
In memoriam

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The vision research community lost a valuable colleague and friend when Ramon (Ray) Dacheux II died on May 30, 2006 at his home in Birmingham, Alabama. Ray was 58 and had been ill with a brain tumor for two years.

Ray was born in York, Pennsylvania in 1947. When he was sixteen, his father, a career non-commissioned officer in the Navy, purchased a farm in Dillsburg, Pennsylvania and Ray, as the oldest of eight children, was expected to run the farm and make it profitable, because his father was stationed away from home for long periods of time. Ray had to learn farming from scratch and, with his younger brothers, brought the farm into a profitable enterprise with long working hours before and after school. Although Ray was expected by his parents to carry on with the farming, he was intent on going to college. He was an outstanding wrestler in high school and received several scholarship offers for wrestling and football, including one from Penn State. Ray, however, wanted to make sure that if he were injured; his scholarship would remain in force: Lycoming College in Williamsport, Pennsylvania, agreed to this condition and he entered this college in the fall of 1965. Ray's preoccupation for completing a college education turned out to be prophetic, as he was injured in his freshman year and was unable to continue in the sport. At Lycoming, Ray met his wife Sandy, whom he married after they graduated in 1969. Ray and Sandy formed an inseparable partnership and had two daughters Tina and Stacy.

The elimination of the demands of varsity sports freed Ray to pursue academic subjects and he discovered a consuming interest in experimental biology. He started to work in the laboratory of Robert Angstadt, a professor of biological sciences at Lycoming, on auditory imprinting in chicks and discovered that these birds could be imprinted to sound before the time of hatching. He developed a close working relationship with his mentor and carried this senior research project far beyond what was expected of an undergraduate. Ray obtained his B.A. with Honors in Biology in 1969 with a project entitled, "Intracerebral Injections of Puromycin and the Effects on Imprinting in Domestic Fowl." Angstadt, who later became a lifelong friend of Ray, saw in him the potential to become a career scientist and encouraged him to apply to graduate school.

Indeed, Ray was admitted to the graduate program of the Physiology Department in the State University of New York at Buffalo (SUNYAB), thanks to the enthusiastic support of Werner Noell, a member of the admissions committee and the head of the Neurosensory Laboratory, who had been impressed by Ray's undergraduate experience. After two years of graduate work, Ray

earned his Master's Degree in 1971 and began to look for a position as a research assistant. It was during the final year of his studies that Robert Miller, one of the authors of this article, met Ray in the course of his visits to Buffalo. Ray, under Werner's direction, had developed his first superfused rabbit eyecup preparation and was using it for a study of ERG wavelets in the light adapted retina that became the object of his M.A. thesis. Miller began to collaborate with Ray in perfecting the preparation for intracellular recordings and when he became an assistant professor in the Neurosensory Laboratory, hired Ray as a research assistant.

Ray and Miller thus began a long and fruitful collaboration by working alternatively on the rabbit and salamander retina-eyecup preparations. Ray had an insatiable appetite for learning and, as the years went by, Miller realized that he was evolving from a laboratory assistant into a full-fledged collaborator. He therefore convinced Ray to apply to the Ph.D. program in Physiology. Ray was accepted in 1975 and Miller became his thesis advisor. In 1978, Ray obtained his Ph.D. in Physiology by completing a thesis entitled "A Physiological Study of the Ontological Formation of Synaptic Interactions in the Rabbit Retina." Clyde Oyster was Ray's external examiner and became his long-lasting friend. Miller still marvels at the quality of Ray's recordings from his early work, which led to two publications in the *Journal of Comparative Neurology*. In the eight years that Ray spent working with Miller, they published 18 full-length papers, three of which were published in *Science*.

Ray and Miller left Buffalo the same year, the first for a postdoctoral fellowship at Harvard and the second for a professorship at Washington University in St. Louis. Miller still has an enduring memory of the years spent with Ray and of the moment in which he turned out the lights for the last time in his laboratory at Buffalo, seeing the long sheet of paper on the wall with dozens and dozens of entries where they had, over the years, kept the record of their best results and of their competition for bragging rights and Friday beers.

Just before completing his Ph.D. thesis, Ray showed up in the office of Elio Raviola, the other author of this article, in the Department of Anatomy at Harvard Medical School. He wanted to have a postdoctoral experience in Raviola's laboratory to learn electron microscopy at the very time Raviola was looking for a collaborator with Ray's kind of expertise and goals. Raviola was immediately struck by Ray's solidity and motivation and assigned him a tough anatomical project for a beginner: to study the connections between photoreceptors and small bipolar cells with a Landolt club by examining serial sections of Golgi-stained, gold-

toned turtle retinas. He did a wonderful job: he found that the cell was postsynaptic to cones and rods at both invaginating and basal junctions and his electron micrographs of impregnated cells were among the best in the literature. He showed so much skill and independence that the paper was published with his name as a sole author.

After Raviola obtained an NIH a grant entitled "Cell Communication in the Retina," Ray built a setup for visual stimulation and intracellular recordings from the rabbit eyecup and thus began a fruitful collaboration that lasted 14 years. In 1981, when he became an assistant professor, Ray switched to principal investigator in the grant, which continued to be the major support for his research for 27 years. He became an associate professor of Anatomy in 1985.

Throughout the years of their collaboration, twice a week Ray and Raviola were recording from rabbit retinal cells and injecting them afterwards with horseradish peroxidase (HRP). The experiments lasted from early in the morning through most of the following night and Ray would then sleep at Raviola's house. They spent more time together than both of them did with their families and became intimate friends. Ray was remarkable both as a scientist and as a man: he combined his imagination and encyclopedic knowledge of the literature with precision, patience, and persistence. He was a virtuoso of the job well done and was endowed with unusual manual skills: his were the hands of an artist. Very few things in life are as rewarding as sharing the joys and frustrations of scientific discovery with a trusted colleague of great intellectual integrity and the same aspiration for excellence. When the work becomes a silent, graceful, well-choreographed ballet of precise, perfect gestures; when there is little or no need to speak and the collaborators complete each other's sentences because they have become one with their object of study.

Ray and Raviola devoted their investigations to the neural network encoding the signals of rod photoreceptors in the rabbit retina: they analyzed the structure of rods isolated from the adult retina (1988, with Ellen Townes-Anderson, now a professor of Neuroscience at New Jersey Medical School) and correlated the response properties of horizontal cells with their morphology and synaptic connections with the photoreceptors (1982, 1990). They obtained the first electrophysiological recordings from H1 horizontal cells in the rhesus macaque and showed that these cells were homologous to the axon-bearing horizontal cells of other mammalian retinas (1990). At the time, there were uncertainties on the response properties of rod bipolars, until Dacheux and Raviola showed that they responded to light with a transient-sustained depolarization dominated by rods and had a center-surround organization of their receptive field. Therefore, the dyad synapse established by rod bipolars with the two depolarizing amacrine cells postsynaptic to them (A2 and A17 or S1/S2) was excitatory and sign-conserving (1986, 1987, and 1989).

This work was followed by a series of papers with Enrica Strettoi (now a senior investigator in the Institute of Neurophysiology of the National Research Council in Pisa, Italy), in which the synaptic connections of the neurons that carry rod signals to ganglion cells, rod bipolars, A2 amacrine and cone bipolars, were reconstructed from continuous series of thin sections analyzed with the electron microscope. These papers established unequivocally that "the rod pathway in the rabbit piggybacks the cone pathway and thus gains access to a single set of ganglion cells that are driven by both rods and cones" (1990, 1992, 1994).

During the years at Harvard, Ray undertook other studies that were either published or concluded after his move to the Univer-

sity of Alabama in Birmingham. He participated in the supervision of John Heussy, a premedical student at Harvard (now a practicing psychoanalyst in New York), as he prepared Golgi-stained rabbit retinas and made marvelous drawings of impregnated bipolar and amacrine cells (see cover of this issue) that were subsequently used for a classification of these cell types in collaboration with Julie Sandell and Richard Masland (1999, 2004). A paper in collaboration with Adalberto Merighi (now Chairman of the Department of Anatomy, Biochemistry and Physiology of the School of Veterinary Medicine in Turin, Italy) showed that two types of OFF-cone bipolar cells received cone input exclusively at basal junctions (1996). Finally, Ray built a setup for patch clamp recordings from retinal slices that was used by his M.D.-Ph.D. student, Michael Gillette (now a Cancer Program Scientist at the Broad Institute) in a study of GABA-activated currents in rod bipolars. They showed that in these cells both vasoactive intestinal peptide (VIP) and a PKC-mediated mechanism decreased the GABA-activated Cl^- current (1995, 1996).

Ray joined the Department of Ophthalmology of the University of Alabama at Birmingham as an associate professor in 1992 and became a professor in 1995, with secondary appointments in the Department of Physiological Optics and the Visual Science Research Center. During this time, he contributed important work on bipolar cells: he identified six anatomically distinct types of cone bipolars whose axon stratifies in the OFF-sublamina of the inner plexiform layer (IPL). In all of them the glutamate agonist kainate opened cation channels and none of them responded to an agonist at the metabotropic glutamate receptor. Thus, all could be identified as OFF-bipolars. Additionally, six types of cone bipolars sent their axonal arborization to the ON-sublamina of the IPL: in all of them an agonist at the metabotropic glutamate receptor mGluR6 closed channels, identifying them as ON-bipolars (with G.S. McGillem, 2001). Cone bipolar cells exhibited different sensitivities to the inhibitory transmitters GABA and glycine; interestingly, three types of OFF-bipolars had larger glycine- than GABA-activated currents, suggesting that they received A2 amacrine cell input, and therefore carried OFF rod signals (with C. Zhou, 2005). This work was accompanied by an exhaustive analysis of the transmitter-gated currents of A2 amacrine cells (with C. Zhou, 2004).

In a series of works on rabbit ganglion cells, Ray confirmed the classic observation by Barlow, Fitzhugh and Kuffler in the cat that alpha ganglion cells lose their antagonistic surround in dark adaptation (with J. F. Muller, 1997). He identified two pharmacological types of ON-alpha ganglion cells, based on the relative amplitude of GABA- and glycine-activated currents, whereas both types expressed GABA_C receptors. In contrast, glycine-activated currents were consistently larger than GABA-activated currents in OFF-alpha ganglion cells and these neurons expressed GABA_B but not GABA_C receptors (with T.C. Rotolo, 2003). He finally analyzed with the electron microscope the synaptic connections of a serially sectioned, HRP-injected, ON-OFF directionally selective ganglion cells, describing a microcircuit potentially mediating feed forward inhibition (with Chimento and Amthor, 2003).

In Birmingham, Ray revealed a new aspect of his personality: this reserved man transformed himself into a successful fundraiser and developed an impressive research operation in the Department of Ophthalmology. Devoted to his department, he felt that he also had to bring a contribution to solving problems more immediately related to human health. He compared gene expression in Müller cells from avascular and vascular retinas and discovered that during experimental proliferative vitreoretinopathy epiretinal mem-

branes caused changes in the cytoskeletal proteins of the Müller cells in the underlying retina (with G.S. McGillem 1998, 1999). He also became interested in the pathophysiology of glaucomas. In his last published paper, he induced a transient increase in intraocular pressure in the rabbit by injecting latex beads into the anterior chamber. He showed that the increase in pressure caused retinal ganglion cell damage and thinning of the nerve fiber layer of the retina. In addition, the lactate concentration in the vitreous body, as determined by NMR spectroscopy, increased and returned to baseline in parallel with the changes in pressure, a phenomenon possibly related to neuroprotection.

To his skills as a scientist, Ray combined intense commitment, a constant effort to improve himself and a remarkable dedication to duty. An important example of these qualities was his teaching activity at Harvard: to become a faculty member in an Anatomy Department, he had to become proficient in teaching human anatomy and histology to medical students, both subjects he had never been exposed to before. In two years, he managed to become an outstanding dissector and one of the favorite teachers of his demanding students, who appreciated his competence, professionalism and dedication.

Ray was a profoundly devoted husband and father. Both authors of this article witnessed in the course of his illness the extraordinary bond between Ray and Sandy: on the one hand, he mustered superhuman strength in facing adversity, hopelessness and pain, trying to spare Sandy from the worst of his suffering. On

the other hand, we equally admired the strength and dedication of his life companion who continued to comfort him with serenity and tenderness until the last moment of his life.

We scientists are reluctant to sing the praises of our work or colleagues, because we are aware of how short life is, how long is our art, how difficult it is to discover the secrets of nature, how brief is the joy or success, how frequent the defeat. Yet, it is amazing how universal has been the admiration and love of his peers for this unusual man. As a student, a postdoctoral fellow and a friend, Ray never let down either of his two mentors: this is most that one can ask of a pupil, an intellectual partner, a friend or a family member. We are missing him with all our hearts.

When the idea first surfaced that Ray's contribution to retina research should be honored by establishing a Ramon Dacheux II ARVO Memorial Travel Fund, a fund drive was established within the ARVO foundation, to raise the required \$30,000 endowment, a level that has to be reached before a travel grant can be awarded. In an expression of appreciation for Ray's many contributions to the UAB Ophthalmology Department and vision research community there, the International Retina Research Foundation (IRRF) funded the entire endowment that made the travel award available for the 2007 ARVO meeting and for ARVO meetings in perpetuity. We sincerely thank director Michael Callahan and Sandy Blackwood of the IRRF, for the generous support provided to establish this memorial remembrance for all of us whose lives were touched by Ray Dacheux.