

XXI. NEUROPHYSIOLOGY

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RESEARCH OBJECTIVES AND SUMMARY OF RESEARCH

As in past years, our objectives are diverse. Our long-range goal is to understand nervous action as it relates to mental action (e.g., perception, memory, and so forth). We are exploring different avenues into the problem, some of which prove to be blind alleys, or occasionally paths into research unrelated to our main purpose. In the coming year we expect to study the following subjects.

1. Membrane Processes

National Institutes of Health (Grants 5 RO1 EY01149-02 and 1 T01 EY00090-01)
Bell Telephone Laboratories, Inc. (Grant)

Stephen A. Raymond, Michael J. Binder, Louis L. Odette, Jerome Y. Lettvin

Dr. Raymond has constructed and begun to use a device for measuring nerve threshold. Contrary to common expectation, we have found that the threshold for single myelinated fibers in sciatic nerve of frog is constant under temperature changes of $\pm 6^\circ\text{C}$ and probably over a much wider range. (Threshold, however, must be both carefully defined and measured.) As would be predicted, the small noise of threshold fluctuation does seem to change with temperature. We have not checked whether this noise is $\frac{1}{f}$, as it would be according to theory. After testing a few pharmacologic agents, it is evident that the measure is not only sensitive but reliable.

The opportunities for research that open up are twofold. First, there is the development of a theory for threshold. No such theory exists at present. The Hodgkin-Huxley equations express clearly enough the current-voltage-time relations of the nonlinear conductances whose actions account for the nervous impulse. But no theory of actual threshold issues for the same reasons as would prohibit such a theory in a triggered nonlinear oscillator where only the current-voltage-time relations are known. (The problem is familiar to anyone who remembers M.I.T.'s Whirlwind computer.) One must know the pulse generator in terms of which parts exchange heat and materials, and how dimensions change, and so forth. In nerve there are several processes strongly and explicitly involved in setting threshold, and these are not given in the Hodgkin-Huxley equations. One is the so-called ionic pump that is driven metabolically. Another is the change of internal (and external) composition of fluids at the axon. A third is pH, as well as trace ion composition, etc. Since it is unlikely that we can gain enough information about all of these variables, our theory will be crudely empirical at best, taking account only of those most obvious processes that change threshold greatly and systematically. Of these the pump is immensely important.

The second opportunity has great practical consequence. It appears that under

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suitable arrangement one can study and actually fingerprint on peripheral nerve the action of drugs that are best known for central influence. The method is being patented. Of scholarly interest in the procedure is that, with it, one can make qualitative inferences heretofore available only from voltage-clamp data.

Besides the threshold studies, we are also pursuing a generating function for m, n, and h as determined by Ca^{++} adsorption and desorption.

2. Metabolic Processes in Nervous Tissue

National Institutes of Health (Grants 5 RO1 EY01149-02 and 1 TO1 EY00090-01)
Bell Telephone Laboratories, Inc. (Grant)

Edward R. Gruberg, Jerome Y. Lettvin

In 1955, O. H. Lowry, in a remarkable paper, found that the glycolytic and oxidative metabolisms are differently distributed in retina. At the same time various anatomists began exploring the localization of enzymes in tissue by staining for them. Most interesting, as well as frustrating, among their tools are the tetrazolium salts, beastly to prepare, capricious to use, and ambiguous in action. These tetrazolium salts accept hydrogen ions in redox reaction with dehydrogenases and their flavoproteins, and precipitate out as insoluble formazans, some of which are either intrinsically electron dense or easily take on, say, osmium to be viewed by electron microscope. Thus they compete with cytochromes in the process by which carbohydrates power a cell — and the theater of action is where the enzymes cluster, in the mitochondria.

Most important among the reliable findings with the tetrazoliums in the retina is that succinic dehydrogenase is seen only in the ellipsoids and paraboloids of the rods and cones and nowhere else. So clean, so marked is this finding that Jay Enoch (Department of Ophthalmology, Washington University School of Medicine, St. Louis, Missouri) was able to use it in making a "photographic" plate out of the receptor layer of retina in succinate medium. He found that the stain occurred earlier where the receptors are light-adapted.

How different is the distribution for the dehydrogenases of malate, lactate, and glutamate, from that of succinate! We find their investments in the plexiform layers and optic nerve layer, in those processes that the pigment epithelium insinuates between the outer segments of rods down to the outer limiting membrane, and in the Müller cells. All of these places are rich in mitochondria, but in mitochondria that, very suprisingly, lack succinic dehydrogenase. Furthermore, these nonsuccinic dehydrogenases also seem to differ among themselves with respect to distribution, the most oddly varied being the one that handles glutamate. We cannot yet perform the necessary microethology of oxidative and glycolytic chains, but, as Lowry himself established by more accepted methods, they are indeed differently distributed through the retina.

One of the most mysterious anatomical features of the retina is its lack of an intrinsic circulation. No capillaries dip into its substance. And, indeed, its major circulation, the choroidal bed, is divorced from retina itself by a syncytial sheet, the pigment epithelium that generates a large, constantly flowing electric current through the retina by some ionic pump. The retina is a thick structure many cell layers deep and with an extremely large cell-surface/cell-volume ratio, if one takes the plexiform layers into account. It is one of the most actively metabolizing tissues in the body and, together with the pigment epithelium, needs all the circulation it can get. An obvious riddle, therefore, is how the cells deepest from the choroid, e.g., the ganglion cells, are nourished. Certainly the Müller cells are admirable pipes and profuse enough. Given diffusion through their substance, aided, perhaps, by the standing current from pigment epithelium, one might imagine the exponential falloff of nutriment with depth and the

collateral exponential rise of excretion products to be diminished in space constant by flow through Müller cells. An additional mechanism, however, also suggests itself, that is, the sharing of metabolism between cells. One way of insuring an equitable source of energy to structures further from the choroid would be to have their food the excreta of cells closer to the choroid. That is to say, if the metabolism in the earlier cells is incomplete, intermediary metabolites issuing from them would sustain later cells.

Such a theory, although still hypothetical, has some attractive consequences. The first is that not only the retina but the brain is, in a sense, undersupplied by its capillary circulation on the basis of cell-surface/cell-volume ratio, using other active tissues such as liver or glands as a guide. And while glia are everywhere in the brain, pedunculated to capillaries and shaping the spaces between nervous elements, there just is too much surface around to make the plumbing intuitively plausible. (Of course our intuitions are almost always faulty, but let the observation stand.) Metabolic sharing in the brain as much as in the retina would be an attractive way of distributing the load. The idea of metabolic sharing would force a new view of the rash of "transmitter substances," all intermediary metabolites, which are now so favored by pharmacologists. As the situation stands now, the experimental neuropharmacologist plunges the equivalent of a large standpipe through the close-woven fretwork of neuropil in the brain and through it forces a solution of some chemical that he suspects of synaptic action. He looks for changes in behavior of the animal that suggest an excitement or depression of that part of the brain. These effects he interprets as an "imitation of actual nervous transmission" in that region. But, were the sharing of metabolism a tenable notion, then no surprise would be occasioned by the changed activity of neurons when their food supply is suddenly enriched. Given the sharing, none of the cells, in the ordinary case, would be running replete, but rather partly starved, since the rate process governance would be by the turnover from other cells. There should be, then, no local controls on consumption, and, therefore, the ability to overdrive cells by enlarging their food supply could be a consequence. These considerations are not wholly fantasy. For example, a new-born rat (or human) is extremely sensitive to circulating glutamate; in fact, a single dose can kill off many retinal ganglion cells and certain other cells in the hypothalamus. Oddly enough, the optic nerve fibers from the ganglion cells stain heavily for glutamic dehydrogenase. But such evidence, rather than only suggesting that glutamate is transmitter to the ganglion cells, points also to the possibility that the cells live, in part, by glutamate turnover. We intend to explore this possibility, using the molecular beam analysis devised by Professor John G. King and his group, especially Dr. James C. Weaver, whereby to some extent one can map enzyme activity in the tissue localizing down to a 10- μ m diameter.

3. Substantia Gelatinosa in Spinal Cord

National Institutes of Health (Grant 5 RO1 EY01149-02)

Ian D. Hentall

It now appears that the cells of the substantia gelatinosa in the spinal cord of the cat are not so difficult to record as previous published investigations have indicated. Laminae II and III can be tapped reliably and over long periods. The cells there share, with lamina I, a coding of noxious stimuli to skin and deeper tissues, but they also respond nicely to nonnoxious modalities, notably hair movement in the fur on the skin. Their receptive fields can be plotted. An account of these cells will form Ian D. Hentall's doctoral thesis.

The activity of units in this region may be excited antidromically by applying local stimulating currents to Lissauer's tract although difficulties exist in interpretation of such experiments because of the unpure and diffuse nature of this tract. Only cells in laminae II and III respond in such a way, although an entraining effect on the spontaneous

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activity of lamina IV cells has been observed. The concomitant field potential similarly appears to originate in the substantia gelatinosa. These are the conclusions of preliminary experiments, but they remain to be substantiated in full.

4. Behavior of Pigment Epithelium of Frog

National Institutes of Health (Grant 5 TO1 GM00778-19)

Mark Lurie, Eric Newman

We are hoping that Mark Lurie will return to advance his study of interaction between retina and pigment epithelium, which was the subject of his Ph.D. thesis research.¹ The first part of his findings is being prepared for publication, and is too extensive to abstract here. Suffice it to say that the activity of the pigment epithelium not only expresses the state of dark adaptation of the eye, but the current that it generates is directly implicated in governing the activity of certain ganglion cells.

References

1. Mark Lurie, "Aspects of Interaction between Retina and Pigment Epithelium: An Electrophysiological Study of the Circulated Frog Eye," Ph.D. Thesis, Department of Biology, M.I.T., June 1973.

5. Electrophysiological Study of Behavior in Stentor

National Institutes of Health (Grant 5 TO1 GM00778-19)

Eric Newman

Stentor coeruleus is a highly contractile ciliated protozoan that displays a wide range of behavioral characteristics. Preliminary experiments indicate that variations in the membrane potential of Stentor are related to changes in the behavior of the cell. Intracellular microelectrode recordings will be made in order to investigate the relation of membrane potential to cell contraction, stalk extension and shrinkage, membranellar and ciliary reversal, oral pouch contraction, and sensitivity to mechanical, chemical, and photopic stimuli.

6. Color Vision

National Institutes of Health (Grants 1 TO1 EY00090-01 and 5 RO1 EY01149-02)

Bell Telephone Laboratories, Inc. (Grant)

Michael H. Brill, Lynette L. Linden, Jerome Y. Lettvin

We have extended our earlier notions of color vision to account for the space of subjective color, that is, the colors actually seen and named. This extension involves a transformation from the space of color matching (which has a metric) to the space of color seeing (which has no metric). The details are both controversial and not completely worked out.

7. Visual System in Pigeons

National Institutes of Health (Grants 1 TO1 EY00090-01 and 5 RO1 EY01149-02)
Bell Telephone Laboratories, Inc. (Grant)

Edward R. Gruberg, Jerome Y. Lettvin

We are now preparing to study the optic nerve, diencephalic and mesencephalic structures in birds. Two subsystems are the subject of our main attack, the nucleus rotundus and the nucleus isthi parvocellularis that Dr. H. Karten has plotted anatomically. Our approach will cover two main features, first, the complex coding of visual processes in single fibers, easily seen and dissected in pigeons; and second, the modes of generating complex receptive fields in tectum and diencephalon.

8. Mechanism and Occasion of Color Change in Flounder

National Institutes of Health (Grant 5 TO1 GM01555-08)

William M. Saidel

Flounders, as classically known, alter the shading of their outer surface to conform to the texture of their surround. The change in color is rapid, for a vertebrate, and is said by some observers to be most vivacious in tropical species. Of the several interesting problems that this phenomenon brings to mind, two have become the subject for research at this laboratory. First, what is the mechanism and control of the color change, and second, what is the strategy of the change, and can it be given abstractly? We are exploring both questions before deciding which to pursue most deeply.

a. The scales are possessed of chromatophores, melanin-containing cells, that, in contrast with the chromatophores of the cephalopods, do not vary in their external shape. Instead the pigment is either withdrawn to a tight cluster intracellularly or migrates out along definite intracellular paths. Whether the pigment is withdrawn or migrates out seems to depend on a complementary innervation to the chromatophores, the neurons issuing from sympathetic ganglia. Neither the anatomy nor the peripheral junctional pharmacology is known. We are exploring this control now by dissection and stimulation of skin nerves.

b. The chromatophores are not distributed uniformly and under independent control. Instead, very much as reported for octopus, the surface looks as if there are positionally fixed "dark" centers more or less widely spaced and "light" centers also spaced. The strategy of coloration is to change the dark tone of the dark centers and spread the dark tone into the surround of each center and, similarly, change the light centers. It is surprising to see how much the arbitrary textures of environment can be imitated by control of the low and high spatial frequency components where the lowest spatial frequencies are fixed. We are testing a variety of ideas that all have to do with the nature of seen texture, a basic problem that is under active study both in perceptual psychology and in Artificial Intelligence.

9. Prosthetic Vocal Cords

Bell Telephone Laboratories, Inc. (Grant)

Don W. Schoendorfer, Stephen A. Raymond

Currently available means of production of artificial speech for laryngectomized patients are clinically and socially inadequate. Progress has been made in the design

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and construction of new artificial vocal cords. In collaboration with Dr. Donald Shedd, of Roswell Park Memorial Hospital, Buffalo, New York, these new cords are being evaluated by patients.

Specifically, the new design employs the use of an old reed-fistula technique. Changes have thus far been made only on the vocal cord design, but we intend to redesign the complete system eventually. The problem that we are now working on is twofold. First, the shape of the air pulse through natural cords for natural speech must be determined. Second, this determined shape must be matched by the artificial cords.