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Tribute

From string galvanometer to computer: Haldan Keffer Hartline (1903–1983)

H. Keffer Hartline was a pioneer in research on the visual system. Beginning in the 1920s with a string galvanometer and later using a digital computer, he explored almost every aspect of vision. Focusing his efforts on relatively simple model systems, he uncovered basic mechanisms of visual function common to many animals, including man. In recognition of his outstanding contributions, he was awarded the Nobel Prize for Physiology or Medicine in 1967 with Ragner Granit and George Wald. He died in 1983 in his 80th year.

A walk in the woods was his daily outing. During his last years, Keffer, as he was known to his friends, spent many enjoyable hours exploring the woods at Turtlewood, his home near Hydes, Maryland. Visiting colleagues who joined him found it was anything but a leisurely stroll as Keffer led the way up steep slopes and down ravines stopping only to catch the song of a bird or inspect the tracks of other inhabitants. By keeping up, one was rewarded with lively conversation ranging from the wonders of nature to the values of basic research. His favorite perch, the 'think bench' (photo), often provided a refreshing pause during the vigorous venture*.

Keffer's love of nature and the outdoors can be traced back to the close relationship he had with his father, a biology teacher. During his early years, Keffer and his father hiked together in the mountains near their home in Bloomsburg, Pennsylvania, and he accompanied his father on many class field trips. Keffer considered his father his first and best teacher. He also had a very high regard for Beverly W. Kunkel, Professor of Biology at Lafayette College, and credited him with stimulating his interest in basic research.

After entering Lafayette in 1920, Keffer quickly drew the attention of Professor Kunkel who was impressed by his prowess and range of knowledge. To get him involved in lab work, Kunkel suggested that land isopods (pill bugs) might be interesting creatures to study. Keffer quickly collected a number of them from the nearby woods and established small colonies. After several weeks of observation, he reported to Kunkel that he was not certain what could be learned from the isopods. Kunkel laughed and suggested Keffer return to the laboratory. Eventually he noticed the isopods tended to avoid light and thought this behavior might be interesting to study. With meticulous care that was to typify his later work, Keffer investigated their visually guided behavior, and at age 20 wrote his first paper[1]. He concluded, 'These experiments seem to show a relationship between certain photochemical laws and the phototropism of animals'. This study instilled in him a keen interest in the neural events leading from photochemical reactions in the eye to changes in an animal's behavior. It set the stage for his career in vision research.

While still a college student, Keffer encountered both Selig Hecht and Jacques Loeb, the two leading investigators whose prevailing theories of visual function had played important roles in his just-completed study of isopods. Building on Kühne's discovery of a light-sensitive pigment in the eye, Hecht[2] set forth in 1919 a photochemical theory to explain light-induced changes in visual sensitivity. A year earlier Loeb[3] had proposed a phototrophic theory to explain how light might control an animal's behavior. The three met at the Marine Biological Laboratory in Woods Hole, Massachusetts. Both Hecht and Loeb were impressed with Keffer's attempt to cast his results in terms of their theories. Aware of Keffer's desire to pursue basic research, Loeb advised him to enroll in a medical school.

The following year, 1923, Keffer entered the Johns Hopkins Medical School brimming with enthusiasm for laboratory work. Much to his dismay, the medical courses proved very time consuming, and those experiments he could manage to do were impeded by mechanical and electrical interference. Encouraged by E. K. Marshall and C. D. Snyder, he worked out a schedule for lecture work during the day and experimental work at night when, to his delight, the sources of interference were minimal. He modified an Einthoven string galvanometer belonging to Snyder to improve its speed of response and began studying the electrical responses of the eye. He was fascinated with an evoked retinal potential, the electroretinogram (ERG), which he was able to record from frog, cat, and human. Although others had recorded such responses, he detected for the first time the individual components of the human ERG, and put to rest a long-time theory of the origin of the vertebrate ERG[4]. He was indeed heartened to be studying retinal signals generated by light stimulation, but in time he grew weary of the complex wave form of the vertebrate ERG.

Armed with Snyder's modified galvanometer, he returned to Woods Hole in 1926 in search of a simpler visual system, one that would be an appropriate model for studying the cellular events that follow visual excitation. After testing a wide range of marine species, he decided that the horseshoe crab, *Limulus polyphemus*, which he had briefly encountered during his isopod studies, yielded the simplest ERG wave form. He formulated a set of experiments based on the Bunsen-Roscoe law that related the intensity and duration of a light stimulus for photochemical reactions. He found that the *Limulus* ERG obeyed this relationship, and concluded that the response resulted from a chain of unknown cellular events triggered by a photochemical reaction in the retina[5].

This reductionist approach to retinal function characterized all of Keffer's research. He constantly tried to interpret biological mechanisms in the simplest terms – according to the laws of physics and chemistry. Convinced of the value of these disciplines, he travelled to Germany after medical school to study physics as a Johnson Research Fellow. He attended lectures by Heisenberg in Leipzig, Sommerfeld in Munich, and Einstein in Berlin – an awesome trio for the newly graduated

*Unless otherwise noted, quotations and personal accounts are taken from letters and conversations between Hartline and the author.

medical student. Keffer found it very tough going but managed with long walks in the Alps to endure the rigors of theoretical physics for more than a year before returning to the States. Detlev Bronk returned at about the same time from Cambridge University, England, to assume the directorship of the E. R. Johnson Foundation at the University of Pennsylvania, and offered Keffer the position of Fellow in Medical Physics. He readily accepted. The emerging field of biophysics had a new advocate.

As Keffer strove to understand the nature of evoked responses in the retina, a revolution was under way in neurophysiology. It started in 1921 with Gasser and Newcomer's design of a three-stage vacuum tube amplifier capable of detecting action potentials in the phrenic nerve[6]. A year later, Erlanger[7] developed a cathode-ray tube that could display action potentials, and then Forbes[8] recorded nerve impulses in response to muscle stretch. Across the Atlantic, Adrian and Zotterman[9] isolated single-nerve fibers from stretch receptors, Adrian and Bronk[10] recorded responses from single motoneurons, and Matthews[11] fabricated a reed oscillograph to facilitate the analysis of trains of nerve impulses. By the early 1930s it was clear that Adrian's laboratory had launched a new era in neurophysiology: the analysis of neural activity of single cells.

Adrian's pioneering approach to neural function had a major impact on Keffer's work. After learning of the rapid advances taking place in Cambridge, England, Keffer quickly acquired a vacuum tube and constructed an amplifier to feed a moving reed oscillograph designed by Matthews. He returned to Woods Hole in 1931 with Clarence Graham in the hope of repeating on the *Limulus* optic nerve what Adrian and Bronk had done with the rabbit phrenic nerve, i.e. record from single nerve fibers. They were optimistic because several years earlier, at Woods Hole, Keffer had detected discrete electrical events with a string galvanometer when he placed the recording electrode on the animal's optic nerve. They found they could easily record mass discharges of nerve impulses from the optic nerve trunk of juveniles but could not isolate the response of a single fiber. With only a few days of summer remaining, Keffer tried, out of frustration, to record from the eye of a large adult that happened to be the last animal in the aquarium. His success was immediate.

The outpouring of results from the single fiber experiments with *Limulus* was enormous[12]. They touched on almost every aspect of vision and led to the formulation of basic mechanisms of retinal function applicable to many species. It was clear to Keffer that a wide range of visual phenomena, such as light and dark adaptation, flicker fusion, and spectral sensitivity, originated in the retina of this primitive animal. He felt *Limulus* was not unique: important visual characteristics probably originated in the retinas of all animals, including man.

Keffer viewed his work on the eye of the horseshoe crab as a stepping stone to studying the vertebrate visual system. However, before touching upon this aspect of his research, it is interesting to consider his early behavioral studies of the shadow reactions of barnacles, scallops, and tube worms[13]. When Keffer was at the Marine Biological Laboratory in 1925, Hecht had just published experiments on the reaction of the clam, *Mya*, to flashes of light. Recalling that summer's work, Keffer wrote, 'I thought I'd try flashes of darkness. Of the many animals that respond to shading, I looked into three – barnacles, tube annelids, and the scallop, *Pecten*. All gave good responses, but the scallop proved the easiest to study. I clamped one valve in a dish of seawater, hooked a wire on the upper valve, so that when the shell snapped shut, I could have it close a switch and so measure the reaction time. I got consistent results – latency varying with intensity. The work never came to much – but it was the interest I developed that led to the later work on the 'on' and 'off' discharges in the optic nerves of those beautiful little eyes.' (From a letter to the author; *Pecten* optic nerve recordings were published in Ref. 14.) The 'off' discharges in response to 'flashes of darkness' intrigued him. They played a key role in his studies of the vertebrate retina.

Haldan Keffer Hartline

In selecting a 'model' vertebrate retina, Keffer was reminded of the magnificent ERGs he had earlier recorded from the frog eye. He decided to try to record responses from single optic nerve fibers of this eye using the same techniques that had proved so successful with the *Limulus* eye. He attempted many times to desheath the optic nerve trunk and dissect from it a single fiber to place on a cotton wick electrode. The cottage cheese consistency of the optic nerve defied this approach; it simply disintegrated instead of dividing. While driving home one day, he wondered, 'Why in the devil do I want to dissect the optic nerve? It is already dissected for me – the fibers are all spread out on the vitreous surface of the retina. So I turned the car around and went back to my lab. But, of course, it's one thing to think of that and another to do it. It was a long time before I finally got the technique down'.

(From letter to the author.) His persistence was richly rewarded.

Keffer's studies of single optic nerve responses in frog changed the course of vision research. They established new concepts regarding the retinal mechanisms that process visual information. He found that some fibers responded to the onset of light, others to the offset, and some to both[15]. Such a diversity of responses termed 'on', 'on–off' and 'off', clearly indicated to him that the retina does more than just relay to the brain a neural picture of the outside world. It integrates the responses of photoreceptor cells, producing complex neural signals that it then transmits to the brain.

A single fiber's response was not only complex, but it could be elicited by light stimuli located in a number of positions in front of the animal. Keffer found that each fiber had associated with it a specific region in the visual field in which flashes of light could elicit a discharge of nerve impulses, whether they be 'on', 'on–off', or 'off'[16,17]. He defined this region as the 'receptive field' of the optic nerve fiber using the term Adrian introduced several years earlier to describe the area of skin innervated by a single afferent fiber[18]. Near the turn of the century, Sherrington had used a similar term, 'reflexive receptive field', to indicate the region of the body that could influence the activity of a single motoneuron in the spinal cord[19]. Keffer found strong parallels between his work and that of both Adrian and Sherrington. The concepts of neural integration set forth by Sherrington for the spinal cord were particularly relevant to Keffer's work on the retina.

The visual system integrates spatial information. Keffer's studies with frogs clearly showed that the integration begins in the retina. This result had a profound impact on the field. Horace Barlow extended Keffer's work on the frog retina and discovered that the response of a single optic nerve fiber to stimulation in the center of its receptor field could be inhibited by illumination of surrounding areas[20]. Although Keffer had noted that 'on–off' responses could be suppressed by nearby stimuli, he did not detect the center–surround configuration uncovered by Barlow in 1953. In the same year, Stephen Kuffler found that surround inhibition was a prominent feature of cat optic nerve fibers[21]. These pioneering studies established conceptual foundations for much of the current research in vision.

Although the frog retina yielded new and exciting results, Keffer eventually found the complexity of the single fiber responses as disturbing as that of the ERG he had studied several years earlier. During the time he experimented on the frog retina, he also continued studies of the *Limulus* eye. Countless times he had noticed that turning on room lights decreased the response of a single nerve fiber of the *Limulus* eye, but did not appreciate its significance. Since his initial work with Graham, he felt that one of the reasons the *Limulus* eye was a fortunate choice of material was that the photoreceptors functioned independently of one another. Why he was suddenly alerted to the effect of room light is not clear; perhaps his recent work with the vertebrate retina was influential. Whatever the case, he finally grasped its meaning: neighboring receptors in the *Limulus* eye inhibit one another[22].

Keffer's discovery of lateral inhibition in the *Limulus* eye initiated a truly remarkable line of research extending over three decades[12]. The advantages of the *Limulus* eye over that of the frog were clear. The interactions among *Limulus* receptor cells were purely inhibitory, and it was possible to record the response of several neighboring cells while they were individually illuminated. In addition, stable optic nerve responses could be recorded from the excised *Limulus* eye for many hours. Taking full advantage of these characteristics, Keffer and his co-worker Floyd Ratliff found that the steady responses of individual optic nerve fibers could be quantitatively expressed in terms of the algebraic sum of inhibitory influences of neighboring receptors. This achievement stands today as the only complete quantitative analysis of neural integration among a matrix of sensory receptors. The well-known Hartline–Ratliff formulation has been the starting point for a number of treatments of information processing in more complex neural systems.

The discovery of lateral inhibition in the *Limulus* eye had immediate consequences for understanding the mechanisms of human vision. Nearly 100 years earlier, Ernst Mach had hypothesized that the ability of the human visual system to enhance contrast could be simply explained by mutual inhibitory interactions in the retina[23]. There was no need to attribute all contrast effects to high-level neural processing. Physiological support of Mach's idea waited many years. It came from a visual sytem far simpler than our own.

As the studies of lateral inhibition progressed in the late 1940s and early 1950s, Keffer's laboratory was also making significant strides in understanding the early neural events in visual excitation. In 1935, Keffer detected an 'action current' with external electrodes placed on the surface of an exposed ommatidium of the *Limulus* eye[24]. Its coincidence with the propagated impulses of the optic nerve suggested to him that the 'action current' could initiate the impulses. Ten years later, with the advent of glass micropipettes capable of penetrating single cells[25], Keffer and his colleagues returned to the problem of photoreceptor excitation. They successfully impaled a single retinal cell and recorded for the first time a depolarizing potential that appeared to be 'intimately related to the initiation of nerve impulses[26]'. Soon after joining Keffer's laboratory, Tsuneo Tomita[27] together with E. F. (Ted) MacNichol[28], showed that the so-called 'generator potential' results from an increase in cell membrane conductance and is indeed related to the generation of nerve impulses. These germinal studies, continued in many laboratories throughout the world, have led to a detailed understanding of the early excitatory events in the process of phototransduction in both invertebrate and vertebrate retinas.

By the late 1950s, it was clear to Keffer and his colleagues that significant advances in understanding mechanisms of neural integration in the retina required a convenient method for collecting and analysing large numbers of nerve impulses. Enter the digital computer. It was a large, awkward machine with little memory and virtually no software, which filled an entire laboratory. With much diligence and self-taught programming skills, Keffer and his students put the computer to use analysing the temporal characteristics of the inhibitory interactions in the *Limulus* eye. Inclusion of the temporal properties of retinal integration significantly increased the complexity of the theoretical formulations. Nevertheless, they succeeded in analysing with precision patterns of optic nerve activity in response to dynamic patterns of illumination of the retina and incorporating the results in a tractable theoretical framework[29] – an extension of the original steady-state

formulation described above. The success of these studies was indeed satisfying to Fred Dodge, Bruce Knight, Norman Milkman, Jun-ichi Toyoda, and others of the group. They realized that more complex neural networks may preclude an equally comprehensive analysis, but hoped their work would shed light on possible mechanisms underlying dynamic interactions in other nervous systems. These studies were concluded in 1974. They brought to a close Keffer's active participation in laboratory research.

Keffer's distinguished career extended over 50 years from the embryonic day of neurophysiology when signals were amplified mechanically to the modern era of computer technology. Throughout, he pursued problems that interested him with great imagination and a gentle, unassuming style that inspired those around him.

He was the consummate scientist. The laboratory was his home. He enjoyed the long solitary hours of dissecting single fibers from the frog retina as much as hiking in the Grand Tetons, his favorite mountain region. In the early years he generally worked alone, however after World War II his collaborative research activities expanded at the Johnson Foundation. His administrative activities also expanded when in 1949 he accepted Detlev Bronk's invitation to chair the newly created T. C. Jenkins Department of Biophysics at the Johns Hopkins University and become its first Professor of Biophysics. He was joined by Lloyd Beidler, John Hervey, Ted MacNichol, William Miller, Floyd Ratliff, Lorrin Riggs, Tsuneo Tomita, Henry Wagner, Myron Wolbarsht and Stephen Yeandle – a truly formidable group. After four years at Hopkins, he moved to the Rockefeller Institute, again at the invitation of Det Bronk, its new president. Bronk knew Keffer and his productive group would help convert the Institute to a graduate university. The university flourished as did Keffer's laboratory. He maintained a solid group of senior scientists and took on a number of students. I was fortunate to be one of them.

The atmosphere of Keffer's laboratory has been accurately described as 'an extremely fertile chaos'. One never had the impression that the course of research was following a carefully laid out plan. With a gentle hand, Keffer encouraged everyone, co-workers and students alike, to follow their own noses wherever they lead. This approach, as I can attest, occasionally caused apprehensions in students who sought his advice in selecting an appropriate problem for their dissertation research. Possibly influenced by his interactions with Professor Kunkel as an undergraduate, Keffer invariably declined to decide for the students and instead encouraged them to return to the laboratory hoping they would arrive at the 'appropriate' decision themselves. Sometimes they did.

Keffer's hands-off approach to the research of others in his laboratory could have been disconcerting. He rarely peered over the shoulders of colleagues and students to inquire about their progress. However, any thought that this represented a lack of interest in your work was immediately dispelled when you asked him to look at something you had running in the lab. His enthusiasm was infectious. On a number of such occasions we spent hours on end working together with an experimental preparation. It was a joy to observe him manipulating delicate retinal tissues. He had a rare gift for such work, as anyone who tries to repeat his experiment on *Pecten* and frog eyes will quickly realize. As the clock approached the wee hours of the morning, it was never clear who would give up first – Keffer, me, or the experiment. Totally exhausted, we finally would stop . . . cherished moments indeed.

Keffer's interactions with his colleagues and students reflected his overall philosophy regarding basic research. He strongly believed that 'significant advances come from scientists who are free to work out their own ideas. Directing basic research is counterproductive.' Regarding research in vision, he felt that a thorough understanding requires a broad picture because vision is almost universal throughout the animal kingdom. 'It is unsound to confine your attention to just a few species.' The legacy of his work underscores this philosophy.

Although awarded science's highest accolade[30], Keffer was never at home with the fame that it brought. He requested that there be no formal memorial service or fund established in his memory. However, he said he would not object to a memorial concert as long as it included his favorite composers – the four B's. Typical of his humor, they were Bach, Beethoven, B'Mozart, and SchuBert. In the summer of 1985 a public concert, organized by his family and friends, was performed at the Marine Biological Laboratory where he began his physiological studies 60 years earlier.

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