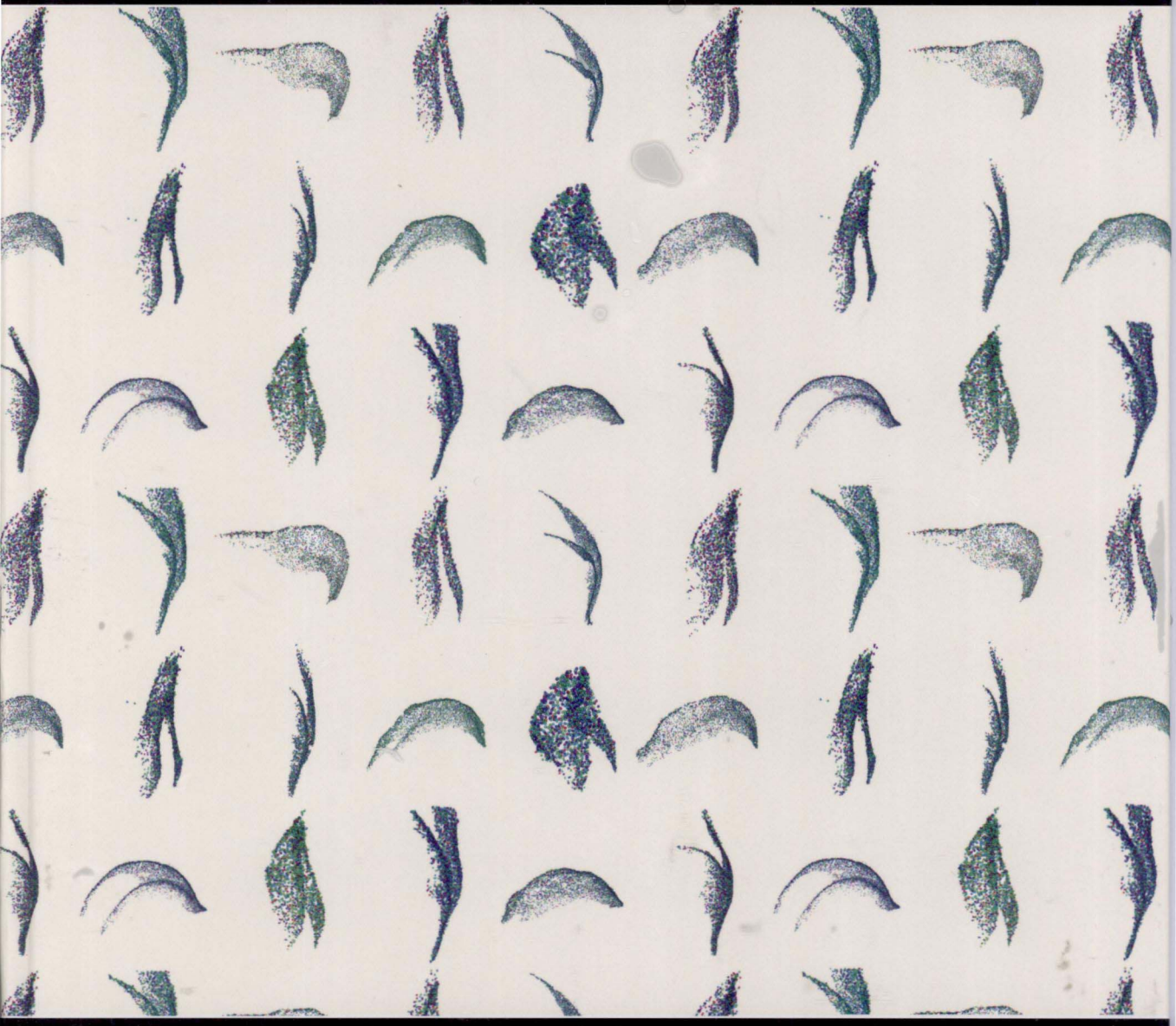
COLD SPRING HARBOR PERSPECTIVES IN BIOLOGY

Neurogenesis



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A subject collection from *Cold Spring Harbor Perspectives in Biology*

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Front cover artwork: Just as we have seen displays in glass-covered boxes in natural history museums, a collection of dentate gyri is here arranged for display. Each representation of a dentate gyrus is a three-dimensional cloud built up from thousands of single points. These points indicate the exact position where a neural precursor cell has been identified previously in a microscopic image. Novel microscopic techniques allow image acquisition of entire anatomical regions on one end, while still assuring resolution beyond the level of a single cell on the other end. The image represents how a collective of thousands of cells create a defined anatomical region. Still, every dentate gyrus is unique and is never identical to another. (Image created by Steffen Vogler, DZNE Dresden.)

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Preface

THE IDEA FOR THE FIRST EDITION of this book had originated from a symposium held at the Banbury Conference Center at Cold Spring Harbor Laboratory in February 2006. In the secluded and intimate setting of this event, the organizers sought to assemble an overview of the field as it stood at the time. The likely impermanence of this contribution did not deter us because it seemed necessary to bring together a number of leading researchers to make an attempt to define our growing field. The great success of the conference made it clear that the conclusions from the meeting should be disseminated to a wider audience in the form of a book. This decision also allowed us to expand the range of topics beyond those covered in the meeting and recruit more colleagues who had made important contributions to the field.

Since then, it has turned out that our attempt was well received and that the resulting book became a popular resource for students, researchers, clinicians, and just anybody who is interested in a one-stop introduction to the field of adult neurogenesis. But time has not stood still and our field has progressed and changed considerably over the past few years.

The new version of the book is not simply a second edition although all chapters have been updated. Several new chapters have been added. Others have been restructured or completely rewritten. A few items were dropped as perspectives have changed over the years. And we have also brought in new experts and topics, because new issues have become central to the field.

The 31 chapters in this volume provide a still incomplete yet hopefully valuable overview of the field of adult neurogenesis research at the current time. As before, we teamed up authors on related topics who had either not yet worked together or had done so in the past but are not currently working together. Our aim was to help integrate the field by mapping its current scope and its diverging ideas, and we hope our selections do not reflect too much of our personal opinions and biases.

We would like to thank the editorial staff at Cold Spring Harbor Press for their advice and diligence, particularly Barbara Acosta. We would also like to thank the authors of all the chapters in the monograph for their thoughtful and scholarly presentations of often controversial and still emerging concepts surrounding this new field of adult neurogenesis.

FRED H. GAGE
GERD KEMPERMANN
HONGJUN SONG

Contents

COMPARATIVE NEUROGENESIS AND EVOLUTIONARY ASPECTS

Neurogenesis: A Prologue

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New ideas pass through a series of stages, from initial rejection to skepticism, to reluctant acceptance (without true belief in its importance), to a final casual acknowledgment of the obvious. It is fair to say that the acceptance of the idea that new neurons are generated in the adult brain of all mammals has been a slow process and, along the way, the idea has been met with skepticism and resistance. And although adult neurogenesis has entered popular press, is still not yet casually accepted as obvious in many scientific contexts. Rather, adult neurogenesis remains, for some, in the stage of reluctant acceptance, without a full understanding of its importance, but the search for its function and its fundamental implications is in full gear. On the other hand, somewhat ironically, we face the danger of a new dogma, implying that new neurons can be used to explain anything that remains mysterious about the brain. Our brains still regenerate as poorly as before adult neurogenesis was discovered. The big step lies in appreciating a previously underestimated mechanism of plasticity—with all its potentials, but also with its limitations.

Joseph Altman's original observations in the 1960s were met with curiosity, but ultimately also with significant reservation, as were attempted confirmations by a handful of investigators in the following 20 years. Somehow, Fernando Nottebohm and Steve Goldman's observation of neurogenesis in the brains of adult canaries was received more positively but—because it took place in birds—was not considered as much of a threat to the prevailing belief (often even termed "dogma") that there are no new neurons in the adult mammalian brain.

Why this resistance to the capacity of the adult brain to generate new neurons? It was well accepted that other systems, like blood, liver, and skin, could generate new cells, so why not the brain? The most straightforward explanation is that the brain is not just any organ. At a philosophical and metaphysical level, the brain is thought to be the place where the very essence of the individual resides. If neurons were being added to this structure, the reasoning might have gone, the consequence would be an instability of this "essence." Who we are would change with time and development. Furthermore, one of the structures where neurogenesis persists is the hippocampus, a structure involved in learning and memory: Would not new neurons that are being added to the structure destabilize memories or their recall? In the 1960s–1980s, much of conceptual modeling of brain function used computers as an analogy, with their complex wiring, feedforward/feedback mechanisms, and oscillations. The idea of structural plasticity provided more of a problem

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to these types of models rather than a resolution. On top of all these forces working against the acceptance of neurogenesis, there was the more practical problem that neurons are big and complex with thousands of connections. Furthermore, neurons are "postmitotic" (a judgment that still holds). How could they divide to make a new neuron? Where could the new cells come from if not from a dividing neuron? The idea of stem cells in the brain, to say nothing of their proven existence throughout adulthood, was not even on the horizon. But the problem of dividing neurons was solved between 1992 and 1995, when it was discovered that the subventricular zone (SVZ) of the lateral ventricles and the subgranular zone (SGZ) of the hippocampal dentate gyrus contained self-renewing stem cells from which the newly born neurons were generated. Therefore, although the computer:brain analogy is still dominant (albeit very problematic), the complexity of incorporating neurogenesis into the model is now less difficult than originally considered, because neurogenesis only occurs in two regions of the brain, and the hippocampus (dentate gyrus) is thought to be involved more in forming new memories than in storing them. Therefore, updating the "software" by adding new neurons can fit into the computer model, for those who are so inclined.

The field has grown dramatically during the last 20 years. The growth has been further amplified by evidence of negative and positive regulation of adult neurogenesis through experience and activity. More and more species, including humans, were added to the list of mammals showing adult neurogenesis, with fundamentally similar anatomical features. Links to pathology have been made for several diseases, and the cellular and molecular mechanisms underlying the control of adult neurogenesis are being revealed. Intriguingly, although these mechanisms are similar to those seen in neuronal development during the embryonic and fetal periods, "adult neurogenesis" is also quite distinct from earlier development. It is precisely the interaction with the environment and activity, be it in the physiological or pathological context, which sets adult neurogenesis apart from intrauterine and early postnatal brain development. Furthermore, surprising novel discoveries continue to be made.

This starts to have far-reaching implications to neurobiology, medicine, and scientific as well as popular concepts of the brain and its plasticity. Can one, for example, still maintain that the function of the hippocampus, and hence learning and memory, be fully appreciated without integrating knowledge about adult hippocampal neurogenesis? Can plasticity throughout the brain, be it in health or disease, ever be understood without considering the contribution of stem cells? How must we perceive brain development if we now learn that it actually never ends?

Today, researchers entering the field are faced with an overwhelming number of publications. At the time of writing this prologue, a simple search in PubMed using the key words "adult neurogenesis" generated more than 2047 entries, and the search with the same key words without quotes generated more than 6780 entries, as opposed to roughly 2000 in 2008, when the first version of this book appeared. The list of factors and issues that are brought into connection with "new neurons in the adult brain" continues to increase. But as so often is the case in emerging fields, increasing knowledge is accompanied by less, rather than more, insight. Apparent contradictions remain and speculative beliefs stand beside solidly grounded facts. We have much work to do. But there has been much progress in our field and the past 6 years has provided not only more factual insight but has also started to change our concepts. We now stand at the verge of comprehensive theories about new neurons in the adult brain, where they are coming from, how their development is regulated, and how their function fits into our current understanding of hippocampal and brain function.

Reading through the chapters in this volume, four guiding principles emerge with regard to adult neurogenesis:

1. Adult neurogenesis is neuronal development under the conditions of the adult brain. It is, therefore, a process, not an event. And it is an

exceptional process that, by and large, appears to be limited to only two "canonical" neurogenic regions: the SGZ of the adult hippocampus and the SVZ of the lateral ventricles.

2. Adult neurogenesis is found throughout the animal kingdom and persists throughout life. In mammals, it does not produce great numbers of neurons after early adulthood. Adult neurogenesis is not a mass phenomenon but appears to make a qualitative rather than quantitative contribution.

3. Although the exact function that adult neurogenesis has in normal behavior is still not yet fully clear, it has become obvious that it has at least one role and might have several and that this function is relevant to our understanding of cognition. Given that adult neurogenesis is the one dynamic process in the adult brain that affects the brain's structure in the most comprehensive way (because entire new neurons are formed), understanding the functions of adult neurogenesis will likely provide fundamental insights into how brain plasticity is linked to function in general.

4. Although there is good evidence that adult neurogenesis is stimulated by all kinds of pathologies, there is almost no indication that this neurogenesis is regenerative or even restorative. Rather, the new neurons appear to make a contribution to normal brain function, presumably in the sense of structural plasticity.

The first section of this volume begins with Methods for the Analysis of Neurogenesis. Three chapters lay out the methodological foundations of research on adult neurogenesis and establish the crucial link to neuronal stem cell biology. The different methods that are used to identify newborn neurons are presented and discussed, and their benefits and limitations are highlighted. Because the most commonly used labeling technique is based on the lasting incorporation of thymidine analog bromodeoxyuridine (BrdU), the merits and pitfalls of this method receive particular attention. Peterson et al. cover the issues related to cell cycle, markers, and fate detection, discussing in detail the BrdU techniques that rank around this method, particularly with respect to microscopy. The important link to neural stem cells and neurogenesis in vitro is made by Azari and Reynolds. We acknowledge that neuronal precursor cell biology includes many aspects that are relevant to adult neurogenesis, which cannot be covered here at an appropriate length. We decided to consider "neurogenesis" to stand primarily for the in vivo process. Nevertheless, adult neurogenesis cannot be understood without reference to precursor cell biology. In some sense, adult neurogenesis is an example of applied precursor cell biology. And for more biotechnologically inclined researchers, adult neurogenesis might even serve as a good model system from which to learn how to "make" neurons from neuronal precursor cells.

Beyond BrdU, novel genetic tools based on reporter genes have been developed. Ge et al. discuss the possibilities of using transgenic reporter gene mice in full detail.

Four chapters in the second section (Cellular Mechanisms) cover the phenomenology of adult neurogenesis: in the dentate gyrus of the hippocampus by Gage et al. and in the SVZ and olfactory bulb by Lim and Alvarez-Buylla, Bonaguidi et al. cover the heterogeneity of neural stem cells throughout the brain, and Bonfanti et al. take us to the so-called noncanonical, often controversial sites of neurogenesis throughout the adult mammalian brain.

In the third section (Molecular Mechanisms), ten chapters cover a variety of themes: the relationship between embryonic and adult neurogenesis by Götz et al., the transcriptional regulation of adult neurogenesis by Lie et al., the role of short-range acting molecules by Mira et al., the balance of trophic support and cell death by Kuhn, the issues around functional maturation and integration by Toni and Schinder, genetic and epigenetic considerations by Hsieh and Zhao, and ideas on the engineering of adult neurogenesis by Berninger and Jessberger. The last three chapters of this section cover an actual net regulation of adult neurogenesis by systemic factors, including those elic-

ited by stress (Korosi et al.), activity dependency and aging (Kempermann), and mechanisms dependent on neuronal circuitry (Song et al.).

Three chapters in the fourth section (Functional Significance) attempt to summarize the current and growing knowledge of the functional significance of adult neurogenesis: Lledo and Valley focus on the olfactory system and Abrous and Wojtowicz focus on hippocampal learning. Aimone adds to this section by reviewing the current computational models of adult neurogenesis.

Neurological and Psychiatric Diseases, the fifth section, addresses the possible role of adult neurogenesis in disease. Hen et al. focus on depression anxiety and antidepressant action, whereas Winner and Winkler review the current evidence of an involvement of neurogenesis in neurodegenerative diseases. Ming et al. consider adult neurogenesis in the context of psychiatric disorders. Jessberger and Parent review neurogenesis and epilepsy and Lindvall and Kokaia cover the effects of stroke on adult neurogenesis.

Finally, the section on Comparative Neurogenesis and Evolutionary Aspects provides reviews of the comparative nature of adult neurogenesis: Ganz and Brand review neurogenesis in teleost fish, and Brenowitz and Larson focus on birds. Frisén et al. review the current information on neurogenesis in the adult human brain, including the striatum, and Amrein contrasts the differences in adult neurogenesis seen in a variety of feral and domestic species. Kempermann attempts a synthesis from an evolutionary perspective.

With these 31 chapters, we have again attempted to take another snapshot of the current field of adult neurogenesis, realizing that some areas are covered not completely or really not at all. For example, adult neurogenesis in insects and invertebrates is not included. Furthermore, because this is such a fast-moving field and new data are being published even as we rush this volume to print, we are already inevitably behind in our reviews. As with some of the other monographs in the Cold Spring Harbor Laboratory series, this volume on neurogenesis will likely be updated in the coming years, and perhaps additional meetings at the Banbury Center, Keystone, or other stimulating places throughout the world will catalyze a greater synthesis in the future.

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Detection and Phenotypic Characterization of Adult Neurogenesis

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Studies of adult neurogenesis have greatly expanded in the last decade, largely as a result of improved tools for detecting and quantifying neurogenesis. In this review, we summarize and critically evaluate detection methods for neurogenesis in mammalian and human brain tissue. Besides thymidine analog labeling, cell-cycle markers are discussed, as well as cell stage and lineage commitment markers. Use of these histological tools is critically evaluated in terms of their strengths and limitations, as well as possible artifacts. Finally, we discuss the method of radiocarbon dating for determining cell and tissue turnover in humans.

Detection of neurogenesis in vivo requires the ability to image at a cellular resolution, which currently precludes noninvasive imaging approaches, such as magnetic resonance imaging (MRI). In vivo microscopy, using deeply penetrating UV illumination with multiphoton microscopy, or by the recently available endoscopic confocal microscopy, may provide new opportunities for longitudinal studies of neurogenesis in the living animal with single-cell resolution. These newer microscopy approaches are particularly compelling when coupled with transgenic mice expressing phenotype-specific fluorescent reporter genes. Additionally, an advanced method using ^{14}C carbon dating of postmortem DNA from specific cell populations of the brain revealed insights into adult human neurogenesis. Nevertheless, at present, the predominant approach for studying neurogenesis relies on traditional histological methods of fixation, production of tissue sections, staining, and microscopic analysis.

This review discusses methodological considerations for detection of neurogenesis in the adult brain according to our current state of knowledge. This will include the use of exogenous or endogenous markers of cell cycle, as well as phenotype markers that contribute to resolving stages of neuronal lineage commitment. The accurate analysis of cell phenotype will be discussed, including suggestions for accurate detection and reliable quantification of cell numbers. Finally, we will present the newly developed ^{14}C carbon dating of nuclear DNA for quantitative analysis of neurogenesis in human tissue.

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CELL-CYCLE PROGRESSION

Thymidine Analogs as Exogenous Markers of DNA Replication

The ability to label a cohort of dividing cells has been useful in verifying the existence of adult neurogenesis and in monitoring changes in neurogenesis under different conditions. The original approach was to use autoradiography to detect incorporation of ^{3}H-thymidine into the nuclear DNA during the S phase of the cell cycle. However, this detection was ambiguous, as ^{3}H-thymidine-induced silver grain deposits and immunoperoxidase labels of phenotypic markers were in different focal planes. The use of the thymidine analog bromodeoxyuridine (BrdU) allowed researchers to overcome this problem as its presence in the nucleus was detected by immunohistochemistry rather than autoradiography. This permitted single labeling or multiple labeling with phenotypic markers detected by brightfield or fluorescence microscopy, and use of thicker sections suitable for design-based stereological quantification of BrdU-immunoreactive cells. Thus, administration of thymidine analogs, rather than thymidine, is now used in most in vivo neurogenesis studies. A summary of study design considerations is presented in Table 1.

When given as a pulse and examined soon after administration (within hours), BrdU labeling indicates that the cell has entered S phase. However, when given repeatedly or examined long after administration, BrdU labeling detection of progression into the G_2 phase of cell cycle or the subsequent reentry into additional cell cycles cannot be distinguished (Fig. 1B). To achieve this degree of temporal discrimination, the examination of endogenous cell-cycle proteins is recommended as discussed below. Commonly, a combination of BrdU and endogenous cell-cycle markers has been adopted to calculate cell-cycle kinetics. However, the question of cell-cycle reentry can also be addressed by the use of sequential administration of multiple halogenated thymidine analogs. Replacement of the bromo group with iodo or chloro groups results in thymidine analogs that behave similarly to BrdU, but whose incorporation can be discriminated immunohistochemically (Vega and Peterson 2005). Thus, by timing the delivery of each analog, it is possible to address questions of cell-cycle kinetics, particularly, the frequency to reenter cell cycle in a given cell population (Thomas et al. 2007; see also Fig. 3) or to label separate populations of cells that divide at different times. However, it is critical for the administration of the halogenated thymidine analogs to be given at an equimolar ratio for valid comparisons to be made (Vega and Peterson 2005).

Possible Artifacts to Thymidine Analog Incorporation

Thymidine analog antibodies have been generated from a variety of species as monoclonal and polyclonal antibodies. Binding of the antibody to labeled DNA requires denaturation of the DNA to remove histones, reduce tertiary DNA structures, or even generate single-stranded DNA. Denaturation is usually performed by exposing cells or tissue to hydrochloric acid, heat, enzymatic digestion, or a combination, depending on the specific antibody and tissue (Kass et al. 2000). Some suppliers mention in their antibody specifications a possible cross-reactivity with methylated DNA that could lead to unspecific labeling of all nuclei and, therefore, recommend specific pretreatment.

Several conditions can create false-positive signals, producing sporadic reports of neurogenesis in novel regions or in response to injury, which show the need for careful controls in tissue preparation (Grassi Zucconi and Giuditta 2002; Rakic 2002; Kuan et al. 2004). The specificity of primary antibodies to BrdU-labeled DNA should be determined for each tissue through the use of a 2-h BrdU pulse-chase interval for labeling with the results compared with coexpression with endogenous cell-cycle markers for confirmation (Fig. 1). Through these tests, widespread unspecific labeling could be unmasked.

Incorporation of thymidine analogs also can occur during DNA repair, leading to concerns that some observations of cell proliferation could be a result of this modest incorporation rather than transition through S phase. Clearly,