The History of Neuroscience in Autobiography
Volume 4

Edited by Larry R. Squire
Published by Society for Neuroscience
ISBN: 0-12-660246-8

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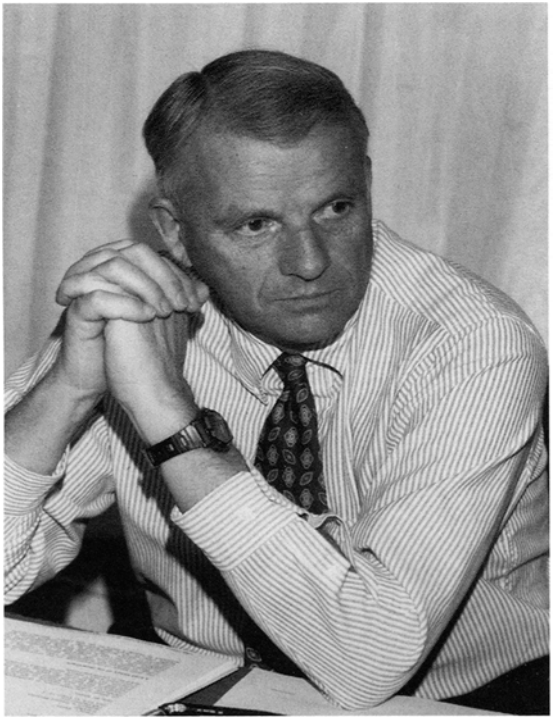
pp. 210–239

https://doi.org/10.1016/S1874-6055(04)80018-5



John E. Dowling

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Harvard College, A.B. (Biology, 1957)
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John Dowling has studied the retina his entire career. He is best known for his detailed analysis of synaptic circuitry of the retina and its functional organization. His laboratory has also focused attention on retinal pharmacology, particularly the role of dopamine in retinal function, and, recently, on retinal development and genetics.

John E. Dowling

I have always enjoyed reading about scientists and their discoveries. As a college student, Sinclair Lewis' *Arrowsmith* left an indelible impression, and then later Cajal's *Recollections of My Life* was inspiring—even exciting—to read. But there are many, many more books—biographies, autobiographies, and even some historical fiction—about scientists that I have devoured. As I sit down to write this brief essay on my own life as a scientist, I ask myself what made these books so much fun to read, and how do I make this piece interesting to others and perhaps as instructive as many of the books I found so fascinating?

Some melding of one's personal and scientific life is important to recount, but beyond that, one's philosophy of doing science, how particular lines of research were taken up, and how one becomes a scientist and manages a scientific life. Perhaps I should start with the latter to put into context what is to come. How did I end up spending a life studying the retina? Clearly, my mentor, George Wald, was instrumental and that story will come. But simple curiosity as to how things work has played an enormous role in directing my scientific life. I have never felt that I am particularly brilliant, but I do find myself mulling things over again and again, seeking an explanation or possible answer to a scientific puzzle and then trying to think of a way to establish my idea. Much of my success, without a doubt, has come from my students and co-workers who arrived in the laboratory with wonderful skills or ideas, and my contribution to their work has been to encourage them, to give them free rein, and to help focus their efforts on the most significant questions. Those working in the laboratory have also learned as much from each other as from me, and I always encourage multidisciplinary approaches and collaborations to crack open a problem. At any one time in the lab, we are carrying out anatomical, physiological, pharmacological, and now genetic approaches to the problems at hand. An interactive laboratory is a happy and productive one, and I count virtually everyone who has been in the laboratory as a good friend. For my 65th birthday, my former students held a symposium in my honor, and about 90 of the 100 or so people who have been in the laboratory over the years attended. That tribute was most gratifying. As a last point for those starting out in science, over the years I have made every effort not to compete with my former students. The projects they undertake in my laboratory are theirs; they take them with them. If my laboratory continues to work on the same or similar problem, it is as a

collaboration or, at the very least, with a continuous exchange of information between the labs. A consequence of this is that my laboratory is always moving on to new areas of research. The downside is that sometimes I feel something is not being finished that was started in the lab. But, that is a penalty I think worth taking—to make sure the next generation has the opportunity to develop an area of research.

The Beginnings: George Wald, Vitamin A Deficiency, and Visual Adaptation

I was born in Rhode Island, the fourth of five children. My father was a physician, an ophthalmologist, and my mother was trained as a chemist. Education was always emphasized in the family, and from the sixth grade onward, I attended a private school. However, studying was not my forte, and I was a mediocre student at best, until my junior and senior years. Chemistry awakened me academically in my junior year—my first A ever—and that then carried over into college.

Curiosity about things and building things consumed much of my time growing up. I had a small room in our basement where I could construct elaborate structures with erector sets; make my electric train layout ever more complicated; and build model boats, cars, and planes with electric or gas engines. Sports, especially competitive ones, were an enduring interest, but a bout with polio when I was 16 left me permanently lame in one leg and altered my activities from baseball, football, hockey, and skiing to golf, squash, and sailing, which I enjoy to this day. Extracurricular activities at school occupied too much of my time, but also may have been the reason I was admitted to Harvard University as an undergraduate. In my senior year of high school I was president of the class, editor-in-chief of the school newspaper, and captain of the golf team. This trait of getting involved in too many things is still with me; I wish I could say "no" more often when asked to serve on a committee or to take on another responsibility.

I arrived at Harvard determined to focus on academics, and it was a new world for me. I had fair success and was pointed toward medicine as a career. But all that changed in my junior year when I took biochemistry from George Wald. He was mesmerizing, especially in the second semester which was devoted to topics in biochemistry. I can still feel the excitement he generated in me when he talked about Albert Szent-Györgyi's famous experiments with glycerinated muscle fibers. That they would contract when ATP was added seemed miraculous to me, getting at the essence of life.

Halfway through that second semester, I asked George (then Professor Wald to me and for many years thereafter) if I could undertake a senior project in his laboratory beginning that summer. I was accepted into the laboratory and that changed my life. George proposed that I work on vitamin A deficiency in rats to determine why after prolonged deficiency, recovery was

often incomplete upon refeeding of vitamin A. Earlier studies by Katherine Tansley and others in England had suggested that photoreceptors degenerate in prolonged vitamin A deficiency, and Ruth Hubbard in the Wald lab had shown that opsin (without attached vitamin A aldehyde) was much less stable than is rhodopsin. George surmised that the lack of complete recovery was due to irreversible photoreceptor degeneration. So I began a series of biochemical measurements, mapping out what happens to a rat on a deficient diet. First, liver stores of vitamin A decrease, then blood stores decline and with that rhodopsin levels. Some time later (~2 weeks), opsin levels begin to decline, and so Wald's hypothesis seemed correct. Opsin is an important structural component of the outer segment of the photoreceptor, and degeneration of photoreceptors occurs as opsin levels decline.

But I was curious as to what effect the loss of rhodopsin had on the rat's vision. How could this be determined? Donald Kennedy, eventually to become Head of the Food and Drug Administration (FDA) and President of Stanford University, was just completing his Ph.D. in the Biology Department, recording the electroretinogram (ERG) of the frog. Don agreed to record the ERG of a rat in his setup, and it was clear that this was the way to go. I inherited much of Don's equipment when he left, moved it upstairs to a darkroom, and began my electrophysiological studies. I found that as the visual pigment levels decline, the light sensitivity of the eye decreases, as one would expect, but it did so logarithmically! This was new and suggested a relationship between visual pigment levels in a photoreceptor and visual sensitivity, a contentious issue at the time. The next question was whether a similar relationship exists during light and dark adaptation, but those measurements were not made for another year. My first paper reporting the studies carried out as an undergraduate were published in the *Proceedings of the National Academy of Sciences (PNAS)* in 1958.

My senior year at Harvard was spent mainly in the Wald laboratory, and I found research immensely satisfying and fun. But I also remained determined to go to medical school and enrolled at the Harvard Medical School in the fall of 1957. I soon found I was missing the Wald lab and so began spending free afternoons in Cambridge. The following summer is when I examined the role of visual pigment levels in light and dark adaptation and also mapped out the exchange of vitamin A between the retina and pigment epithelium during light and dark adaptation. This resulted in a *Nature* paper published in 1960. The second year of medical school was spent split between Cambridge and Boston, and a new observation I made led me to take a year's leave of absence from medical school to work on it.

This was the finding that vitamin A acid (now called retinoic acid) could prevent animals from dying from vitamin A deficiency, but did not prevent them from going blind. In other words, retinoic acid can fulfill all of the somatic functions of vitamin A—growth, tissue maintenance, and

so forth—but cannot be reduced in the body to vitamin A aldehyde (now called retinal) which is essential for vision. Thus, with retinoic acid it was possible to isolate vitamin A deficiency to the eye and photoreceptors. This enabled me to complete elegantly my original project that I began with Wald. I could show that with prolonged vitamin A deficiency, photoreceptors were indeed lost and that in such retinas complete recovery did not occur after vitamin A refeeding.

Again, though, curiosity about an aspect of the project led me to another technique that eventually would play a major role in my research career, namely electron microscopy. What did vitamin A-deficient photoreceptors look like? Ian Gibbons had just joined the Harvard Biology Department and was in charge of a new electron microscope facility. Why not learn how to do electron microscopy and find out? I did just that and thus began my anatomical studies. A *PNAS* paper reporting our retinoic acid studies was published in 1960 and included biochemical, electrophysiological, and electron microscopic observations.

Retinoic acid (RA) has become a most important molecule for understanding development, and 10 years ago we revisited RA in terms of its role in retinal and photoreceptor development. But how did I come to use it in the first place? In 1957, R.A. Morton's classic book on vitamin A appeared, and Ruth Hubbard read in it that whereas vitamin A deficiency symptoms could be reversed in animals dosed with RA, never could one find traces of RA in the animal's tissues. This suggested that RA could not be converted back to retinal or vitamin A in biological tissues, the molecules essential for vision. She suggested I try vitamin A acid in my experiments, and, as they say, the rest is history.

An opportunity arose while in medical school that led me into yet another area of lifetime interest, namely inherited retinal degenerations. Richard Sidman was my Instructor in Neuropathology at Harvard Medical School and he had read my first paper on vitamin A deficiency. He had recently brought into this country rats with an inherited retinal dystrophy—the RCS (Royal College of Surgeons) rats—and he wondered if they might have a deficit in vitamin A metabolism. We began a collaboration that resulted in a *Journal of Cell Biology* paper in 1962. Although we found no evidence that the defect in the RCS rat was caused by a deficit in vitamin A metabolism, several inherited retinal degenerations in man have now been shown to be caused by such genetic defects. But I am now getting ahead of my story.

During my year leave of absence from medical school, as the story of RA unfolded, George Wald suggested that I consider obtaining a Ph.D. Since I had had two years of medical school training, had been an undergraduate in the Biology Department, and had done, in his opinion, enough research for a thesis, George believed I could quickly qualify for the degree. An amusing story resulted from my attempt a few days later to enroll in

the Graduate School of Arts and Sciences (GSAS) at Harvard. When I approached the receptionist asking for the application forms for graduate study, I was told in a somewhat contemptuous voice that since the deadline for admission for the following year was the next day, there was no way I could complete the application process in time. I then said I was not seeking admission for the following year, but for the next semester (only two weeks off!) and that I believed Professor Wald had spoken to the GSAS Dean on my behalf. The receptionist disappeared into the Dean's office, but reappeared a short time later with the forms and a red face! I entered graduate school in February 1960, took qualifying exams in April, and wrote my thesis over the summer and into the fall. The degree was granted in January 1961.

I fully expected to return to medical school the following year, but, again, George intervened. He was undertaking a new introductory undergraduate course in biology at Harvard, designed for both scientists and non-scientists, that would emphasize the unity of life at the molecular level. This course was to replace the traditional botany and zoology courses that focused on the differences among organisms. He asked if I would join him and a small cadre of young biologists to help design and teach the course. The offer was too tempting to refuse, and I extended my leave of absence from medical school—now in its 43rd year!

The course, entitled "The Nature of Living Things," began that year, and I was appointed an Instructor in the Department beginning in February 1961. I was promoted to Assistant Professor in July and given laboratory space adjacent to the Wald laboratory. And so, my independent research career began. Initially, I followed up on and extended the research projects I had begun as an undergraduate and graduate student, as well as completed the study on the RCS rats with Richard Sidman. With Ruth Hubbard, I looked at the formation and utilization of 11-cis vitamin A in eye tissues, as well as the effects of brilliant light flashes on the ERG in light and dark adaptation. With Ian Gibbons, I studied the fine structure of the pigment epithelium, and then, when Richard Cone was visiting from the University of Chicago, I extended my observations on the mechanisms underlying light and dark adaptation, showing that there are both neural and photochemical components at play.

Up to that point (1963), virtually all of my research had been carried out on the rat, a rod-dominated animal. What about cones? Do cones light and dark adapt like rods? How different is their fine structure? Curiosity about these questions was to lead me far beyond photoreceptors, light and dark adaptation, and vitamin A deficiency and to one of the most fruitful collaborations of my career. Ground squirrel retinas contain mainly cones, and I thought they would be ideal animals to study. But, how would I obtain some? Charles Lyman at Harvard Medical School was studying hibernation in ground squirrels and was the obvious source. A call to Charles resulted in the promise of some animals, but he mentioned that a visitor to our department

from England, teaching Jack Welch's Invertebrate Zoology course that year while Jack was on sabbatical leave, was also interested in ground squirrels and why didn't we share animals? That was my introduction to Brian Boycott.

Brian was then interested in synaptic plasticity and wished to follow up on an old observation that spines on cerebellar Purkinje cells change shape during hibernation. His laboratory was just down the hall, and so we began to take the bus over to the medical school to pick up animals and tissues. In my lab, I recorded the ground squirrel ERG during light and dark adaptation, and then I began to study the fine structure of the photoreceptors by electron microscopy. What caught my eye were the photoreceptor terminals and the fact that on occasion I could trace a process back from the photoreceptor terminal to its cell of origin. Photoreceptor-bipolar cell contacts were expected and seen, but I could also identify processes from horizontal cells synapsing with the photoreceptor cells. Horizontal cells were very much a mystery then—indeed, some thought they were glial cells—but the fact that they were receiving input from photoreceptor cells clearly indicated they were neuronal.

What was the neuronal circuitry of the outer retina? I began to discuss this with Brian on our medical school trips and started to learn more about synapses and synaptic circuitry. We mused that it was important to learn more about the horizontal and bipolar cells of the ground squirrel retina by light microscopy and Golgi staining, techniques with which Brian was expert. At the same time, Brian had found significant changes in the cerebellar (Purkinje cell) spines during hibernation at the light microscopic level, but he recognized that these changes needed to be studied by electron microscopy—something I could do. The next step was obvious: we join forces and share each others' expertise.

We made substantial progress on both fronts, but curiously we never formally published either study. (Several years later, Roger West in our laboratory revisited the ground squirrel retina and did publish several papers on its cellular and synaptic organization.) Brian returned to England at the end of the year, and the next year, I moved to The Wilmer Institute at Johns Hopkins University. I had been an Assistant Professor in the Harvard Biology Department for three years, and, as was then the custom, assistant professors were seldom promoted at Harvard. A tempting offer from Ed Maumenee, Chief of Ophthalmology at the Wilmer, to occupy magnificent new space in the Woods Research Building was too good to pass up, and so I moved to Baltimore in June 1964.

The Early Wilmer Years: Retinal Circuitry and Single Cell Recordings

During my last year as an Assistant Professor at Harvard, George Wald was on sabbatical leave in England, and so my teaching responsibilities were

considerable. I did stay in contact with Brian, who was becoming more and more interested in the retina, but who also felt that to make progress with understanding retinal circuitry, the ground squirrel was not the place to start; too little was known about its cells. Since Polyak's 1941 book *The Retina* provided a wealth of material on the primate retina and its cells, we thought perhaps we should begin there. Brian visited the Wilmer in July 1964 for a few weeks, the first of many such visits over the next 10 years. During that first visit we studied what ground squirrel material he had prepared, but also fixed some monkey retinas for both light and electron microscopy, and it was this material that set us on our course.

I first looked at the foveal cones—about which virtually nothing was known at the electron microscopic level. A paper in *Science* early in 1965 described the photoreceptor outer and inner segment structure, but also, tantalizing, the structure of the foveal cone photoreceptor synapses which seemed simple compared to the ground squirrel photoreceptor synapses and, perhaps, easier to analyze. I began to focus on identifying retinal synapses in both plexiform layers. Ribbon synapses in photoreceptors were well known, but ribbon synapses were also present in the inner plexiform layer (IPL). In what cells were they? A particularly well-fixed piece of human retina, provided us by Ed Maumenee from an eye surgically removed for melanoma, gave us the answer and much more. Large bipolar cell terminals could be readily identified in the human IPL, and they made abundant ribbon synapses. Conventional synapses were also seen in the IPL. The obvious question was what cell makes these? Again, the piece of human retina provided the answer. I could follow processes from amacrine cells into the IPL where they made such conventional synapses. We reported these first results at the annual Cold Spring Harbor Laboratory Symposium in the spring of 1965 and with them a tentative diagram of the synaptic circuitry of the IPL (Fig. 1).

Fritoff Sjostrand was Chair of the session at the Cold Spring Harbor meeting at which I presented our findings, and following the presentation, he expressed contempt for our wiring diagram. He chastised us for not carrying out serial section studies (of which he was then master). He said sitting on one's rear end and doing the hard work was necessary before drawing any such diagram. I replied that sitting on one's behind and thinking hard about one's observations was equally valid, which brought down the house but lost me the friendship of Sjostrand. He never spoke to me again.

In London, Brian was making new observations at the light microscope level on the primate retina, whereas in Baltimore, I extended the electron microscopic (EM) observations (even carrying out some limited serial section analyses). Brian was then visiting twice a year, and when he was in Baltimore, it was intense but exciting. We published our electron microscopic studies on the primate retina (with much grander diagrams) in the *Proceedings of the Royal Society* in 1966 (Dowling and Boycott, 1966), but the light

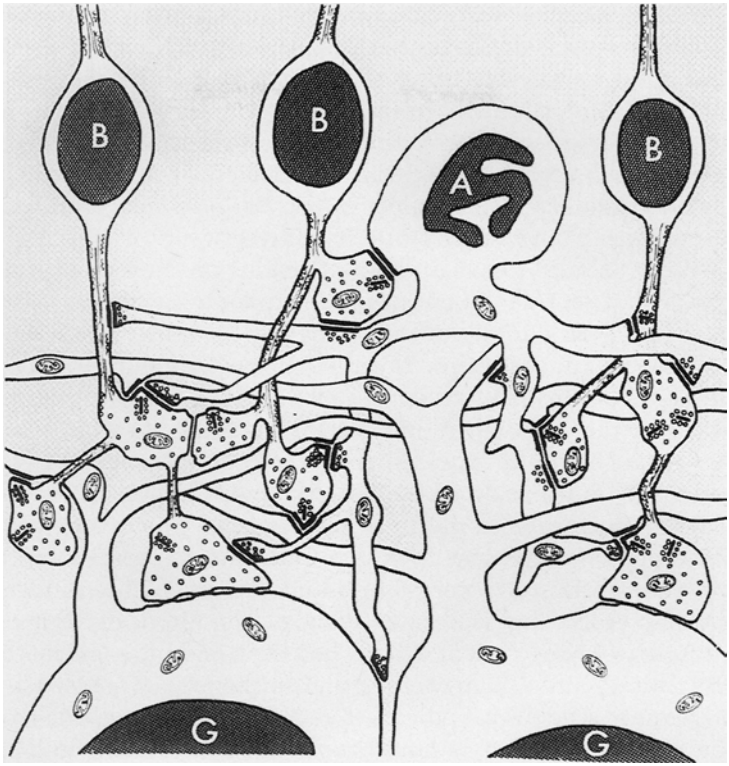

Fig. 1. Our summary diagram of the synaptic organization of the primate IPL layer presented at the 1965 Cold Spring Harbor Symposium. B, bipolar cells; A, amacrine cell; and G, ganglion cells. (From Dowling JE, Boycott BB. *Cold Spring Harbor Symp Quant Biol* 1965;30:393–402. With permission.)

microscopic paper did not appear until 1969. The latter was a massive tome, 75 pages in length, published in the *Physiological Transactions of the Royal Society*. It went through 12 drafts and had over 100 micrographs and figures.

My lab at Johns Hopkins was also growing. George Weinstein, an ophthalmologist, first joined us and carried out a marvelous study on light and dark adaptation of the isolated rat retina, making simultaneous physiological and biochemical measurements. Helga Kolb soon arrived from Geoff Arden's laboratory in London, and she wanted to do anatomical studies. Brian and I thought combining the light and electron microscopic observations by studying Golgi-stained cells in the electron microscope (a technique pioneered by Bill Stell at the National Institutes of Health) was the next logical step to take, and it yielded wonderful results. An early result was the discovery of a second type of midget bipolar cell in the primate retina—the

flat midget cell—which was the first hint that information from photoreceptors to bipolar cells was divided into two pathways, ON and OFF pathways. But at that point we knew nothing of the physiology of bipolar cells so that realization was some time off. Helga studied the connections of all of the outer plexiform layer cells, providing for the first time quantitative data with regard to the number of connections made between photoreceptor and bipolar or horizontal cells. Her paper in the *Philosophical Transactions* in 1970 was submitted for a Ph.D. from the University of Bristol.

Following a lecture I gave at Johns Hopkins on the synaptic organization of the primate retina, a new graduate student, trained as an electrical engineer at MIT, came to my office asking if I thought it possible to build a theoretical model of the retina. My answer was that since we knew virtually nothing of the electrical responses of the retinal cells, it was too early to model the retina, but why didn't he, for his graduate work, make such recordings from the mudpuppy retina. I had been introduced to the mudpuppy retina by Paul Brown of the Wald lab when I was still at Harvard; he was taking advantage of the mudpuppy's large photoreceptor cells for microspectrophotometric measurements. But what impressed me then was that all of the retinal cells were large, and this might be an ideal retina from which to record the responses of single cells. Alexander Bortoff of the State University of New York at Albany had been making some intracellular recordings from the mudpuppy retina, and so the project seemed feasible.

The graduate student called back a few days later to say he would like to try the project and that is how Frank Werblin joined the lab. Frank was soon recording intracellularly from the mudpuppy cells, but the critical step was to stain the recorded cells. This was accomplished with the use of Niagara Sky Blue, and soon Frank identified and characterized the electrical responses of all of the retinal neurons. His was a spectacular thesis reporting for the first time that there are both ON- and OFF-center bipolar cells, that bipolar cells have a center-surround organization, and that many amacrine cells respond transiently at the on and off of illumination. His results were subsequently published in the *Journal of Neurophysiology* in 1969 (Fig. 2).

In the meantime, I was continuing my anatomical studies, first on the frog retina and then on mudpuppy. Clearly, retinas vary in their synaptic circuitry, and I began to explore whether the variations in synaptic circuitry could be correlated with complexity of ganglion cell responses. It had been appreciated for some time that cold-blooded vertebrates such as frogs had many ganglion cells with complex receptive field properties such as movement and direction sensitivity. This must be correlated with differences in circuitry. But how was this done?

Electron microscopic studies soon showed that there were many more amacrine cell (conventional) synapses in the IPL compared to bipolar (ribbon) synapses in frog as compared to primates and that there were abundant

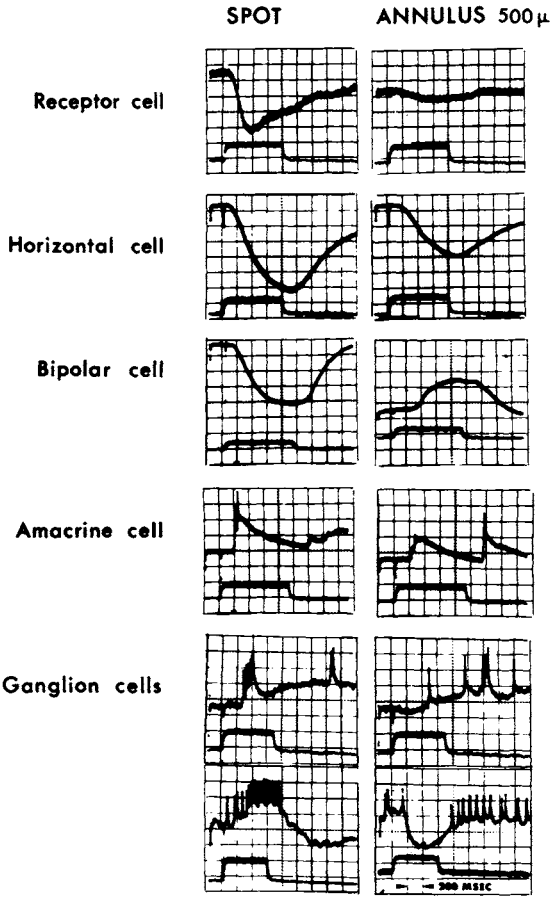

Fig. 2. Intracellular recordings from mudpuppy retinal cells to a spot of light (left) and annulus (right). (From Werblin FS, Dowling JE. *J Neurophysiol* 1969;255:339–355. With permission.)

serial and reciprocal synaptic arrangements made by the amacrine cell processes in the frog retina. This implicated the amacrine cells as playing a pivotal role in generating complex ganglion cell responses such as movement and direction selectivity, and Werblin's recordings from amacrine cells, showing that many of them respond transiently at the onset and offset of illumination—that is, they are highly movement sensitive—strongly supported this interpretation. Mark Dubin, another Johns Hopkins graduate student, eventually extended these studies to a number of species, providing quantitative measurement of synaptic frequencies and densities in the IPL.

The Marine Biological Laboratory: Horseshoe Crabs, Skates, and Teaching

The years at Johns Hopkins were exceptionally productive and fruitful. I did miss teaching, although I was an affiliate member of the Biophysics Department on the Homewood Campus of Johns Hopkins and taught a graduate seminar there. One day, Francis (Spike) Carlson, Chairman of the Biophysics Department, called to say that the Marine Biological Laboratory (MBL) wished to establish a Neurobiology Course and would I consider teaching such a course there? I knew little of the MBL, so Spike suggested I spend a summer in Woods Hole, MA, and become acquainted with the laboratory. I did that, beginning the summer of 1967, and I have been returning to Woods Hole and the MBL ever since.

That first summer I decided to learn how to record intracellularly from neurons. I recorded from photoreceptor (retinular) and second-order (eccentric) cells in horseshoe crab eyes and discovered a second type of discrete potential in the retinular cells. This is a regenerative potential that serves as an amplifying mechanism, ensuring that the absorption of a single photon in the retinular cell results in the generation of a nerve impulse in the eccentric cell. I spent a second summer recording from the *Limulus* eye, but then the following summer began another exceptionally fruitful collaboration with Harris Ripps, then of New York University and now of the University of Illinois School of Medicine in Chicago. Harris is an expert in visual pigment measurements and also has a long-standing interest in light and dark adaptation. We surmised that studying these processes in an animal that had only rods could be useful, and the early literature suggested that certain marine elasmobranches, including dogfish, had only rods. We first looked at dogfish, but discovered they have some cones. However, the other common elasmobranchs in Woods Hole, the winter and summer skates, did turn out to have pure rod retinas, and we spent nearly a decade of summers studying the retina of these animals and mapping out light and dark adaptation processes at all levels of the retina. The photoreceptors in the skate, although being classic rods in the dark-adapted retina, adopt cone-like behaviors when the eye is light adapted. Many of the features of photoreceptor light and dark adaptation were first revealed in the skate, and we showed that there are adaptation mechanisms at play proximal to the receptors. Another important finding we made in the skate was that horizontal cells hyperpolarize and lose light responsiveness when synaptic transmission from the photoreceptors is blocked with Mg^{2+}. This provided direct evidence that the photoreceptors release an excitatory neurotransmitter in the dark and that the hyperpolarizing response of horizontal cells in the light is due to a decrease in transmitter release from the photoreceptors. We eventually published 14 papers based on our summers' collaborative efforts.

Teaching soon became a prominent part of my Woods Hole experience. Michael Bennett of the Albert Einstein School of Medicine and I initiated the Neurobiology Course at the MBL in the summer of 1970, and we taught the course for five years before turning the reins over to others. The Neurobiology Course at the MBL is now one of the mainstays there. In the 1980s I helped David Papermaster establish a training course in vision at the MBL, and then in the mid-1990s Nancy Hopkins from MIT and I began a short course on the Neural Development and Behavior of Zebrafish, my most recent research interest.

I continued to maintain a summer laboratory at the MBL until the mid-1990s, when my laboratory began to raise zebrafish in The Biological Laboratories at Harvard. It then became difficult to justify setting up a summer lab at the MBL when we had more fish at Harvard (~30,000) than were available at any one time at the MBL. I continue to come to the MBL to teach and write, and for the past few years I have been involved administratively at the laboratory, serving at the present time as President of the Corporation.

The Later Wilmer Years: Functional Retinal Organization

A number of students and postdoctoral fellows eventually joined the lab at Johns Hopkins and contributed significantly. Bob Frank, an ophthalmology resident, looked at the effects of rhodopsin photoproducts on visual sensitivity. Dwight Burkhardt discovered a new extracellular potential in the frog retina—the proximal negative response (PNR)—and provided evidence that it derives from amacrine cells. Bob Miller recorded intracellularly from the glial (Müller) cells in the mudpuppy retina, found that they respond when the retina is illuminated, and provided some of the first evidence that Müller cell responses contribute to the ERG.

Les and Steve Fisher explored synaptic circuitry in the tadpole and cat eye, respectively, showing that there are significant changes in retinal synaptic organization during metamorphosis and that cat amacrine cells can make direct somato-somatic synapses on bipolar cells in the IPL. Gus Aguirre, a veterinarian, came to the lab to study dogs with inherited retinal degeneration, and Dick Chappell undertook a study of the dragonfly ocellus thought to be a "simple" retina. He recorded the electrical responses of the ocellar cells, whereas I examined the fine structure of the photoreceptors and synapses made in the ocellar synaptic plexus.

Those were wonderful and heady days at Johns Hopkins. On Thursdays at noon, the lab went *en masse* down to Lombard Street, lined with Jewish delicatessens, and the usual fare was a knish and corned beef sandwich washed down with a bottle of Cel-ray soda. Friday was seminar day, with

lunch provided by Pat Sheppard, about whom I will say more shortly, followed by an informal talk. In the spring and fall, doubles tennis on the courts across the street from the Wilmer was often played, followed by beer and a sandwich at Frank's, a small pub-like bar a few blocks away. Sunday afternoons in the fall were spent watching the Baltimore Colts from the end zone—seats that Mark Dubin obtained by staying up all night at the stadium to ensure our obtaining them.

Pat Sheppard joined our lab shortly after I arrived in Baltimore and served as the lab's technician, accountant, artist, cook, procurer of material, and whatever. She cut marvelous sections for both light and electron microscopy and was responsible for all of the drawings we published. She fed us well before the Friday seminars and always seemed able to find what we needed for our experiments. The only time I saw her flustered was when Brian dashed in one day to ask if she could get him a box of rubbers. She did not know that rubbers in England are erasers. Pat ended up working for me for 27 years, retiring in 1991.

The Association for Research in Vision and Ophthalmology (ARVO) meeting (then the ARO meeting) became a mainstay for the lab beginning in the late 1960s. Until 1968, it was a small, mainly clinical meeting, but the ARO Trustees wanted it to expand. Several of us were asked how this might be done, and we proposed organizing sections that would represent various areas of eye and vision research. Paul Witkovsky from New York University, who had been spending time with us learning electron microscopy, and I organized a section on visual electrophysiology. The ARVO meeting eventually expanded beyond all expectations and now attracts over 8000 vision researchers each year to Ft. Lauderdale, FL, its present meeting site. In 1970, I was given the Friedenwald Award by ARVO, and I presented an overview of our lab's research to the attendees. It was well received and the published paper remains one of the most satisfying I have written.

In mid-1970, I was asked by the Biology Department at Harvard if I would like to return as full professor. It was a hard decision. Johns Hopkins had not only been generous to me, but the research had been going exceptionally well and many excellent students and postdoctoral fellows were coming to the lab. The deciding factor was the opportunity to teach undergraduates again and to be involved in college life. I returned to Harvard in June 1971, and that is where I have been since.

The Harvard Years—1970s: Dopamine, Pharmacology, and Interplexiform Cells

A number of people came with me to Harvard, including Pat Sheppard, Gordon Fain, Jochen Kleinschmidt, and Roger West. Ralph Nelson, a graduate student in Biophysics, decided to stay in Baltimore to finish his degree and his experiments on the electrical properties of mudpuppy

retinal neurons. At Harvard, Gordon recorded intracellularly from the mudpuppy photoreceptors and showed definitively that rods have a higher sensitivity to light than do cones—by about 25 times. Jochen examined adaptation in gecko photoreceptors recorded intracellularly, and Roger undertook an analysis of the photoreceptors and synaptic input onto the ganglion cells in the ground squirrel retina.

In 1973, a visitor from Sweden joined the laboratory for a two-month stay, and once again a marvelous collaboration began that added another dimension to our research that continues to this day. Berndt Ehinger, an ophthalmologist, was interested in retinal pharmacology, particularly the role of monoamines in the retina. He was an expert with the Falk Hillarp method, which causes cells containing monoamines to fluoresce. He and others had observed what appeared to be a new type of cell in fish and New World Monkeys that sits among the amacrine cells, but extends processes into both plexiform layers of the retina. The color of the fluorescence suggested that these cells contain dopamine. But what are their synaptic connections? Berndt came to the lab to find out.

To identify the processes of the cells in the electron microscope, we took advantage of the fact that cells containing monoamines have robust reuptake systems that do not discriminate between the natural transmitter and certain analogs that can alter the fine structural appearance of the synaptic terminals. By feeding fish retinas one such analog, we were able to identify the synapses made by these cells and show that they are centrifugal in nature. They receive input in the IPL, whereas the bulk of their input is in the outer plexiform layer on horizontal cells. These cells were eventually called interplexiform cells, and they and dopamine have been studied in our lab ever since (Fig. 3).

Dopamine acts as a classic neuromodulator on horizontal cells, binding to D1 receptors and activating adenylate cyclase through a G-protein. The resulting increase in cyclic AMP activates protein kinase A (PKA), which modulates both glutamate and gap junctional channels in horizontal cells. Since those early days, the study of dopamine in the retina has become a virtual industry. Every retinal cell type responds to dopamine, and there are a variety of dopamine receptors in the retina whose activation can both increase or decrease cyclic AMP levels. The story gets more complicated yearly, and we still do not have a complete grasp of dopamine's overall role in the retina. Indeed, it appears as if dopamine must play multiple roles. Many people in the lab have studied the effects of dopamine on retinal cells over the years, including Bill Hedden, Rick Lasater, Keith Watling, Rob Van Buskirk, Andy Knapp, Doug McMahon, Stuart Mangel, Denis O'Brien, Pat O'Connor, Xiong-Li Yang, Tina Tornqvist, Osamu Umino and, most recently, Ethan Cohen, and all have added to the story.

Work in the laboratory during the 1970s was not focused entirely on dopamine and dopaminergic mechanisms. Curiosity about other neuroactive

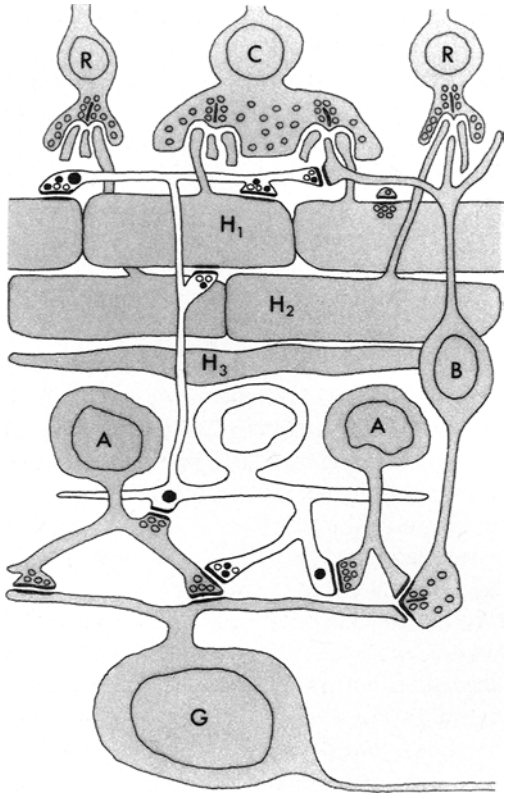

Fig. 3. Synaptic organization of the dopaminergic interplexiform cell in goldfish. The unshaded cell is the interplexiform cell. R, rods; C, cone; H_1, H_2, and H_3, horizontal cells; B, bipolar cell; A, amacrine cell; and G, ganglion cell. (From Dowling JE, Ehinger B. *Science* 1975;188:270–273. With permission.)

substances in the retina led us into studies of the amino acids as the principal excitatory and inhibitory neurotransmitters in the retina. Rick Lasater, Sam Wu, Mickey Ariel, Randy Glickman, Stuart Bloomfield, and Ido Perlman contributed here, as did Berndt Ehinger, who for many years paid us an annual visit. Dave Pepperberg and Stuart Lipton also continued work on photoreceptor adaptation, studying the effects of retinal, Ca^{2+}, and the cyclic nucleotides on these cells, while Geoff Gold, a physics graduate student at Harvard, carried out beautiful experiments, both anatomical and physiological, on the electrical coupling between photoreceptors.

Much of our work during the 1970s was physiological or anatomical and was carried out on fish retinas, both goldfish and skate. As the questions we were asking became more sophisticated, it became clear that we needed to

know more about the molecules involved in synaptic transmission, particularly those involved in retinal neuromodulation. I was eligible for a sabbatical leave in 1978, my first at Harvard, and so I went to Cambridge, England for the year to work in Les Iversen's laboratory. I was awarded a Guggenheim Fellowship for the year and stayed at Churchill College where I was an Overseas Fellow. It was a marvelous year. I worked closely with Keith Watling, a young postdoctoral fellow in Iversen's laboratory, on the generation of cyclic AMP in the retina. Watling came to the other Cambridge to work in my laboratory in the early 1980s, so our collaboration continued for several years.

I had returned to Harvard in 1971 to become involved in undergraduate teaching. I was the only senior neurobiologist in our department at that time, and so in the spring of 1972 I initiated an introductory neurobiology course. Previously, faculty from the Neurobiology Department at Harvard Medical School had taught an upper level neurobiology course at the College, but I wanted to teach a more introductory course that would enable students in their sophomore year to be introduced to the field. The course was called Biology 25 and was equivalent in level to the Department's introductory cell, developmental, and molecular biology courses that students could take after introductory biology and chemistry courses. The course continues to this day, although it has changed somewhat as I will explain later. I was joined in teaching the course initially by Dave Hubel and Richard Sidman. Eventually, Dave retired from the course and then Dick, so for many years I taught it alone.

I also became more involved in administration at Harvard. I was Chair of the Department of Biology from 1975 to 1978 and elected to the Faculty Council in 1976. Henry Rosovsky, Dean of the Faculty of Arts and Sciences, asked if I would serve on the committee to revamp the General Education requirements at Harvard. From this committee came the Core Program which requires that all undergraduates take specially designed "Core" courses in fields outside of their area of specialty. I was concerned principally with the science part of the Core; all students not in a science concentration are required to this day to take two science courses before they graduate: one in the physical sciences and the other in the life sciences. I have team-taught two such courses for many years, first with Carroll Williams, a cell biologist and expert in insect hormones, and then with Howard Berg, a biophysicist interested in bacterial motility. In these courses, we have introduced non-science students to the exciting advances in molecular, cell, and neurobiology.

The 1980s: White Perch and Neuromodulation

It was becoming increasingly clear as our pharmacological experiments progressed that we needed a simpler system than the whole retina to understand

how neuroactive substances were affecting neurons at the cell and molecular level. A few laboratories were beginning to isolate and culture retinal neurons, and this seemed to be a promising approach. We first isolated neurons, especially horizontal cells, from goldfish or carp retinas, but the results were often disappointing. Although the neurons would survive in culture for several days, they would usually round up, and it was difficult to distinguish subtypes of cells. Nevertheless, we were able to make a number of important observations with them. Rick Lasater, for example, showed that cultured horizontal cells respond selectively to L-glutamate and not to L-aspartate, providing some of the early evidence that the photoreceptor transmitter is L-glutamate. Rob Van Buskirk made partially purified carp horizontal cell preparations by velocity sedimentation procedures that enabled him and others in the laboratory to study the effects of dopamine, vasoactive intestinal peptide, and other substances on the generation of cyclic AMP in these cells. In Woods Hole, Lasater, Ripps, and I isolated skate retinal neurons and recorded from both horizontal and bipolar cells, but cultured skate horizontal cells, like carp horizontal cells, tended to round up in culture.

In the summer of 1981, I decided to spend my time at the MBL seeking a fish whose neurons would culture better. We had just bought a house on Oyster Pond, in Woods Hole, and this was our first summer on the pond. Our first weekend there, a friend of my wife was visiting with her 12-year-old son. He had a new fishing rod—a birthday gift—and wanted to try it. From the dock at the rear of the house, we soon found we could readily catch fish of 6–8 in. in length. I wasn't sure what they were, but catching them was great fun. On Monday, my first day in the lab, I decided to culture neurons from the retinas of these fish, now identified as white perch. I'll never forget my first look at the cultured neurons from the white perch—they were spectacular! I could, for example, identify four types of horizontal cells, and the cells maintained their shape for days to weeks (Fig. 4). I spent the rest of the summer trying other fish, but none worked as well as the white perch.

Needless to say, the white perch retina became the mainstay preparation in our laboratory for more than a decade. We would collect 200–300 each fall from Oyster Pond to use throughout the winter and spring. A number of important observations were made both in the 1980s and the early 1990s with isolated retinal neurons, as well as with the intact retina from the white perch. Rick Lasater and I showed that strong electrical coupling occurs between overlapping pairs of horizontal cells of the same morphological type, but not between overlapping pairs of cells of different morphological types, and that dopamine decreased the conductance of the electrical junctions of the coupled cells. Subsequently, Doug McMahon and Andy Knapp demonstrated that the reduced conductance induced by dopamine was the result mainly of a reduced open time of the gap junctional channels.

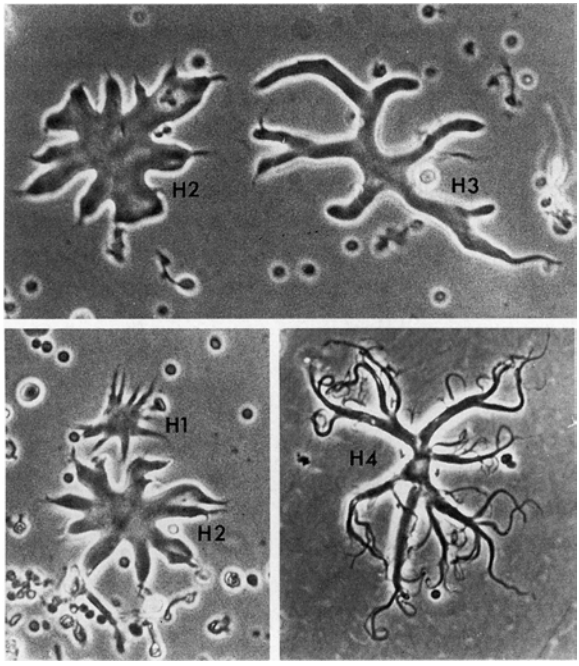

Fig. 4. Subtypes of horizontal cells observed in cultures of the white perch retina. The H_4 cell is connected exclusively to rods. The H_1, H_2, and H_3 cells are cone horizontal cells. (From Dowling JE, Pak MW, Lasater EM. *Brain Res* 1985;360:331–338. With permission.)

In the mid-1980s, it was generally believed that the principal effect of dopamine on horizontal cells was to modify the electrical coupling between adjacent cells. However, earlier Bill Hedden and then Stuart Mangel in our laboratory showed that dopamine reduced the responsiveness of horizontal cells to light in the intact fish retina when full-field illumination was used. This effect of dopamine cannot be explained by reduced coupling between cells, and so Andy Knapp looked to see if dopamine affected the horizontal cells' responsiveness to the photoreceptor transmitter, L-glutamate. He found that dopamine greatly enhances L-glutamate-gated conductances in cultured white perch horizontal cells, the first direct evidence for dopamine modulation of excitatory amino acid neurotransmission in the vertebrate central nervous system (CNS). Such modulation by dopamine has now been found in many parts of the CNS. Subsequently, Knapp and Karl Schmidt showed that dopamine exerts this effect on the glutamate channels by increasing the channel opening probability in response to a given concentration of agonist and also by increasing the duration of the channel open times somewhat. Emily Liman, at about the same

time, showed that the enhancement of the excitatory amino acid currents in the horizontal cells was mediated via a cyclic AMP-dependent kinase (PKA).

The work of Mangel and, subsequently, of Xiong-Li Yang, Tina Tornqvist, and Bill Baldridge stimulated another line of research that continues to this day. Following prolonged dark adaptation, horizontal cell responses are suppressed, and light is needed to sensitize the cells. The extent of dark suppression of horizontal cells is influenced by the time of day. From these observations, Mangel in his own laboratory has demonstrated circadian clock regulation of horizontal cells and other retinal neurons. Dopamine is clearly involved in the circadian regulation of retinal neuronal responses, but the story is as yet incomplete. We do not yet understand very well the significance of dark suppression of horizontal cells, and the effects of dopamine on retinal cells are many and complex; indeed, effects of dopamine on every retinal cell type has been reported.

Two other studies from our laboratory utilizing the white perch retina deserve mention. Using cultured neurons, Haohua Qian discovered a novel GABA response from the rod (H_4) horizontal cells of the perch. This non-desensitizing, bicuculline-resistant GABA response was subsequently shown to be the result of activation of ligand-gated channels made up of rho (ρ) subunits. These ligand-gated channels have been called $GABA_C$ receptors and appear to play a particularly important role in the visual system. Isolation and cloning of the genes for the rho receptors in the white perch have now been accomplished by Qian, first working in our laboratory and later with Harris Ripps in Chicago. George Grant used slices of the white perch retina to study the generation of ON-bipolar responses in the white perch retina. For more than 30 years, it has been known that the ON-bipolar response generated by cones in fish is different from the ON-bipolar response generated by rods. The latter response has been shown to be linked to a metabotropic glutamate receptor and biochemical cascade that results in channel opening in the light, resulting in cell depolarization. But the cone ON-bipolar cells response is different; glutamate released in the dark directly hyperpolarizes the cell and the ON-light response results from relief of the hyperpolarization. Grant found that glutamate hyperpolarizes the bipolar cells by activating a glutamate transporter linked to a Cl^- channel. In the dark, Cl^- enters the bipolar cell, thus hyperpolarizing it. When the transporter is no longer activated by glutamate, i.e., in the light, and Cl^- no longer enters the cell, it depolarizes. This is a novel mechanism of generating a postsynaptic response, and it will be of interest to see if this mechanism occurs elsewhere in the brain. Kwoon Wong, a graduate student in the lab, is presently following up and extending Grant's work.

Teaching in both Bio 25 and the Core courses continued in the 1980s and, on an alternate year basis, I also led a graduate seminar. I was also drawn

more into administration, both at Harvard and elsewhere. In 1980, Dean Rosovsky asked if I would serve as Associate Dean of the faculty to advise him on matters pertaining to the burgeoning biology activities at Harvard. I sat with a small group of Academic Deans, mainly over sandwich lunches in the Rosovsky office on Tuesdays, and we discussed faculty appointments, issues of the day, and new initiatives. It was an interesting experience of four years duration that convinced me that I did not want to go further in academic administration. I did accept in 1981 an administrative assignment as Master of Leverett House at Harvard that proved to be a delight. For the last three of their four years at Harvard, undergraduates live in 1 of the 12 residential units called Houses. They provide a small college atmosphere, having a dining hall, a library, music, theatre, and athletic facilities, and a staff of both academic and administrative personnel to provide advice, letters of recommendation for fellowships, graduate study, and so forth. Each House is led by a senior faculty member and his/her spouse, and they serve as Co-Masters. Judith and I served as Co-Masters of Leverett House for 17 years, until 1998.

Administrative activities outside of Harvard also began to take time. I was elected to the National Academy of Sciences in 1976 and shortly thereafter was asked to serve on the Assembly of Life Sciences, the oversight committee for life science, projects undertaken by the National Research Council at the request of the U.S. Government. I served two terms on the Assembly and then in the mid-1980s served as chair of the committee, then called the Commission of Life Sciences. I also served on the Council of the National Eye Institute, so for a few years I was going to Washington, D.C. once or twice a month. Again, I much enjoyed these experiences, but they also confirmed that I did not want to become a full-time administrator.

I was eligible for another sabbatical in the mid-1980s and decided to use the year to draw together in a book what had been learned about retinal mechanisms over the previous 25 years. I spent seven months in Okazaki, Japan, hosted by Ken Naka at the National Institute of Biology there. It was a memorable time. I wrote in the mornings and spent the afternoons working in the lab with Ken and his colleagues. The choice of Japan as a place to write the book was suggested by my wife Judith, who was becoming increasingly interested in the arts and culture of Japan. She was then in graduate school at Harvard, studying under Harvard's expert in Japanese art, John Rosenfield. She made many friends in Japan that year and brought back some wonderful objects to sell. That turned into a flourishing Asian Art Gallery, first in Cambridge and now in Boston.

I titled my book *The Retina: An Approachable Part of the Brain*, and it was well received. I found writing the book most enjoyable. So I then embarked on writing an introductory textbook on neuroscience, based on my course Biology 25. Called *Neurons and Networks*, it is now in a second edition. A trade book on neuroscience, *Creating Mind*, followed and was

published in the late 1990s. It did not become a best seller, but did get some nice reviews.

1990s: Zebrafish, Genetics, and Development

Toward the end of the 1980s, the white perch in Oyster Pond began to disappear, and so we cast around for a substitute fish. The closest relative to the white perch readily obtainable was the hybrid striped bass. These fish, a cross between striped bass and white bass (essentially landlocked white perch), were being raised for commercial purposes, and a large fishery raising them operated in western Massachusetts. When we began to use them in our experiments, I was astonished at how consistent our results became. Why? The obvious answer was that these fish were all raised under identical conditions and were of the same age and of the same genetic stock. It seemed clear to me that carrying out experiments on animals whose genetics, age, and environment could be controlled was the way to go.

Zebrafish as an experimental model were introduced back in the 1970s by George Streisinger at the University of Oregon, but it was not until the early 1990s that a number of groups recognized their potential. We began to examine the eyes of zebrafish at that time, first looking at the effects of retinoic acid (RA) on retinal development. This work, carried out mainly by George Hyatt, Ellen Schmitt, and Nick Marsh-Armstrong, found that RA is critical for early retinal development, especially of the ventral retina. Too much RA at an early stage of eye development results in an apparent duplication of the retina, whereas block of RA synthesis early on results in an eye with no ventral retina (Fig. 5). These experiments were some of the first to show that the ventral and dorsal parts of the retina are quite distinct, especially with regard to development.

A collaboration with Wally Gilbert's groups at Harvard was undertaken to see if we could mutagenize zebrafish using insertional viral methods. In this we failed, but in the mid-1990s efficient methods for mutagenizing zebrafish chemically became available, and Sue Brockerhoff, Jim Hurley, and Jim Fadool began to look for functional and developmental mutations in zebrafish that were eye specific. To this end, Sue developed a behavioral test, based on the optokinetic reflex (OKR), that enabled her to examine visual function in 5-day-old larval fish. She eventually isolated a number of mutants that were completely or partially blind or even color blind. Jim Fadool isolated several developmental mutants, a number of which were worked on subsequently by newer members of the team, including Brian Link and Tristan Darland. A second behavioral test, based on the escape response, was developed by Lei Li, another postdoctoral fellow, that enables us to examine visual behavior in adult fish. Fish with slow inherited retinal degenerations were found with this test, which also permitted us to study dark adaptation and the effects of the circadian clock on visual responsiveness.

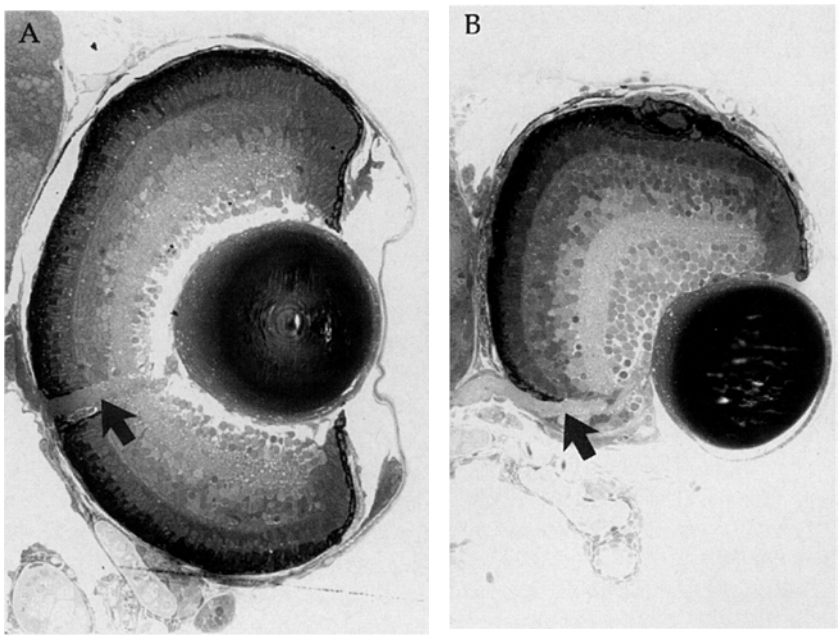

Fig. 5. The effect of blocking RA synthesis by citral on development of the zebrafish eye. In the citral-treated eye (B), no ventral retina is present. The control (A) is a 5-day-old eye. The arrow in each micrograph points to the optic nerve. (From Marsh-Armstrong N, et al. *Proc Natl Acad Sci USA* 1994;91:7286–7290. With permission.)

We continue to work on zebrafish and I remain confident that they have much to teach us. Not only can the ERG be recorded from the zebrafish eye, but slices of the zebrafish retina can be made, making it possible to examine the responses of single retinal neurons. The opportunity to carry out anatomical, physiological, and pharmacological studies on retinas from animals whose genetics can be manipulated presents a wonderful opportunity. But only time will tell us how far the zebrafish can take us.

As one becomes an elder statesman in a field, invitations to join boards pour in and, as I noted earlier, I say "yes" too often. Thus, in the 1990s I was on the board of over 20 organizations at one time or another, and some I still sit on. All were interesting, but they do take time. I also served as President of ARVO in the mid-1990s and on the Council of the National Academy of Sciences. I continued to teach my introductory neurobiology course and core courses and became involved in a new interdisciplinary program at Harvard called Mind, Brain, and Behavior (MBB). This interfaculty initiative was set up by the University President to inquire as to what impact modern neuroscience is having, or might have, on other academic disciplines. I was

primarily involved in establishing an undergraduate program in MBB, and today we have five participating concentrations; Biology, Computer Science, History of Science, Philosophy, and Psychology. My introductory neurobiology course is taken by everyone in the program, and because of the diversity of students taking the course with varied backgrounds, it has broadened considerably and is now entitled "Behavioral Neuroscience."

Epilogue

At this point in my life, I remain active with a full laboratory and teaching schedule. I intend to stay full time for several more years, but then I think it is important to scale back and allow the resources I am using to go to younger scientists. I believe our research is still of high quality, but then I am reminded of something Henry Rosovsky said to me when I was Associate Dean at Harvard: "Whenever I talk to one of our scientists, he tells me he is doing the best work of his career. That can't be true!" There are several reasons why scientists believe the work they are presently doing is the best of their careers, but of course, Henry is correct.

What have I left out of this account? My indebtedness to many people, but especially to my wife of 28 years, Judith, who has been so supportive. Her only complaint has been that I do too many things, and she is almost certainly correct. It is the second marriage for both of us, and we have his, hers, and our own children—all of whom are now grown and doing their own thing (none in science, by the way). I have had an assistant at Harvard, Stephanie Levinson, for more than 25 years, and she has contributed to the laboratory and our research in innumerable ways. Many at Johns Hopkins and Harvard have served us well, and a number are mentioned in this piece. But there are others who have played important roles and they are not forgotten.

It has been a full and fortunate life, with many "ups" and very few "downs." Cajal said in *Recollections of My Life* that "The retina has always shown itself to be generous with me," and I feel exactly the same. Some 30 years ago, I rashly predicted that the retina would be pretty much understood in 25 years. I now recognize how wrong I was, but the field has come far, and I am delighted to have been part of this adventure. When I began, little was known of the mechanisms underlying light and dark adaptation and the relationship, if any, between visual pigment levels and visual sensitivity. Retinoic acid was unappreciated as a biologically important molecule. Retinal synapses and circuitry were virtually unknown, and the electrical responses of the retinal neurons were mysterious, save for those of the ganglion cells.

Today not only has much progress been made on these issues, but many others as well. The major excitatory and inhibitory neurotransmitters in the retina are identified, and we realize that the majority of neuroactive substances in the retina serve as neuromodulators—not initiating retinal

activity but modifying it; in response to light conditions, time of day, and so forth. Clues as to how the retina develops from unspecified precursor cells have emerged and so on and so forth. Many investigators and laboratories have contributed to our present understanding of this tiny piece of the brain. Not that all of the mysteries are solved—far from it—but the progress made over the past 50 years is satisfying. I only wish I could be present in 2050 to hear then of the understanding of this "approachable part of the brain."

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