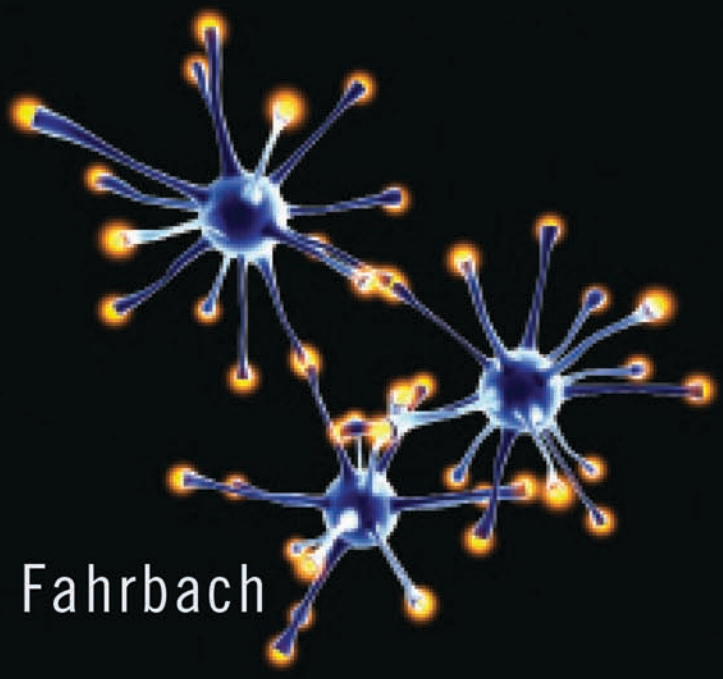
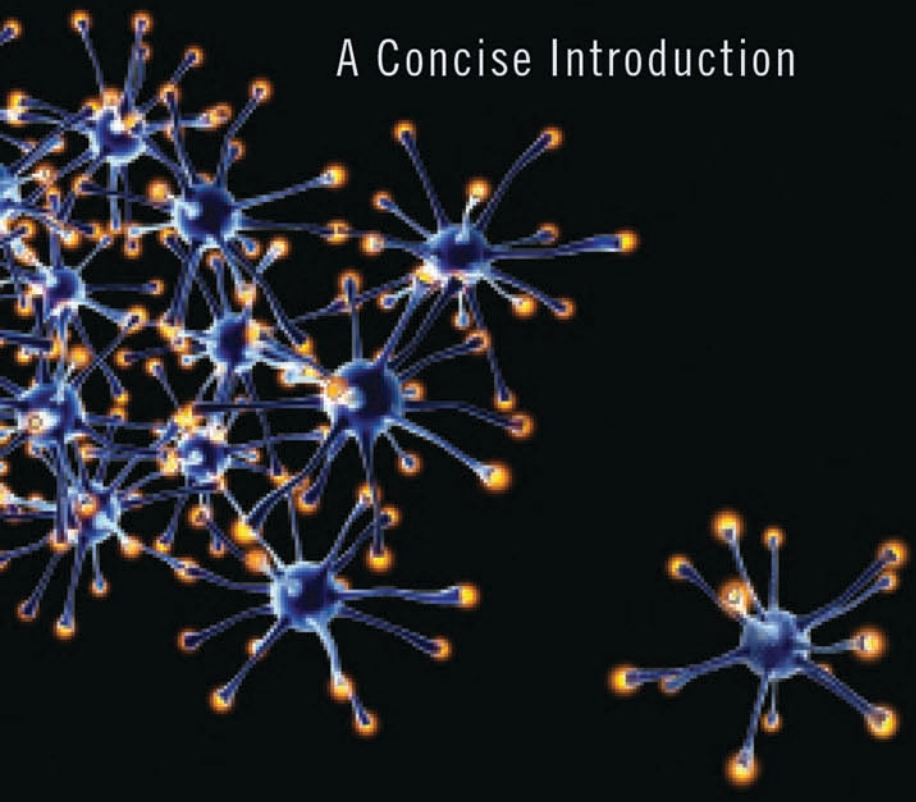


Susan E. Fahrbach

DEVELOPMENTAL NEUROSCIENCE

A Concise Introduction



Developmental Neuroscience

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A CONCISE INTRODUCTION

Susan E. Fahrbach

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To Jon, for reminding me that it's an adventure

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ILLUSTRATIONS

PREFACE

> The best investigators recognize interesting questions that don't fit into a predefined paradigm and follow the biology for its own sake. These curiosity-driven experiments are the ones that lead to truly surprising discoveries. We can expect that studies of seemingly exotic developmental events will continue to provide new perspectives on evolution and human biology.
>
> Anderson and Ingham (2003), 285

It is long-established tradition that beginning biology students learn to identify the four categories of animal tissue: epithelial, connective, muscle, and nervous. The student learns that nervous tissue contains two cell types: neurons and glial cells. The glial cells are then immediately set aside as the focus shifts to the stars of the nervous system, the neurons. It will be noted that neurons are electrically excitable cells that integrate information and transmit it to other cells (most often, to other neurons), primarily by chemical signals; that neurons are unlike other cells in that they possess long, thin extensions of the cytoplasm called axons and dendrites; and that neurons form polarized cell junctions called synapses. Following this reductionist line of thought helps students understand that the study of nervous system development is the story of how newly born cells differentiate a neuronal phenotype: how they come to express voltage-gated ion channels, assemble an extended cytoskeleton, and position synaptic proteins in just the right locations. In the twenty-first century, the story of how cells acquire a neuronal phenotype can be told in terms of molecular signals and the cellular receptors for those signals. An introductory account of these signals fills major parts of the chapters in this book. The molecular story of neuronal differentiation can in fact be told over and over again, with subtle variations and surprising plot twists, because there are so many different types of neurons. It's been estimated that as many as 100 billion neurons make up the human brain, collectively representing thousands, maybe even tens of thousands, of different ways to be a neuron.

But molecular signaling is only part of our story. The ability of the nervous system to integrate environmental cues and internal signals such as hormones with remembered experience to produce thoughts and behavior depends on its wiring diagram. Information flows through the nervous system via polarized neural circuits (by *polarized* I mean simply that there are

distinct input and output sides to the circuit). These sophisticated circuits have built-in feedbacks, delays, and convergences that collectively enable a single circuit to produce multiple outputs. The second part of our story therefore involves understanding how connections within neural circuits are formed and sustained. If the diversity of neuronal phenotypes in the human brain is surprising, the targeting of the estimated 100 trillion connections (synapses) in the brain to form circuits is absolutely astonishing.

The third part of our story is the plasticity of the nervous system. In a sense, the development of the nervous system is a never-ending story. Across the life span, nervous systems respond to internal and external signals by altering neuronal phenotype and refining neural circuits. Familiar examples of neural plasticity are the seasonal behaviors of temperate-zone animals, acquisition of a skill such as playing the violin or a new video game, and formation of long-lasting memories of life events such as our first day of school. Nervous systems also have the capacity to recover from many (but not all) injuries. Does lifelong plasticity reflect reengagement of the mechanisms that supported the formation of the embryonic nervous system? Until we have a fuller understanding of both development and plasticity, this fascinating question is impossible to answer. This book does not avoid topics related to plasticity, but its primary goal is to give the reader a thorough grounding in the earliest stages of development.

While some readers of this book will be interested in learning about the nervous system so that they can better understand brain evolution and animal behavior, others will want this information so that they can be better physicians and educators and, eventually, parents. The latter category of readers may be disappointed that so many chapters focus on species other than humans. These species—some of which play such an important role in studies of development that they are referred to as *model organisms*—have contributed so much to our understanding of development that it would be impossible to write a meaningful book without reference to them. But readers primarily interested in humans can take heart, because advances in our knowledge of the human genome and proteome paired with new techniques of noninvasive brain imaging mean that direct studies of the development of the human nervous system are increasingly informative. For example, studies of teenagers using noninvasive brain imaging have revealed surprising and useful information about brain development during adolescence (Chapter 9).

Some readers of this book may be considering careers in neuroscience research. In the early 1980s, I chose to investigate the changes that occur in insect nervous systems during metamorphosis because I did not foresee the rapidity with which exciting studies of the developing mammalian nervous system would become possible. I love insects and have never been unhappy with my choice, but students interested in research on development of the nervous system can now choose from a longer menu of enticing options.

Many of these new models and areas of research are described in this book. Is it a good time to choose a career in neuroscience research? I think that most neuroscientists would agree with me when I say that the answer to this question is always *yes*.

It is my hope that this introductory account of nervous system development inspires all readers, but it is particularly dedicated to undergraduates encountering the subject of development for the first time. I assume that most such readers will have completed an introductory biology course (or courses) covering the basics of physiology, cell biology, genetics, and molecular biology. Students ready for more information can consult the notes and source lists for each chapter. Many of the references cited are review articles. A well-written review is often fun to read because it provides a concise summary of an interesting topic, but the savvy student appreciates that every such article is also a database. The opinions expressed in a review eventually become dated, but the curated list of references at the end of the article is timeless. In other words, use review articles (the secondary literature) as your portal to the primary literature (research reports published as journal articles).

By the way, my surname is easier to pronounce than to spell: just say *far*-bock, and you've got it.

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Thanks to my teachers, mentors, and colleagues, especially (in order of acquaintance) Paul Rozin, Jane Mellanby, Joan Morrell, Donald Pfaff, Toni Wolinsky, Robert Meisel, Jim Truman, Karen Mesce, Stanley Friedman, Hugh Robertson, Gene Robinson, Sarah Farris, Rodrigo Velarde, and Scott Dobrin. Thanks to Bill Greenough for introducing me to Illinois and fragile X syndrome and to Linda Restifo for her insights into intellectual disability. Thanks to the Z. Smith Reynolds Library of Wake Forest University for easy access to the resources needed to complete this book. Thanks to Jonathan Christman, Maurice Robinson, Nathaniel Robinson, and Kenneth Fahrbach for encouragement, never overdone but always supplied when needed. Thanks to Maggie Christman for the daiquiris by the pool.

WHAT ARE INVESTIGATIVE READING QUESTIONS?

Investigative reading questions are found at the end of each chapter. These questions offer students the chance to test their understanding by thinking about experiments based on material presented in the chapter. The most difficult of these questions are highlighted as *Challenge Questions*. Answers can be checked by carefully reading the recommended article that is provided, although you should be open to the possibility that you will come up with a better answer than the original investigators did. Full citations for each article are given at the end of the book. All of the articles used as the basis of investigative reading questions can be accessed free of charge by anyone with an Internet connection, regardless of institutional affiliation. Some of the articles are accessible because the journal publishers (in particular, scientific societies) make their archives freely available. Many articles are archived in PubMed Central (PMC), a free, full-text online library of over 2 million biomedical and life sciences articles maintained by the U.S. National Institutes of Health's National Library of Medicine (http://www.ncbi.nlm.nih.gov/pmc/). All research supported by the National Institutes of Health, the major public funder of research in the United States, is required to be made available to the public via PMC no later than 12 months after initial publication. Other articles that serve as the basis for investigative reading questions were originally published by choice of the authors in an Open Access format. That is, some publishers routinely restrict access to newly published material to subscription holders but give authors the option of paying an Open Access fee to make their articles immediately available to all.

TEACHING USING THE PRIMARY LITERATURE AND INVESTIGATIVE READING QUESTIONS TO COMPLEMENT THE TEXT

Many neuroscience instructors introduce primary literature into their undergraduate classes, but the fact that this practice is common does not mean that it is easy for either instructor or student. My own teaching of the primary literature has been heavily influenced by the C.R.E.A.T.E. method developed by Sally Hoskins and colleagues. C.R.E.A.T.E. stands for **C**onsider, **R**ead, **E**lucidate the hypotheses, **A**nalyze and interpret the data, and **T**hink of the next **E**xperiment. This method has been described in several journal articles, and useful sample teaching modules are available on the C.R.E.A.T.E. Web site (www.teachcreate.org). Another effective approach modifies the familiar journal club format to teach undergraduates a systematic method for reading primary literature. A method to accomplish this, described by Katherine Robertson (2012), can be incorporated into existing courses and takes about four class sessions to complete.

Teaching References

Hoskins, Sally G. 2008. "Using a paradigm shift to teach neurobiology and the nature of science—a C.R.E.A.T.E.–based approach." *Journal of Undergraduate Neuroscience Education* 6: A40–52.

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Developmental Neuroscience

ONE

Introduction

What Do We Mean When We Say "Neural Development"?

Development unfolds smoothly over time but can be divided for experimental analysis into successive stages, each with its own defining events. Some of these events have clear beginnings and endings, although others may be protracted, sometimes unexpectedly so. For example, myelination of axons in the human brain, a key event that supports behavioral development by increasing the rate of action potential transmission, begins approximately 24 weeks after conception and then continues for decades. In general, the earliest events are most easily categorized as discrete stages shared by almost all members of a species, whereas later events are best described as ongoing processes, the exact details of which are unique to each brain. This is particularly true in long-lived species such as humans, but the inherent ability of nervous systems to refine neural circuitry across the life span is evident even in short-lived invertebrates. The neuroscientist Martin Heisenberg and colleagues were reflecting on data obtained from neuroanatomical studies of fruit fly brains, not human brains, when they were inspired to write, "An individual's life experience can . . . be encoded in the volume of selected neuropil regions."[1]

What Is in This Book and How to Use It

After a brief presentation of methods (this chapter), an overview of human development (Chapter 2), and an introduction to animal models (Chapter 3), the subsequent chapters consider the molecular mechanisms of selected earlier and later events (Chapters 4 and 6), neurogenesis (Chapter 5), and formation of synapses (Chapter 7). Glial cells are the focus of Chapter 8. Chapter 9 describes the postembryonic maturation of the nervous system via metamorphosis in some species and adolescence in others. In Chapter 10 the focus shifts to human intellectual disabilities. This chapter attempts to build a case that at least some forms of human intellectual disability reflect reversible differences in developmental processes rather than permanent

deficits. This chapter was inspired by my personal connections with two outstanding neuroscientists—William T. Greenough at the University of Illinois at Urbana-Champaign and Linda L. Restifo of the University of Arizona, a researcher who is also a physician. Many other outstanding investigators work in this field, but it was Greenough's studies of the fragile X protein in the context of his life's work on experience-driven brain plasticity and Restifo's studies of mental retardation genes in *Drosophila* that forced me to rethink my views on human intellectual disability.

Each chapter has notes. Some provide additional background information on the topic being discussed. This information may be useful and/or interesting, but it has been placed in the notes because I believe it is not essential for an understanding of neural development. The notes are probably most helpful if they are consulted the first time you read the chapter. Some notes link specific results recounted in the text to specific references. The full references can be found in the chapter-by-chapter reference lists that appear at the end of the book. These reference lists also include pertinent reviews and commentaries that provide additional context if you are interested in the history of developmental neuroscience. Short chapter-by-chapter lists of trustworthy online resources can also be found at the end of the book. These are intended to provide additional graphic material and technical details as well as links to selected patient information web sites. This material is also nonessential. It is included to allow you to follow up a specific interest, either as you read or in the future.

Students who want to go further will find that there are numerous points of entry into the research literature. The student can begin with the end-of-chapter suggestions for *Investigative Reading*. Each of these readings is introduced by a short question based on the chapter. The answer to the question (one answer; the student may well come up with a superior alternative) is contained in the recommended reading. Students are encouraged to try to answer the questions on their own before going online to retrieve the article. Note that only partial citations are provided at the end of each chapter. This is because the titles of the articles often give the answers away! The journal articles listed in the Investigative Reading sections are freely accessible online, and full citations are provided at the end of the book.

This text is designed to provide a concise introduction to nervous system development. This goal will be achieved, in part, by a nearly exclusive focus on the central nervous system (the brain and spinal cord in vertebrates, the brain and nerve cord in invertebrates). We'll venture into the peripheral nervous system primarily in Chapter 7, where I use the neuromuscular junction to describe how synapses form. Topics intentionally shortchanged for the sake of brevity include the history of embryology, the neural crest, development of vertebrate sense organs, and the emerging story of microRNAs (miRNAs) as posttranscriptional regulators of development.[2] In addition,

many of the signal transduction pathways described in this book have been pruned for clarity. Even in the garden, pruning is a tricky business. I apologize in advance if I inadvertently clipped your favorite branches.

Methods for Studying Development of the Nervous System

The modern neuroscientist's tool kit is stocked with powerful tools for studying the structure and function of the nervous system. While the tools of the electrophysiologist (intra- and extracellular recordings of neuronal electrical activity) and the neuroanatomist (many variants of microscopy) are still in heavy use, many developmental neuroscientists routinely incorporate measures of gene expression and functional brain imaging into their studies. Others just as routinely use genetically engineered (transgenic) animals.

The following sections introduce key techniques used to study nervous system development. Some researchers specialize in a particular technique, but many investigators work in teams and combine multiple approaches to answer research questions. Students who delve into the primary research literature are often amazed at the number of techniques required to generate the data contained in a single paper. This is one of the reasons that many modern research papers feature lengthy author lists.

Birthdating

All cells, including neurons, are produced by division of other cells. The time at which the cell division occurs that produces a particular neuron is referred to as that neuron's birthdate. Knowing neuronal birthdates is important for understanding the sequence of events that builds a neural circuit or a brain. Birthdating is also important for exploring the capacity of mature brains to add new neurons. Neurons themselves do not divide—part of becoming a neuron involves saying farewell to the cell cycle—so the challenge to the developmental neuroscientist wishing to determine a birthdate is to catch the neuron in the act of being produced by a progenitor cell that by definition is not itself a neuron.

If an animal is small and transparent, the process of cell division can be observed directly using a microscope. Otherwise, developing tissues may be fixed (preserved by chemical treatment), sectioned into thin slices (section thickness is typically measured in micrometers, μm), and attached to glass slides for viewing with a microscope. Stains may be applied to the sections to enhance detection of dividing cells. A combination of hematoxylin and eosin reveals key features of many tissues, including nervous tissue, because hematoxylin stains nuclei blue and eosin stains most other structures red or pink. DNA stains aid the identification of mitotic profiles by making condensed metaphase chromosomes readily visible. The Feulgen stain is tradi-

tionally used to mark DNA for viewing with a standard bright-field microscope. Modern biologists with access to a fluorescence microscope can choose from an array of colorful dyes that bind to DNA.

A drawback to searching for mitotic profiles in tissue is that the window for detecting these profiles is often so brief that the likelihood of catching a neuron in the act of being born is small. An alternative approach also relies on detection of DNA, but instead of staining all of the nuclear DNA present in a tissue, the investigator labels only new DNA. This is accomplished by providing special DNA precursors to cells as they copy their nuclear DNA prior to cell division. These precursors do not occur naturally in cells. Because the precursor provided is incorporated into new DNA, any neurons born during the time the precursor was present contain labeled DNA and can therefore be distinguished from cells born when the precursor was not present.

In classic studies, living animals were injected with the nucleoside thymidine linked to a radioactive atom (a nucleoside is a purine or pyrimidine base attached to a ribose sugar molecule; a radioisotope commonly used to label nucleosides is tritium, a radioactive isotope of hydrogen). The distribution of radioactivity in a tissue section prepared from the treated animal was subsequently detected by applying the section to a photographic emulsion. The radioactive decay particles emitted from the radioisotope exposed the film. At the end of an exposure period typically measured in months, the location of nuclei with radiolabeled DNA was revealed by developing the emulsion using darkroom chemicals. This method of detecting the distribution of a radioisotope in tissue is known as autoradiography.

Tritiated thymidine (^{3}H-thymidine) was used in neuronal birthdating studies through the 1970s. Its use has been superseded by a method based on detection of bromodeoxyuridine, a synthetic nucleoside that is an analog of thymidine. Antibodies can be purchased that bind specifically to bromodeoxyuridine. Labels attached to these antibodies make the position of bromodeoxyuridine within a tissue section readily evident using standard techniques of light microscopy (fig. 1.1). Bromodeoxyuridine is commonly referred to by its nickname, BrdU, pronounced bee-are-dee-you. Oval spots representing BrdU-labeled nuclei flash before the mind's eye of a neuroscientist who hears the term *neuronal birthdating*.

Birthdating methods that rely on incorporated nucleosides work only when the investigator can introduce the marker at the appropriate stage without perturbing normal development. Depending on the species, this may be accomplished by injecting or feeding or by immersing the entire animal in a solution containing BrdU. An alternative approach relies on immunodetection of endogenous molecules expressed by dividing cells. This circumvents the need to introduce a marker. Antibodies are available that recognize proteins expressed during the cell cycle. These include antibodies that bind to proliferating cell nuclear antigen (PCNA) and a nuclear protein

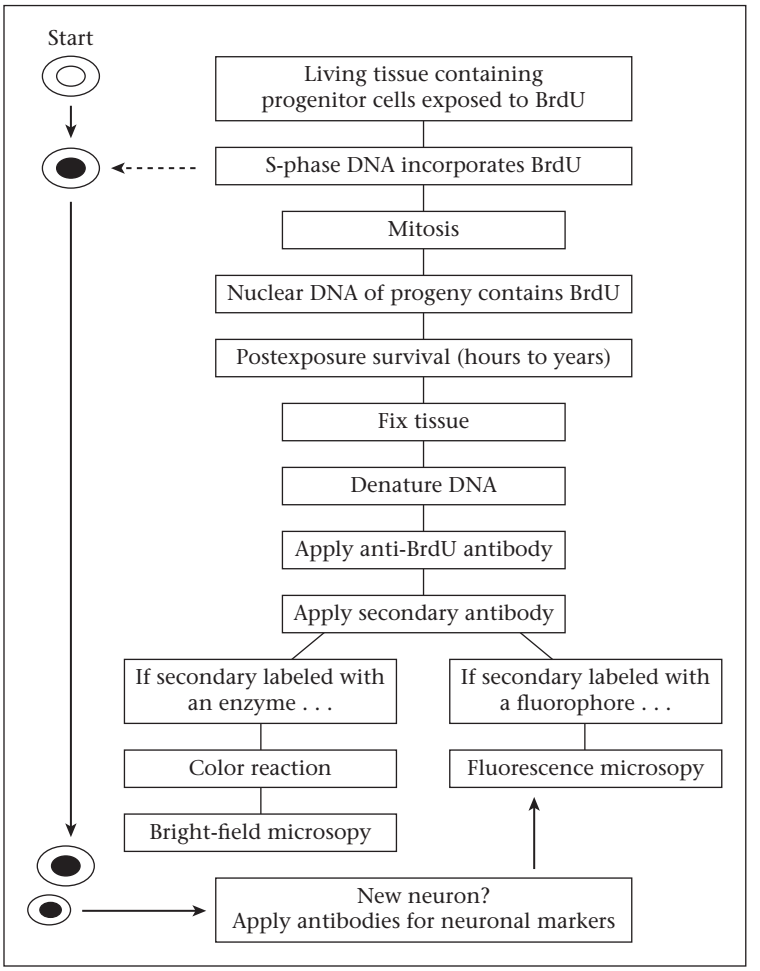

Figure 1.1. Determining neuronal birthdates by immunodetection of BrdU. DNA synthesized in the presence of the synthetic nucleoside BrdU can be detected in progenitor cells and their progeny. In the event depicted here on the left, a stem cell has completed a round of mitosis that regenerated the stem cell (the larger cell at the lower left) and produced a neural progenitor cell (the smaller cell at the lower left). An example of such an event is the production of a ganglion mother cell by a neuroblast in the developing ventral nerve cord of the fruit fly *Drosophila melanogaster* (Chapter 5). If the postexposure survival had been longer, three labeled nuclei would have been detected: the neuroblast and the two neuronal progeny of the ganglion mother cell.

called Ki-67. These proteins are not expressed by mature, postmitotic neurons, but they are good markers for progenitor cells and for newborn neurons, as they persist for several hours after mitosis before being metabolized.

One disadvantage of relying on the immunolabeling of endogenous proteins as markers for mitosis is that the antibodies used in these studies may not recognize proteins from a broad range of species. For example, antibod-