CONCEPTS AND CHALLENGES IN RETINAL BIOLOGY

EDITED BY

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Preface

In late August 2000 a Festschrift was held at the Marine Biological Laboratory, Woods Hole, Massachusetts, to celebrate the career of Professor John E. Dowling on the occasion of his 65th birthday. The fact that John's 65th coincided with the dawn of the new millennium made it a particularly propitious time to take stock of the remarkable advances that have been made in retinal biology, many of which we owe to John, his students and his co-workers. In addition, it provided an opportunity to consider the directions one might anticipate retinal research will take in the future. Thus, this special event was notable not only as a celebration of a fine scientist and wonderful human being, but also because it brought together an outstanding group of individuals whose careers were shaped and enhanced through their interactions with John Dowling. The list of about 150 researchers who worked at his side, whether as undergraduate, grad student, post-doc or co-investigator, is not merely impressive, it constitutes the Who's Who of the vision research community, representing institutions in every corner of the globe. All have benefited from John's guiding hand, and his incredible foresight in identifying avenues of investigation where significant advances were to be made. Although there was time to hear from only a limited number of John's many colleagues in attendance, more than 50 of them have contributed chapters to this volume. The appropriateness of the Marine Biological Laboratory as the venue for the meeting is also noteworthy. John has been associated with the MBL for more than 35 years, during which time he has been a member of the Corporation, and a Neurobiology course director. He has served on advisory councils and numerous committees, was a member of the Board of Trustees, and he is currently the President of the MBL Corporation.

The impact of John's research on the field of visual neuroscience has been monumental, and he has championed virtually every aspect of this remarkably diverse discipline. Indeed, there is a striking correlation between the areas of investigation that John pioneered, and those that remain among the most intensively studied to this day. For example, while still a medical student, John was awarded the Soma Weiss prize for his studies on "The biological activity of retinoic acid," a molecule that continues to grow in interest because of its key role in ocular development and intercellular signaling, not to mention its widespread use in dermatology and the cosmetic industry. Thereafter, as a junior investigator in George Wald's laboratory, he described the exchange of retinoids between the visual cells and the retinal pigment epithelium, a vital and seemingly simple process that is still not fully understood. His innovative work on the identification of retinal cell types and their electrical signatures, the structural and functional organization of the retina, and the neuroactive substances that transmit and modulate neuronal signals, provide the foundation upon which we continue to attempt to build a better understanding of the retina and the means by which it analyzes and extracts meaningful information from a complex visual environment. John rightfully

refers to the retina as an "approachable part of the brain," and through his exemplary research has set the stage for the subject of this meeting: "Concepts and Challenges in Retinal Biology."

In his charming little volume *Imagined Worlds*, Freeman Dyson makes mention of a rule formulated by his war-time associate, Reuben Smeed. Smeed's Rule says that "you can either get something done, or get the credit for it, but not both." As we know, there are exceptions to every rule, and John is clearly that exception. He has accomplished much, and he has been showered with the accolades and awards he so richly deserves. Among them are the coveted Friedenwald Award of the Association for Research in Vision and Ophthalmology, the Award of Merit of the Retina Research Foundation, and most recently the Helen Keller Prize for Vision Research. In 1976, about the time of his 40th birthday, John was elected to the National Academy of Sciences, and in 1992 to the American Philosophical Society. In the intervening years, John, who chose to abandon medical school for a career in research, was given the title of honorary Doctor of Medicine by the Faculty of Medicine of the University of Lund, Sweden, and in 1987 he was appointed Maria Moors Cabot Professor of the Natural Sciences at Harvard University.

But honors and awards do not reflect fully the influence John has had on young men and women in every walk of life. During his 18-year tenure as Master of Leverett House, John and his lovely wife Judy, who served with equal responsibility as Co-Master, have helped to guide, nurture and counsel thousands of Harvard undergrads. The organization of this Festschrift and the scientific contributions incorporated in this volume are a tribute to John, and they have afforded his colleagues and former students the opportunity to express their admiration and gratitude.

In addition to providing a memento of the occasion, we hope this book will serve as a fund of basic reference material for future researchers in retinal biology. The volume is divided somewhat arbitrarily into seven areas of retinal research containing chapters that present in some cases a broad overview of a particular topic, and in others an account of current research and studies in progress. Although we have attempted to unify each section in terms of the scientific question being addressed, not all chapters fit neatly into one or another of the selected categories, whereas others bridge more than one field of research. Nevertheless, the chapters that follow exemplify the richness, diversity, and excitement of contemporary retinal research. They also remind us of how much more needs to be done before we understand fully the interrelationship between retinal neurons, the complex interactions between neurons and glial cells, and the mechanisms that govern retinal development.

Cellular Organization and Synaptic Circuitry: This section deals with the organization and synaptology of retinal neurons, and includes the classification of their multiple subtypes, the synaptic connections and neural circuits that underlie signal processing within the retina, and the microcircuitry subserving ON–OFF and directionally-selective pathways. Comparative studies of the structure and function of retinas in diurnal vs. nocturnal animals are presented, and some of the interesting parallels that can be drawn between invertebrates and vertebrates regarding strategies for feedforward and feedback interactions are considered. In addition, the specialized neural chains that allow neuromodulators such as dopamine to influence the retinal message are discussed, and recent studies on gap-junctional channels and the effects of nitric oxide and other agents on junctional communication are described.

Functional Organization: The chapters in this section summarize current views on the mechanisms of synaptic transmission, the formation of discrete functional pathways, and the processes by which visual information is encoded into neural signals. The calcium-dependent chemical synapses that regulate signal transmission from photoreceptors to second-order retinal cells are discussed, and a description is provided of the parallel pathways through which various cell types segregate rod- vs. cone-mediated signals, and sustained versus transient responses. Also included in this section is an account of the responses to complex patterns of light stimuli, suggesting how different sublaminae of the inner retina represent selective aspects of the visual image.

Neurotransmission and Neuromodulation: This section considers the physiological and pharmacological properties of retinal neurotransmitters and their postsynaptic receptors, as well as the uptake mechanisms by which transmitter action is terminated. The effects on neuronal responses of several neurotransmitters and neuromodulators that play key functional roles in signal processing are presented, with emphasis on the three amino acids, glutamate, GABA and glycine, that are used at the vast majority of chemical synapses in the vertebrate retina. The special functions of individual synapses are analyzed in terms of the multiple types of postsynaptic receptors through which these neurotransmitters exert their action.

Photoreceptors, Visual Adaptation and the ERG: The chapters in this section address some of the fundamental issues in photochemistry, describe components of the photoreceptor machinery that regulate light- and dark-adaptation, and describe novel approaches in the use of the electroretinogram for the non-invasive study of retinal disorders. Other chapters consider the ambient light levels that determine the adaptive state of the eye, postreceptoral mechanisms governing contrast sensitivity and network adaptation, and the action of Müller cells in regulating the potassium concentration of the neuronal environment.

Circadian Rhythms: Both structural and functional changes in retinal organization are mediated by circadian oscillators that modulate sensitivity in anticipation of daily changes in ambient illumination. This section deals with the nature of these events in vertebrate and invertebrate retinae, the purported loci from which they originate, and the chemical agents that induce and modulate the periodicity of this behavior. Of considerable interest are the functional consequences of the circadian clock on metabolic activity, and its influence in determining the dominance of pathways mediating rod versus cone signals.

Retinal Development and Genetics: The zebrafish is rapidly becoming the animal model of choice for the study of retinal development and genetically-mediated visual system abnormalities. Several chapters in this section discuss gene regulation of development and differentiation in this teleost, and others describe the techniques of screening for visual abnormalities, the molecular approach being utilized to study the various mutants that are identified, and the cellular mechanisms that have been implicated in disease processes. Development of the vertebrate retina can also be studied in mammals, particularly in rodents and rabbits, and included in this section are recent advances on the roles of neurotransmitter systems and cellular activity in early retinal development, and on the regulation by retinoic acid of ocular development and the expression of important retina-specific proteins.

Retinal Degenerations: The genetic basis of retinal degenerations has long been a topic of intensive study. Chapters in this section describe proteins of the phototransduction cascade that have been associated with various forms of retinitis

pigmentosa, and the use of animal models as well as organ culture for the analysis of defective retinal function. Evidence is presented of rod–cone interaction as a cause of cell death induced by rod-specific mutations, and trophic factors and other agents that inhibit the degenerative process are discussed. Other chapters deal with the merits of studying canine models of photoreceptor degenerations, novel approaches toward understanding the sequence of cell biological reactions that lead to the pathological changes associated with retinal detachment, and the origins of free radical-mediated oxidative stress in the aging eye and its relation to age-related macular degeneration. The final chapter in this section considers a mechanism by which elevated intraocular pressure causes ganglion cell death presumably by overstimulation of the NMDA subtype of glutamate receptor; the resultant rise in intracellular calcium and its effect on mitochondria can lead, in turn, to the production of free radicals and cytotoxicity.

Reflections and Comments: A final chapter contributed by John Dowling provides an overview of past accomplishments, and offers some future perspectives on retinal research in the 21st century.

There are two appendices in this volume. The first documents all graduate students, postdoctoral fellows, visiting scholars, collaborators, staff and undergraduate senior thesis students of John Dowling's laboratory during the past 35 years. The second appendix shows a collection of historical and recent photographs of retinal researchers.

We are saddened by the loss of three members of the Dowling "family" earlier last year, Brian Boycott, Pat Sheppard and Geoff Gold. This book is dedicated to the memory of these three individuals.

Helga Kolb,
Harris Ripps and
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Introduction

An historical perspective by Robert Miller and Harris Ripps

The chapters in this volume deal with a broad array of topics, representing the remarkable number of research areas to which John Dowling contributed his insight and interpretive powers during his highly productive scientific career. The volume also commemorates the single most creative and productive historical period in biological research, attributable in large measure to the strong postwar support for research funded by the federal government and private foundations. In the past 40 years we have achieved an unparalleled level of understanding about the structural and functional organization of the retina, the principles of sensory transduction, and the cellular and subcellular mechanisms mediating neuronal communication. Perhaps more than any other scientist in the latter half of the twentieth century, John Dowling helped to create this new level of knowledge and forge it into our contemporary views about retina and brain function. Because this compilation of papers documents John's influence on our present understanding of retinal biology, it seems appropriate to provide a brief historical survey of the key events in visual science that set the stage for John's entry into the field, and place in context the range of investigative science he promoted.

John was an undergraduate at Harvard when, in 1956, he elected to do a research project in the laboratory of George Wald, an inspirational teacher and superb scientist. This experience ignited his interest in science, and led him to abandon medicine eventually and enter graduate school, where he obtained his Ph.D. with Wald in 1961. During the early Harvard years he had the opportunity to work with Ruth Hubbard and Richard Cone on photochemical mechanisms of visual adaptation, with Richard Sidman on inherited retinal dystrophy in rats, and with Ian Gibbons on retinal electron microscopy. With each of these co-workers he acquired new skills that he would utilize to the fullest in these and related areas of research.

The discoveries made in his very first studies in Wald's laboratory almost immediately propelled John's reputation into a position of leadership in retina research. Although the basic biochemistry of visual pigments was fairly well known by that time, there was no information as to the role these pigments played in determining visual sensitivity during light- and dark-adaptation. One common assumption was that Weber's Law, the relationship between the level of background (ambient) illumination and the magnitude of the light stimulus necessary to reach visual threshold, was simply a function of the reduced amount of available photopigment as the background light intensity increased. On this view, a two-fold rise in threshold occurred when 50% of the visual pigment had been bleached. But John showed that this was patently wrong. By raising rats on a vitamin A deficient diet, and feeding them vitamin A acid (retinoic acid) to maintain them in reasonably good health, John was able to relate retinal sensitivity, using the electroretinogram, to the rhodopsin concentration in the eye.

What John discovered was that pigment concentration was correlated with the *logarithm* of the visual threshold, indicating that the rise in visual threshold is far greater than can be accounted for by the loss of quantal absorption. In other experiments, John discovered that if the rhodopsin content of the normal eye was substantially depleted by a bright adapting light, the recovery of log visual sensitivity followed the regeneration of rhodopsin over its full time course, thereby accounting for the classic dark adaptation curve first reported in humans. On the other hand, if the eye had first been exposed to a weak background light that significantly raised visual threshold but bleached a trivial amount of photopigment, the dark adaptation curve showed a rapid fall in threshold with no detectable change in visual pigment concentration. This phenomenon was termed "neural" adaptation to distinguish it from the "photochemical" adaptation observed following intense light stimulation that bleaches a significant fraction of the visual pigment. The two different components of visual adaptation would continue to interest Dowling and his colleagues throughout his career, and some of his work with Harris Ripps, done in the summers at the MBL, was devoted to the mechanisms of adaptation and their role in retinal function. Today, we understand that at least part of the neural adaptation process occurs in the photoreceptors themselves, but there are clearly post-receptor adaptation mechanisms, some of which are only now beginning to be elucidated.

Although John's initial studies dealt primarily with visual photochemistry, his love of the history of science led him to the monumental work of Ramón y Cajal published more than a century ago. Using the silver staining technique described earlier by Camillo Golgi, Cajal was the first to appreciate that the retina is a true nervous center, and a tissue from which one could learn fundamental concepts of brain organization and function. He identified the major cell types in the retina, formulated ideas about the functional polarization of nerve cells, and appreciated the fact that in the retina one knew in which direction the information was moving, something that you could not intuit when looking, for example, at the Purkinje cells of the cerebellum. Cajal also identified amacrine cells as axonless cell types, but was unable to speculate on their role in retinal processing. In Dowling's early exploration of the ultrastructure of the retina, he identified the numerous dendrodendritic synapses which amacrine cells make, as well as their feedback, feedforward, and serial synaptic connections.

Retinal research from the time of Cajal up to the 1960s focused largely on the biochemistry of visual pigments pioneered by George Wald, the electroretinographic studies of Ragnar Granit, and the single unit recordings from the *Limulus* lateral eye performed by H.K. Hartline. The work of these three vision researchers, who later shared the Nobel Prize, and the innovative psychophysical studies of Selig Hecht on the quantum sensitivity of the retina, and those of W.S. Stiles on light- and dark-adaptation greatly advanced the field of retina research. During this period, Hartline recorded the impulse activity of the single fibers he painstakingly dissected from the frog's optic nerve, Barlow and Kuffler discovered that ON- and OFF-center cells have antagonistic surrounds, and later work by Enroth-Cugell and Robson showed that in the cat retina these can be segregated into X and Y subtypes based on whether the response is sustained (X) or transient (Y). The elucidation of the complexities in the center-surround organization of ganglion cell receptive fields points to a spatial organization that cannot be accounted for by a simple wiring structure of the retina, confirming Cajal's contention that the retina is a processing nerve center and does not treat visual space as would a simple camera.

Many other important studies emerged during the latter part of this period. J.Y. Lettvin and his colleagues at M.I.T. were the first to demonstrate that stimuli which would be of behavioral interest to a frog, such as a moving fly, strongly activated a subset of ganglion cells in the animal's retina. These findings prompted other workers to study receptive field properties using more varied stimuli, including moving targets and differently shaped objects. Indeed, this concept led to studies by Barlow and Levick that first revealed the presence of directionally-selective cells in the rabbit retina, and the approach that Hubel and Weisel would later take in studying receptive fields in the cortex of the cat and primate. It was already well established from earlier anatomical and psychophysical work that the photoreceptor population was species-variant; some animals had rich color vision capabilities, while for others with less well-developed cone systems, color was a less important visual cue. However, the physiological data argued for species differences in retinal processing as well. In animals like the frog, which lack binocular vision, the retina handles a great deal of visual information processing, whereas in primates a comparatively greater proportion of their visual processing machinery resides in the visual cortex. Thus retinal processing in primates consists mainly of mechanisms for contrast enhancement and the segregation of rod–cone pathways; binocularity and more demanding discriminations are dealt with by the complex and hypercomplex cells of the visual cortex.

In 1964, as these new lines of investigation were emerging, John joined the Ophthalmology Department at Johns Hopkins, and it was there that John's research career gathered momentum. He was given spacious laboratory facilities where he could accommodate a number of trainees, and he quickly acquired a national reputation for excellence in research. His easy-going manner, leadership qualities, and productive research program began to attract an outstanding group of students and postdoctoral fellows, among them Gus Aguirre, Dwight Burkhardt, Richard Chappell, Mark Dubin, Robert Frank, Helga Kolb, Robert Miller, George Weinstein, Frank Werblin and Paul Witkovsky.

At about this time John shifted his attention from photoreceptor mechanisms to an analysis of retinal structure and function, and he began a lifelong, fruitful collaboration with Brian Boycott, whose work with the Golgi technique had provided new insights into the organization of the retina at the level of the light microscope. Although the Golgi method enabled one to visualize the fundamental cell types of the retina, their interactions through chemical synapses and gap junctions required ultrastructural identification and classification. This involved the use of high resolution electron microscopy, just emerging as a powerful method for interpreting the structural features and synaptology of the nervous system. The light- and electron-microscopic studies of Dowling and Boycott, and their subsequent work with Helga Kolb, remain to this day among the most definitive accounts of the structural organization of the primate retina. John's move to Hopkins also marked the beginning of a new phase in his research career, which included summer trips to Woods Hole, where he began a fruitful and productive collaboration with Harris Ripps. They produced a significant body of work on the adaptive properties of the all-rod skate retina and forged a working relationship that continues to this day. John also did electrophysiological work on *Limulus* in Woods Hole and produced new findings related to the transduction mechanism of the *Limulus* lateral eye. Interest in the invertebrate visual system would continue to capture his attention, particularly when he worked with Richard Chappell and later Ian Meinertzhagen on the dragonfly eye.

Comparative studies of the retina and the need to account for species differences in the degree of retinal processing continued to be of great interest to John. In collaboration with Max Cowan, he identified by electron microscopy the centrifugal fibers in the pigeon retina and showed that they terminated primarily on amacrine cells, and with his student Mark Dubin, John compared the synaptic neuropil of the frog with that of primates. They concluded that the additional complexity of retinal processing in the frog could be accounted for by the relatively greater ratio of amacrine to bipolar synapses; i.e., the simple retina of the primate had relatively few amacrine cell synapses, whereas the retina of the frog achieved its greater complexity with more amacrine synapses. This idea remains as viable today as when it was first stated, and with the discovery of inhibition in the retina, attributed largely to amacrine cells, this view continues to provide a plausible basis for species differences in retinal processing. However, more recent observations, suggesting that ribbon synapses of bipolar cells have multiple active zones for synaptic vesicle release compared to amacrine synapses, may impact on the ultimate interpretation of this observation.

Our understanding of how the retina processes information lagged far behind our knowledge of the outcome of these interactions (ganglion cell output) for many years. The reasons for this were simple. Among the retinal neurons, only ganglion cells could be reliably studied with extracellular electrophysiological techniques. To study preganglionic neurons, the more difficult technique of intracellular recording was required, and reliable methods for obtaining cell penetration and stable recordings from retinal cells had not yet been achieved. All that was to change in the decade of the 1960s. Svaetichin had earlier produced the first intracellular recordings from fish horizontal cells, and Tomita, using superfine microelectrodes and the ingenious "jolting" technique he had developed, accomplished the feat in the cones of the carp retina. It was now clear that some neurons in the distal retina generate slow, graded potentials, but no one had yet described in a systematic way the response patterns of the full complement of neurons in a vertebrate retina. Recordings from the smaller retinal neurons seemed unattainable until the landmark studies of Werblin and Dowling in the mudpuppy, a beast with a tiny eyeball containing a retina with relatively large neurons. Not only did they record from every cell type in this vertebrate retina, they incorporated within the electrode a visible dye so that the cell from which a recording was made could be filled and later identified microscopically to verify the cell type, its anatomical characteristics, and its location within the retinal laminae.

This achievement typifies John's attitude and approach to science. Rather than focus on the difficulty of a research problem, which can sometimes be paralytic, John's approach has been to focus on the solution, and determine the most likely road to a successful outcome. If the problem seems intractable, John takes the view that it has not been tackled with sufficient intensity, or conceivably, in the right species. So if the cells of the retina are too small, find an animal with large cells, and the retina of *Necturus* seemed ideally suited for this approach. The results reported in two 1969 publications with his graduate student Frank Werblin are widely considered the most defining work in retinal neurocircuitry of the last half of the twentieth century. In these two papers, the basic outline of the functional anatomy and neurophysiology of the retina was established, and a plausible model of the interactions which formed the receptive fields was established. In addition to having recorded from and marked every basic cell type, they identified two types of bipolar cells (hyperpolarizing and depolarizing) which, logically, formed the basis of the ON- and OFF-center responses of ganglion cells; they

demonstrated the presence of an antagonistic surround in the bipolars, implying that their connections with ganglion cells would provide the center-surround organization that Kuffler had described; and they showed the transient spike activity of amacrine cells, most of which responded both to the onset and offset of illumination. Although nothing was known about the neurotransmitters which were utilized by the different cell types, and the distinction between excitation and inhibition was yet to be discovered, the work of Werblin and Dowling provided the single most important advance in our understanding of the structural and functional organization of the vertebrate retina.

A few years after the Werblin and Dowling work, Müller and Dowling, who continued to work on the mudpuppy retina, published their observations suggesting that the b-wave of the ERG was generated by the Müller cells of the retina, the prominent, radial glial cells that are similar to astrocytes. Although this general conclusion has recently been challenged, these early observations elevated the Müller cells to a research status equivalent to that of the neurons and served to stimulate a great deal of fruitful insights into the many roles which we now understand the Müller cells to play in retinal function and homeostasis.

In 1971, John was given the opportunity to return to Harvard, and eager to return to his roots and engage in undergraduate teaching as George Wald had done before him, he accepted a full professorship in the Department of Biology. The decade of the 1970s ushered in a new era in retinal physiology and anatomy. While many details of retinal neurocircuitry would continue to be filled in, attention shifted to the mechanisms by which neurons communicate. At the time, little was known about synaptic transmission in the retina and brain. Two inhibitory neurotransmitters, γ-aminobutyric acid (GABA) and glycine, had been associated with inhibition in the CNS, but inhibition in the retina was not discovered until the mid-1970s and virtually nothing was known about excitatory neurotransmitters or other substances that would be identified subsequently as "modulators." One of the first key steps towards understanding intercellular signalling between retinal neurons was to determine the nature of the photoreceptor neurotransmitter and its effect on second-order neurons. An important contribution came from Dowling and Ripps in their *Nature* paper of 1973, where they showed that blocking synaptic transmission with high Mg^{++} caused the horizontal cell to hyperpolarize and lose its ability to respond (hyperpolarize) to light. Since both transmitter blockade and photic stimulation cause the cell to hyperpolarize, the only possible explanation for their finding was that the photoreceptor released an excitatory transmitter in the dark that depolarized the horizontal cell: light reduced the rate of transmitter release and caused the horizontal cell to hyperpolarize. In a similar way, the photoreceptor transmitter caused one bipolar tissue to hyperpolarize (OFF) and the other to depolarize (ON) on stimulation with light. The basis for the two channels of OFF and ON bipolar pathways was discovered to be a result of ionotropic and metabotropic receptor channels respectively, by one of John's first postdoctoral students Robert Miller with his colleague, Malcolm Slaughter. In this work, the first metabotropic glutamate receptor in the whole central nervous system was characterized and later identified as mGluR6.

Immunohistochemistry now provided a new way of identifying neurotransmitters, and in a relatively short time, a large array of transmitter candidates, including many peptides, were localized to different neurons and proposed as possible neurotransmitters. As the search for neurotransmitters emerged as a central topic, Dowling began a collaboration with Berndt Ehinger, in which dopamine-containing interplexiform cells