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Development of nerve cells and their connections

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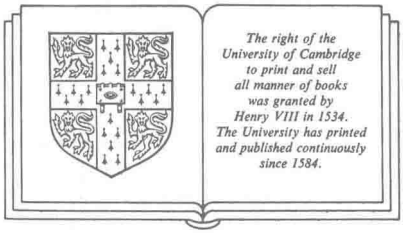

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Preface

This short book gives an account of the development of the nervous system, from the early embryonic stages when nerve cells first appear through to the adult, where interactions between nerve cells are the basis of learning, memory and recovery from injury. Vertebrates and invertebrates are considered.

The book is intended to be used both as a text for undergraduates and graduates, and as a source book for those teaching courses on neuronal development. Scientists who wish to fill in gaps in their knowledge of this rapidly expanding field should also find this book particularly useful. Key references are provided for those wanting to read in greater detail about the issues discussed. We have assumed that readers have an elementary knowledge of basic neurophysiology and neuroanatomy.

Acknowledgements

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Contents

PART I
Introduction

The nervous system is arguably the most complex structure known to man, and certainly the most complex organ system in the body. The complexity of the nervous system is due in part to its large number of cells (about 10^{12} neurons in man and at least as many glial cells), and in part to its large number of different kinds of cell (probably several hundreds or possibly even thousands). In both these respects other organ systems can be compared with the nervous system: for example, there are as many cells in the digestive system, and at least as many different kinds of cell in the immune system. However, the nervous system differs from any other part of the body in that neurons make precise patterns of connections onto other neurons, and it is this unique property that generates the complexity of the nervous system and the diversity of its actions.

The development of the nervous system is a remarkably complex process and not surprisingly many features of it are still mysterious. In recent years an increasingly large number of scientists have become involved with research on developmental neurobiology, and it is probable that newly developed techniques will solve many of the problems that have daunted previous generations of neurobiologists. Investigation of neuronal development, as is the case in most other fields, is carried out both because of the intrinsic curiosity of those concerned and because a better understanding of the mechanisms involved will have benefits for man. Abnormalities arising in development and as a result of disease or accident later in life may be alleviated by application of the new knowledge, and a better understanding of brain development will probably provide a better understanding of normal brain function. Indeed, normal function and the development of a normal brain are intimately linked processes, as will be shown in part IV.

In the following introductory chapter there is a very brief outline of the normal sequence of events in brain development and then the methods and the design of experiments used in research in this field are given.

I.1

A short outline of neuronal development

In the vertebrate the cells whose descendants contribute predominantly to nervous tissue are first identified as the neural ectoderm, a disc-shaped part of the ectoderm on the surface of the sphere of embryonic cells. This disc elongates and rounds up to form a tube within the ectoderm. Further regional elongation, outpocketing, folding and thickening of this tube produces the gross anatomical divisions of the central nervous system. At the time of closure of the neural tube some cells break away to form a transient structure called the neural crest. The cells of the crest migrate away from the neural tube and form the ganglia of the peripheral nervous system. The fibre tracts and peripheral nerves are produced when nerve cells send out axons to their targets.

At the cellular level three major stages in brain development can be identified. These follow each other more or less consecutively, and we have made these the basis for the three main subdivisions (parts II, III and IV) of this book (see table I.1).

Part II is concerned with the origin of nerve cells. In vertebrates nerve cells proliferate in several distinct germinal areas. Post-mitotic neurons then migrate, sometimes over considerable distances, to their permanent positions. At some time near the proliferation and migration stages the nerve cells become committed to developing particular biochemical and

Table I.1. *Stages in the growth of vertebrate nerve cells*

Origin of nerve cells	Proliferation, specification and migration
Establishing connections	Axon growth, dendritic growth, synapse formation
Modifying connections	Nerve cell death, reorganisation of initial inputs, adult connectivity changes

physiological properties and connectivity patterns, i.e. their phenotype is specified.

Part III deals with the ways neurons send their axons to establish connections with one another and with peripheral targets. The tip of the growing axon forms a structure called the growth cone, which guides the axon towards its target, and which also recognises its appropriate target cells. When axons reach their target synapses form, a process involving structural and functional modification of pre- and post-synaptic membrane.

As synapses develop, a considerable proportion of nerve cells die. The connections from the remaining nerve cells are subject to extensive further remodelling as excess axon branches are removed and favourable connections are expanded and stabilised. The properties of the neuron and its target are also modified as innervation matures. Functional and structural connectivity changes can continue in the normal adult and can be induced by injury. All these modifications are considered in part IV.

The invertebrate nervous system consists of interconnected ganglia positioned usually along the midline of the animal, and also sensory neurons in the periphery. All the nerve cells develop from ectodermal cells without phases of active movement or migration. The subsequent developmental stages are similar to those of vertebrates. The results of studies on selected invertebrates (usually annelids or arthropods) are dealt with briefly where appropriate in the following chapters. For a comprehensive review of neuronal development in invertebrates see Anderson, Edwards & Palka (1980).

Background reading

Short articles and a book of collected papers giving useful overviews on developmental neurobiology are:

Anderson, H., Edwards, J.S. & Palka, J. (1980). Developmental neurobiology of invertebrates. *Annual Review of Neuroscience*, **3**, 97–139.

Cowan, W.M. (1979). The development of the brain. *Scientific American*, **241** (September), 56–69.

Hamburger, V. (1981). Historical landmarks in neurogenesis. *Trends in Neurosciences*, **4**, 151–5.

Patterson, P. & Purves, D. (1982). *Readings in Developmental Neurobiology*. Cold Spring Harbor: Cold Spring Harbor Laboratory.

I.2
Methods and techniques

Histological methods

The greatest technical problem in developmental neurobiology has always been to make neurons visible. Indeed, the science began with Ramon y Cajal's use of a nerve-specific silver stain which was discovered last century by Golgi and which is still widely used today. A short summary of methods used for identifying neurons and neuron processes in developmental studies is given below.

Silver stains. These are either of the Golgi type, which stain completely a small proportion of neurons, or of the reduced silver type, which stain all nerve processes with variable efficiency.

Electron microscopy. This has provided most of the knowledge about cellular structures important in cell migration, axon growth, synaptogenesis and remodelling of connections. Many of the methods devised for identifying particular neurons in the light microscope have been adapted to the electron microscope, although many questions remain to be answered at the level of light microscopy.

Histochemistry. Some neurons can be visualised by means of their transmitter or transmitter enzymes. Catecholamines fluoresce in ultraviolet light in formaldehyde-fixed tissue, and this has been very useful in assaying the presence of adrenergic neurons. Cholinergic neurons can be detected by the presence of accompanying acetylcholinesterase.

Immunohistochemistry. Sera, or monoclonal antibodies specific for particular cell antigens, are bound to tissues and then revealed 'indirectly' by binding anti-antibodies coupled with horseradish peroxidase or fluorescent markers.

Horseradish peroxidase (*HRP*). This enzyme is transported within

cells, both towards and away from the cell body. It can be injected or cells will take it up spontaneously. It is used to trace pathways or identify cell bodies when fixed sections of tissue are incubated with appropriate substrate. HRP is also retained in the descendants of dividing cells and can be used to identify clones.

Fluorescent markers. These are used like HRP but are visualised with ultraviolet microscopy. Other dyes and substances such as cobalt have also been used to trace axons.

Autoradiographic tracing. Radioactive substances are transported along nerves in the same way as HRP, and are visualised by autoradiography of sectioned material. In some cases the labelled material crosses synapses and delineates further pathways (transneuronal autoradiography).

Chimeras. Embryonic neural cells (and their descendants) from one species transplanted to another are subsequently identified in the chimera through interspecies differences in histology of cells, e.g. structure of the nucleolus. Cells transplanted from an animal of the same species can be identified in a chimera if they are prelabelled with an intracellular marker, such as HRP.

Birth-dating of neurons. A pulse of tritiated thymidine is incorporated into the DNA of the dividing precursors of nerve and glial cells and detected in these cells or their descendants by autoradiography.

Physiological methods

Patterns of central connections and changes in these patterns have been assayed by extracellular recording of action potentials from single cells or small clusters of cells, using wire or glass microelectrodes. Intracellular recording has been used to detect development of innervation and the changes in inputs to individual cells in the peripheral and central nervous system. Connections can also be determined by detecting biochemical changes arising as a result of activity in particular axons or pathways. In muscle, the fibres comprising a single motor unit can be identified histochemically if their glycogen levels are depleted by prior activation. Neurons can be identified by a non-metabolisable glucose analogue (2-deoxyglucose) which accumulates in active cells and can be detected autoradiographically.

Biochemical methods

Levels of transmitter and transmitter enzymes in some tissues have been used to determine changes in innervation. Effects of innervation or

denervation on transmitter metabolism and RNA and protein synthesis in the nerve cell bodies have also been monitored in this way.

Experimental design

Armed with technical expertise, what kinds of experiment can the developmental neurobiologists do? There are three basic designs.

1. *Description of events in the normal animal.* The first stage in any study is to observe to the limit of the techniques available what happens in the normal developing animal. Such phenomenological observations give clues to underlying cellular and subcellular mechanisms.

2. *Description of events after experimental manipulations.* A hypothesis about mechanisms acting in the intact animal can be tested by altering conditions in an experimental animal and comparing the observed outcome with that predicted by the hypothesis. Procedures for altering conditions include either removing or translocating nerves or their targets, cutting or crushing axons and allowing them to regenerate, and changing the level of substances (e.g. hormones, transmitters, growth factors) thought to be important for an observed developmental phenomenon.

Observations on development in animals with single gene mutations affecting the central nervous system also belong to this category of experimental design. One of the drawbacks with these mutants is that a mutation in a single gene may affect several kinds of cell and several closely related developmental events, and this makes it difficult to disentangle causes and effects of the observed defects.

3. *Analysis of events in tissues* in vitro. The study of development in organ or tissue culture offers the possibility of testing a hypothesis by altering conditions in ways which are not practical *in vivo*. Conditions in culture are sufficiently different from those in the animal to leave a doubt about the relevance of events seen in culture to those that occur in the animal. Notwithstanding this doubt, the culture approach has been particularly valuable in demonstrating the importance of chemical factors in guiding neuron growth and in keeping neurons alive. It has also been useful in determining some of the mechanisms of synaptogenesis and neuronal differentiation. The chick embryo chorioallantoic membrane is an organ culture environment more closely approximating that of the whole animal, and has been used to study the development of neurons of the peripheral nervous system.

Most publications report the results of work on a single animal species, chosen from considerations of economy, availability and ease of experimentation for the particular developmental phenomenon being studied. Generally the findings should not be assumed to apply more widely until they are confirmed in other species.

PART II

Genesis of nerve cells

Synopsis

In invertebrate species it has been possible to follow the development of specific, identified neurons from earlier precursor cells. These studies have revealed stereotyped patterns (lineages) of cell division and cell differentiation, suggesting that the characteristics (phenotypes) of particular neurons are determined at the time of mitosis of precursor cells by asymmetrical cell division. Some neuronal phenotypes in invertebrates are also determined by the action of local environmental signals on uncommitted precursor cells, a process known as induction. The signals that determine phenotypes in either lineage or inductive specification have not yet been identified.

In vertebrates commitment of embryonic cells to neuronal development has long been thought to begin at the gastrula stage when mesoderm, migrating under the ectoderm, induces it to become neural ectoderm. However, it has been claimed recently that this classical theory of primary neuronal induction is incorrect and that specification of the major subdivisions of the nervous system may begin at the 512-cell blastula stage.

Little is known about the origins of the specific nerve cell phenotypes in the vertebrate central nervous system, but investigations of the origins of the peripheral nervous system have been more successful. Peripheral neurons are derived from the neural crest, a transient aggregate of cells that detaches from the edge of the neural ectoderm at the time of closure of the neural tube. The crest cells migrate out into the periphery, where specific phenotypes are induced by local influences.

Proliferation of nerve cells after neural tube closure occurs only along the inner surface of the neural tube, and later at a few sites on the outer surface of the brain. Post-mitotic neurons migrate radially through adjoining