 [465]

**Willy Haefely,** Pharmaceutical Research Department, F. Hoffmann-La Roche & Co., Ltd., CH-4002 Basle, Switzerland [629]

**I. Hanbauer,** National Heart, Lung and Blood Institute, Hypertension–Endocrine Branch, Bethesda, MD 20205 [453]

**Anders Härfstrand,** Department of Histology, Karolinska Institute, S-10401 Stockholm, Sweden [137]

**Pauli Helén,** Departments of Biomedical Sciences and Public Health, University of Tampere, SF-33101 Tampere, Finland, and Laboratory of Neurosciences, National Institute of Aging, National Institutes of Health, Bethesda, MD 20205 [569]

**Antti Hervonen,** Departments of Biomedical Sciences and Public Health, University of Tampere, SF-33101 Tampere, Finland, and Laboratory of Neurosciences, National Institute of Aging, National Institutes of Health, Bethesda, MD 20205 [569]

**Heikki Hervonen,** Department of Anatomy, University of Helsinki, SF-00170 Helsinki, Finland [443]

**Christine Heym,** Anatomisches Institut der Universität Heidelberg, D-6900 Heidelberg, Federal Republic of Germany [493]

**Tomas Hökfelt,** Department of Histology, Karolinska Institute, S-10401 Stockholm, Sweden [xv,331]

**B. Hwang,** Department of Anatomy, The Milton S. Hershey Medical Center, The Pennsylvania State University, Hershey, PA 17033 [21]

**John E. Johnson, Jr.,** Section of Experimental Morphology, Gerontology Research Center, Baltimore, MD 20824 [569]

**Y. Kataoka,** Laboratory of Preclinical Pharmacology, National Institute of Mental Health, Saint Elizabeth's Hospital, Washington, DC 20032 [453]

**George B. Koelle,** Department of Pharmacology, Medical School/G3, University of Pennsylvania, Philadelphia, PA 19104 [175]

**Jari Koistinaho,** Departments of Biomedical Sciences and Public Health, University of Tampere, SF-33101 Tampere, Finland [569]

**Rudolf Lang,** Pharmakologisches Institut der Universität Heidelberg, D-6900 Heidelberg, Federal Republic of Germany [493]

**Lars-Inge Larsson,** Unit of Histochemistry, University Institute of Pathology, DK-2100 Copenhagen Ø, Denmark [527]

**J. Lehtosalo,** Department of Anatomy, Eye Research Laboratory, University of Helsinki, SF-00170 Helsinki, Finland **[587]**

**C.-T. Lin,** Department of Physiology, The Milton S. Hershey Medical Center, The Pennsylvania State University, Hershey, PA 17033 **[21]**

**H.S. Lin,** Department of Physiology, The Milton S. Hershey Medical Center, The Pennsylvania State University, Hershey, PA 17033 **[21]**

**F.M. MacMillan,** Department of Human Anatomy and Sir William Dunn School of Pathology, Oxford University, Oxford OX1 3QX, United Kingdom **[49]**

**E.G. McGeer,** Kinsmen Laboratory of Neurological Research, Department of Psychiatry, University of British Columbia, Vancouver, British Columbia V6T 1W5, Canada **[355]**

**P.L. McGeer,** Kinsmen Laboratory of Neurological Research, Department of Psychiatry, University of British Columbia, Vancouver, British Columbia V6T 1W5, Canada **[355]**

**Rob Milner,** Division of Preclinical Neuroscience and Endocrinology, Scripps Clinic and Research Foundation, La Jolla, CA 92037 **[3]**

**Hanns Möhler,** Pharmaceutical Research Department, F. Hoffmann-La Roche & Co., Ltd., CH-4002 Basle, Switzerland **[629]**

**Norton H. Neff,** Department of Pharmacology, Ohio State University, Columbus, OH 43210 **[465]**

**Ole P. Ottersen,** Anatomical Institute, University of Oslo, 0162 Oslo 1, Norway **[107]**

**Heikki Päivärinta,** Department of Anatomy, University of Helsinki, SF-00170 Helsinki, Finland **[xix, 227]**

**A. Palkama,** Department of Anatomy, Eye Research Laboratory, University of Helsinki, SF-01700 Helsinki, Finland **[587]**

**Pertti Panula,** Department of Anatomy, University of Helsinki, SF-00170 Helsinki, Finland **[xix, 425]**

**Matti Partanen,** Departments of Biomedical Sciences and Public Health, University of Tampere, SF-33101 Tampere, Finland, and Laboratory of Neuroscience, National Institute of Aging, National Institutes of Health, Bethesda, MD 20205 **[569]**

**J.H. Peng,** Kinsmen Laboratory of Neurological Research, Department of Psychiatry, University of British Columbia, Vancouver, British Columbia V6T 1W5, Canada **[355]**

**Virginia M. Pickel,** Laboratory of Neurobiology, Department of Neurology, Cornell University Medical College, New York, NY 10021 **[397]**

**Leena Rechardt,** Department of Anatomy, University of Helsinki, SF-00170 Helsinki, Finland **[443]**

**Grayson Richards,** Pharmaceutical Research Department, F. Hoffmann-La Roche & Co., Ltd., CH-4002 Basle, Switzerland **[629]**

**Robert M. Santer,** Department of Anatomy, University College, Cardiff CF1 1XL, United Kingdom **[569]**

**J. Schipper,** Department of Pharmacology, Duphar B.V., 1380 AA Weesp, The Netherlands **[75]**

**Marianne Schultzberg,** Department of Histology, Karolinska Institute, S-10401 Stockholm, Sweden **[477]**

**Åke Seiger,** Department of Histology, Karolinska Institute, S-10401 Stockholm, Sweden **[273]**

**Yahe Shiotani,** Department of Neuroanatomy, Institute of Higher Nervous Activity, Osaka University Medical School, 4-3-57 Nakanoshima, Kitaku, Osaka 530, Japan **[295]**

**Seppo Soinila,** Department of Anatomy, University of Helsinki, SF-00170 Helsinki, Finland **[xix, 207]**

**H.W.M. Steinbusch,** Department of Pharmacology, Faculty of Medicine, Free University, 1081 BT Amsterdam, The Netherlands **[75]**

**Jon Storm-Mathisen,** Anatomical Institute, University of Oslo, 0162 Oslo 1, Norway **[107]**

**Greg Sutcliffe,** Department of Molecular Biology, Scripps Clinic and Research Foundation, La Jolla, CA 92037 **[3]**

**Lars Terenius,** Department of Pharmacology, Biomedical Center, University of Uppsala, 75123 Uppsala, Sweden **[137]**

**Masaya Tohyama,** Department of Neuroanatomy, Institute of Higher Nervous Activity, Osaka University Medical School, 4-3-57 Nakanoshima, Kitaku, Osaka 530, Japan **[295]**

**Roberto Toni,** Department of Human Physiology, University of Modena, 41100 Modena, Italy **[137]**

**Klaus Unsicker,** Department of Anatomy and Cell Biology, University of Marburg, D-3550 Marburg, Federal Republic of Germany **[183]**

**H. Uusitalo,** Department of Anatomy, Eye Research Laboratory, University of Helsinki, SF-00170 Helsinki, Finland **[587]**

**J. de Vente,** Department of Pharmacology, Faculty of Medicine, Free University, 1081 BT Amsterdam, The Netherlands **[75]**

**Steven R. Vincent,** Division of Neurological Sciences, Department of Psychiatry, University of British Columbia, Vancouver, British Columbia V6T 1W5, Canada **[375]**

**S. Wei,** Department of Physiology, The Milton S. Hershey Medical Center, The Pennsylvania State University, Hershey, PA 17033 **[21]**

**J.-Y. Wu,** Department of Physiology, The Milton S. Hershey Medical Center, The Pennsylvania State University, Hershey, PA 17033 **[21]**

**Michele Zoli,** Department of Human Physiology, University of Modena, 41100 Modena, Italy **[137]**

# In Memoriam

## OLAVI ERÄNKÖ

In the beginning of the 1950s, Olavi Eränkö discovered that treatment of adrenal glands with liquid formalin makes it possible to induce fluorescence in the cells of the medulla with ultraviolet light. These experiments introduced a new era in the field of histochemistry and, as it turns out, in endocrinology and neurobiology. During the 30 years that have elapsed since then, we have witnessed a remarkable development of histochemical methods for the analysis of endocrine and neuronal tissues. Much new important information has been collected and morphological techniques have been established as basic and valuable tools in biomedical research.

The VIIth International Congress of Histochemistry and Cytochemistry, organized by Olavi Eränkö and his colleagues and held in Helsinki in August 1984 under the patronship of Dr. Mauno Koivisto, the President of Finland, summarized much of the progress in the field of neurohistochemistry, highlighting the most recent advances in this research area. It was very appropriate and fortunate that the pioneer Olavi Eränkö, together with his young enthusiastic collaborators in Helsinki, had been chosen to head this congress, which would emerge as his last important contribution to the field. Although we all recognized the seriousness of his long lasting illness, we were all shocked to hear about his death. Retrospectively I think we, who participated, were all happy that we were there and were able in this way to pay a tribute to Olavi Eränkö and to his achievements. I think we are all convinced that Olavi was happy with the outcome of the conference, a task he had struggled with for several years and which he could lead to a great success.

Against this background, it may be appropriate at this time to recall some of Olavi Eränkö's major achievements as a man and a scientist. He was born in Helsinki, Finland, in 1924. His father was a mathematician. He has four children and much of his scientific work was carried out in collaboration with his darling wife, Liisa. Olavi Eränkö served in the Army during the war, after

which he started his medical studies and achieved his medical license in 1950 and his medical degree in 1952. He became Professor of Anatomy in 1956 and was the Chairman of the Department from 1964. He was Vice Dean of the Medical Faculty of the University of Helsinki 1978–1981 and Dean 1981–1984. He spent several periods abroad, for example as a British Council Scholar at the University of Edinburgh, 1952–1953. He was Senior Research Fellow at the University of Melbourne in 1971–1972 and Fogarty Scholar-in-Residence at the National Institutes of Health in Bethesda, Maryland, USA, periodically between 1973 and 1977. Olavi Eränkö was founder and member of many learned societies and received honorary degrees at several universities. He was President of the Societies for Histochemistry and Cell Biology, the Anatomici Fenniae, the Brain Research Society of Finland and of the International Federation of Societies for Histochemistry and Cytochemistry. He was an honorary Fellow or Member of the Royal Microscopical Society (U.K.), the Histochemical Society (USA), the American Association of Anatomists, the Anatomical Society of Australia and New Zealand and of Kungliga Fysiologiska Sällskapet in Lund (Sweden). He was a member of Deutsche Akademie der Naturforscher Leopoldina since 1980 and of the Finnish Academy of Sciences and Letters since 1973. He received several honors, including war medals, the Olympic Cross of Merit II, and was a Commander of the Order of the Lion of Finland (1970) and of the Order of the White Rose of Finland (1980).

Olavi Eränkö had a long and successful scientific career. He started his research during his medical studies and his first publication appeared in 1947 with subsequent papers originating from the Pharmacological and Physiological Departments. In fact, Olavi Eränkö published several physiological papers during the first part of his career and he received competence for a Professorship in Physiology. His early histochemical work dealt with several tissues including the central nervous system. But soon he turned his interest towards the adrenal gland, initially carrying out enzyme histochemistry for acid phosphatase, where he was able to describe two types of gland cells. It was in this tissue that he made the fundamental observation that a subpopulation of gland cells exhibited a strong fluorescence upon ultraviolet light exposure after fixation with formaldehyde solution. Eränkö immediately realized the importance of these results, and to be able to pursue and explain these findings he joined Marthe Vogt and Sir John Gaddum in Edinburgh, who had developed specific and sensitive methods for quantitative measurements of the catecholamines adrenaline and noradrenaline. In 1955, he had further improved these techniques and was able to compare the distribution of amines in microdissected specimens of freeze dried tissue (so much ahead of his time!) with the distribution of the fluorescent cells. In this way he could directly

demonstrate that the strongly fluorescent cell islands contained noradrenaline, whereas adrenaline was present in the nonfluorescent cells. In a large number of experiments and papers he extended these fundamental studies to the adrenal gland of other animals, as well as to analysis of the neuronal and humoral control of the adrenal gland cells. This work was collected in 1958 in his second thesis "Studies on the Adrenal Medulla", which he defended at the University of Edinburgh to be awarded the degree Doctor of Philosophy.

On the basis of Eränkö's fundamental achievements, methods were developed for the demonstration of catecholamines in nervous tissue, notably in Sweden by Hillarp, Falck, Carlsson and their associates. Eränkö turned his attention towards the peripheral nervous system and especially to sympathetic ganglia. He was able to define two different subcellular storage sites at the light microscopic level and made the intriguing observation that sympathetic fibers in the pineal gland were also positive for acetylcholinesterase, suggesting interesting mechanisms for interactions between the adrenergic and cholinergic systems.

A major discovery was the finding that the sympathetic ganglia were heterogeneous with regard to the types of cells found in them. Thus, in addition to the principal ganglion cells, Eränkö and collaborators demonstrated the presence of small cells exhibiting very strong formaldehyde-induced fluorescence. These small, intensely fluorescent (SIF) cells then became very much the main interest of Olavi and Liisa Eränkö and their associates, approaching the problems both at the light and electron microscopic levels. In fact, Olavi Eränkö has edited two books about SIF cells. Based on his fundamental work, experiments have been carried out in many laboratories all over the world. It has been established that the SIF cells represent inhibitory interneurons, but that they may also have a neuroendocrine function and be sensitive to chemical stimuli. A particularly interesting observation was the dramatic increase in the number of SIF cells in the sympathetic ganglia after treatment of newborn rats with glucocorticoids. With an arsenal of techniques including light and electron microscopy, organ culture, transplantation to the anterior chamber of the eye and surgical procedures, this phenomenon has now been investigated in detail.

Although Olavi Eränkö is no longer among us, the analysis of the sympathetic nervous system with histochemical techniques is being continued by his co-workers and students. One of the major achievements of Olavi Eränkö was his ability to interest young people in scientific problems. Thus, he has during his 25 years as a Professor guided more than 40 young scientists to their thesis and they are now valuable members of the Finnish scientific and medical communities. In this way, Olavi Eränkö is the founder of a whole school of histochemists working on endocrine tissues and the nervous system. His in-

fluence is, however, by no means limited to his home country but extends all over the world. Swedish histochemists and neurobiologists do indeed value his contributions, from which they have drawn inspiration and important information for their work.

Finally, let us extend our thanks not only to Olavi Eränkö for his scientific contributions and for the VII International Congress, but also to his wife Liisa for the hospitality that many of us have experienced upon visits to Helsinki during the years. Liisa and Olavi Eränkö and their Finnish colleagues have offered the warmest hospitality of a type rarely experienced. Let us join in wishing them luck in carrying on this tradition and their research, which is surely the best way to honour the memory of Olavi Eränkö.

**Tomas Hökfelt**

# Preface

Advancement in the field of neuroscience during recent years has been characterized by isolation and identification of a large number of chemical substances that have profound effects on the nervous tissue. Some of these substances are well known as hormones of the classical endocrine system which now have been found also in the nervous tissue. Others are totally novel compounds. This extensive work, carried out by neurochemists, has led to intensive search of the structures in which these substances are located. Such information, pursued by neurohistochemists, is the prerequisite for understanding how the neuroactive substances affect nervous transmission in normal and pathological conditions. This volume summarizes the present state of knowledge concerning newly developed histochemical techniques, as well as their applications on studies of neuronal differentiation. In addition, the anatomy of some recently characterized neurotransmitter systems in the central and peripheral nervous systems is described in detail.

The rapid development of neurohistochemical methods during the last ten years is featured in the first chapter of the book. Now that highly purified synthesizing enzymes of neurotransmitters have become available, it has been possible to reveal the distribution of specific neuronal systems within the brain and peripheral nervous system using antibodies against these enzymes. A number of new neuroactive peptides have been found using the methods of molecular biology. Monoclonal antibodies made against neuropeptides and neurotransmitter-synthesizing enzymes have made it possible to raise specific antibodies against substances which have been difficult to purify completely. Specific antibodies against small neurotransmitter substances like amines and amino acids have expanded the possibilities to reveal new neuronal systems within the nervous tissue. Quantitative analysis of the immunocytochemical results has become a rapid and reliable tool in neuroanatomical research when suitable automatic systems have been applied.

The second section deals with differentiation and development of the nervous tissue. It is evident that neurons show great plasticity in their developmental capabilities and are critically dependent on environmental factors.

Lack of thyroid hormone during development leads to permanent disturbances in different brain regions. Glucocorticoids cause the expression of adrenergic properties in developing sympathetic cells. Purification and characterization of new neuronotrophic and neurite growth-promoting factors, sophisticated histochemical methods, intraocular transplantation technique, and tissue culture experiments have brought new understanding on the differentiation and development of the nervous system.

The third section aims at integration of the present knowledge on the location of some neurotransmitters with functional aspects. An overview is given to the extensive progress from the development of acetylcholinesterase histochemistry to modern immunocytochemical localization of a large number of putative neurotransmitters. Some neurotransmitter systems of the brain are described in detail, including the classical transmitters acetylcholine and catecholamines as well as the novel transmitter candidate histamine. Significance of the enzyme histochemical methods localizing NADPH-diaphorase and adenylate cyclase is discussed. Several articles address the current questions in the research of the peripheral nervous system, such as localization and function of dopamine and gamma-aminobutyric acid, and transmitter coexistence in sympathetic ganglia, autonomically innervated target organs, neuroendocrine, and paraganglionic tissues. The sympathetic ganglion is also presented as model tissue for studies dealing with general neuronal aging.

This volume is of interest to basic neuroscientists including anatomists, physiologists, pharmacologists, and biologists. Clinicians working in the fields of neurology, psychiatry, and endocrinology may also find it beneficial. It is hoped that this volume will stimulate research in these fields and thereby gradually advance our knowledge on how single neurons using various transmitters are organized to form the extreme complexity of neuronal circuits regulating our functions from simple reflexes to higher mental activities.

This book is dedicated to the memory of Olavi Eränkö, Professor of Anatomy at the University of Helsinki during the years 1956–1984, and one of the pioneer scientists in catecholamine histochemistry. His life's work is appraised in the foreword written by his long-time friend and colleague, Professor Tomas Hökfelt. The Editors, all students of Olavi Eränkö, wish to express their gratitude to the Authors for their willing contributions which make this volume represent the highest expertise in the fields of modern neurohistochemistry.

**Pertti Panula**
**Heikki Päivärinta**
**Seppo Soinila**

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