XIII. NEUROPHYSIOLOGY*

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A. COMPUTATION IN THE PRESENCE OF NOISE

We have recently applied information theory to the problem of achieving reliable computation in the presence of noise, and have shown that the information measure $\mathscr{I}[X;Y] \underset{\text{df}}{=} \sum_X \sum_Y \Pr\{x,y\} \log \frac{\Pr\{x,y\}}{\Pr\{x\}\Pr\{y\}}$ has valid application to this problem.

In particular, we have shown that noisy computing channels possess definable capacities. Nets of formal neurons were considered as examples of such channels, and codes were constructed that realized nonzero rates for reliable computation. The resulting nets are quite dissimilar to the multiplexed nets of von Neumann (1).

J. D. Cowan, S. Winograd

References

1. J. von Neumann, Probabilistic logics and the synthesis of reliable organisms from unreliable components, Automata Studies, edited by C. E. Shannon and J. McCarthy (Princeton University Press, Princeton, N.J., 1956).

B. ERRATA

In the report entitled "Infallible Nets of Fallible Formal Neurons," Quarterly Progress Report No. 54, July 15, 1959, pages 189-196, the number of nondegenerate infallible nets of two ranks that was stated (pp. 189-192) is an underestimate, but we still cannot put a reasonable upper bound on them.

The example of an infallible net of three ranks given in Fig. XVI-4 (p. 193) is wrong; it only improves, but is not infallible.

W. S. McCulloch

C. NOTE ON THE SYNTHESIS OF INFALLIBLE NETWORKS

Only infallible nets of two ranks are considered. The first rank consists of n fallible neurons, each of which receives information from all of the n binary input lines. The

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second rank consists of one fallible neuron that receives information from the n neurons of the first rank. Each neuron is represented by a Venn diagram with 2^n spaces. In each space a one, a zero, or a dash indicates the output of the neuron for the input configuration corresponding to that space. A one indicates that the output is "on"; a zero, "off"; and a dash shows that the output is uncertain. These dashes represent the fallibility of the neuron. The input-output relation of the net is represented by a single Venn diagram V_r with 2^n spaces.

For an infallible net, the spaces of V_r contain only ones and zeros, say, α ones and $\beta = 2^n - \alpha$ zeros. A community set in a rank of n Venn diagrams is the set of n spaces, one from each Venn diagram, which represents the same input configuration to the Venn diagrams. There are α community sets I in the first rank V_1 corresponding to the α spaces in V_r that contain a one; and $\beta = 2^n - \alpha$ community sets II in V_1 corresponding with the β zeros in V_r. The n Venn diagrams of V_1 are ordered by subscripts. V_1: $\{V_{1,1}, V_{1,2}, V_{1,3}, \ldots, V_{1,n}\}$.

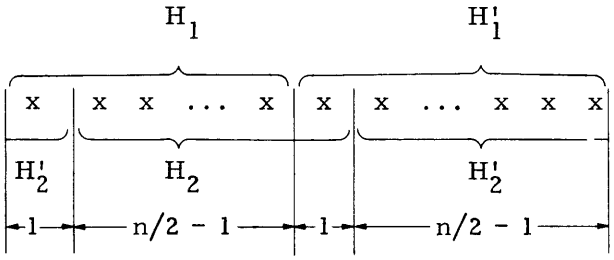


Fig. XIII-1. Division of V_1 into two subsets in two ways: H_1, H_1', and H_2, H_2' if n is even.

Consider the case for n even: Divide V_1 into two equal subsets in two ways. The first division yields the subsets H_1: $\{V_{1,1}, V_{1,2}, \ldots, V_{1,n/2}\}$ and H_1': $\{V_{1,n/2+1}, \ldots, V_{1,n}\}$. The second division yields the subsets H_2: $\{V_{1,2}, V_{1,3}, \ldots, V_{1,n/2+1}\}$ and H_2': $\{V_{1,1}, V_{1,n/2+2}, \ldots, V_{1,n}\}$. These divisions are indicated in Fig. XIII-1. It will become clear that these two divisions must be such that H_1 and H_1' each contain at least one element belonging to H_2, and at least one element belonging to H_2'. Similarly, H_2 and H_2' must each contain at least one element of H_1, and at least one of H_1'. Under these conditions, the two divisions can be made if $n \geq 4$. In $[\alpha/2]$, which indicates the integer in the number $\alpha/2$, of the community sets of I put ones in the spaces belonging to H_1, and dashes in those belonging to H_1'. In the remaining $\alpha - [\alpha/2]$ community sets of I place dashes in the spaces of H_1, and ones in the spaces of H_1'. The α community sets of I in V_1 then give rise to $2^{n/2+1} - 1$ possible input configurations for the Venn diagram of the second rank V_0. The appropriate $2^{n/2+1} - 1$ spaces of V_0 must contain ones if the net is to be infallible. Now in $[\beta/2]$ of the community sets of II place zeros

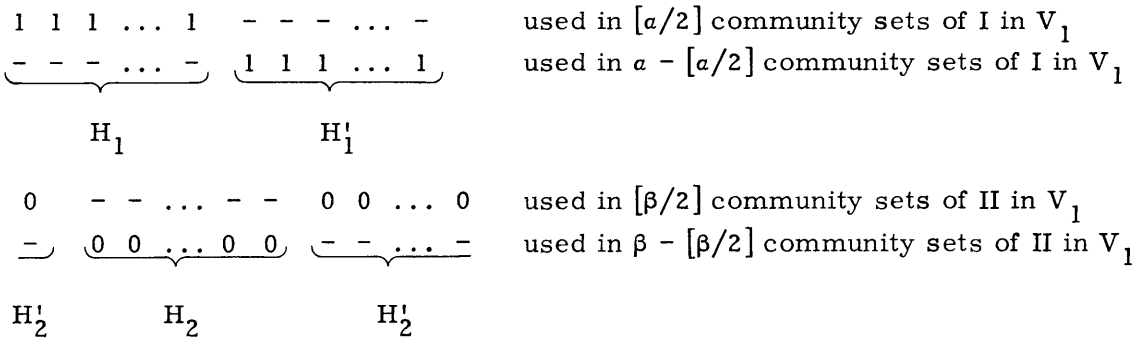


Fig. XIII-2. Scheme for placing ones, zeros, and dashes in V_1.

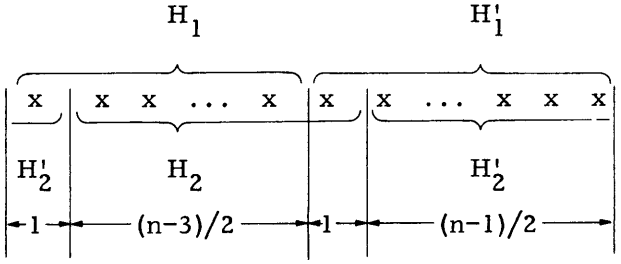


Fig. XIII-3. Division of V_1 into two subsets in two ways: H_1, H_1', and H_2, H_2' if n is odd.

in the spaces of H_2', and dashes in the spaces of H_2. In the other $\beta - [\beta/2]$ community sets of II put dashes in the spaces of H_2', and zeros in those of H_2. The configurations of zeros and dashes in the β community sets II of V_1 give rise to $2^{n/2+1} - 1$ possible input configurations for V_o. Infallibility of the net requires zeros in the $2^{n/2+1} - 1$ spaces of V_o corresponding to these configurations. Figure XIII-2 indicates the described construction.

The rank V_o must certainly be able to distinguish output configurations of V_1 for which it must fire from those for which it must not fire if the net is to be infallible. This method of construction guarantees this to be always possible by requiring that for any community set i of I, and any community set j of II, i always have a one in at least one space in which j has a zero.

The number of dashes in the Venn diagrams V_1 can now be counted. Substituting $\beta = 2^n - \alpha$, the number of dashes in $V_{1,1}$ is

$$\alpha - [\alpha/2] + \beta - [\beta/2] = 2^{n-1} \text{ for } \alpha \text{ even}$$
$$= 2^{n-1} + 1 \text{ for } \alpha \text{ odd}$$

in $V_{1,n/2+1}$ the number is

$$[\alpha/2] + [\beta/2] = 2^{n-1} \text{ for } \alpha \text{ even}$$
$$= 2^{n-1} - 1 \text{ for } \alpha \text{ odd}$$

and in the other n - 2 Venn diagrams, $\alpha - [\alpha/2] + [\beta/2] = [\alpha/2] + \beta - [\beta/2] = 2^{n-1}$.

The rank V_o contains $2^{n/2+1} - 1$ ones and $2^{n/2+1} - 1$ zeros. Its other spaces may be filled by dashes, since inputs to V_o corresponding to these spaces never occur. The number of dashes in V_o is thus $2^n + 2 - 2^{n/2+2}$.

For n odd, the division of V_1 into subsets has to be altered. As indicated in Fig. XIII-3 the subsets are H_1: $\{V_{1,1}, \ldots, V_{1,(n-1)/2}\}$, H_1': $\{V_{1,(n+1)/2}, \ldots, V_{1,n}\}$ and H_2: $\{V_{1,2}, \ldots, V_{1,(n+1)/2}\}$, H_2': $\{V_{1,1}, V_{1,(n+3)/2}, \ldots, V_{1,n}\}$. This partitioning is possible if $n \geq 5$. The algorithm for placing ones, zeros, and dashes in the α community sets I and in the $\beta = 2^n - \alpha$ community sets II is the same as for n even. Figure XIII-2 indicates the result. A simple computation gives for the number of dashes in $V_{1,1}$,

$$\alpha - [\alpha/2] + \beta - [\beta/2] = 2^{n-1} \text{ for } \alpha \text{ even}$$
$$= 2^{n-1} + 1 \text{ for } \alpha \text{ odd}$$

for $V_{1,(n+1)/2}$,

$$[\alpha/2] + [\beta/2] = 2^{n-1} \text{ for } \alpha \text{ even}$$
$$= 2^{n-1} - 1 \text{ for } \alpha \text{ odd}$$

and for the other n - 2 Venn diagrams, $\alpha - [\alpha/2] + [\beta/2] = [\alpha/2] + \beta - [\beta/2] = 2^{n-1}$.

Table XIII-1. The number of dashes in V_o and the ratio of this number to the number of spaces in V_o expressed as a percentage for several values of n.

n	Number of Dashes in V_o	Ratio (per cent)
4	2	12.5
5	8	25.0
6	34	53.1
7	80	62.5
8	194	75.8
9	416	81.3
10	898	87.7
11	1856	90.6
12	3842	93.8
13	7810	95.3
14	15874	96.9
15	32002	97.7

The ones and dashes in the α community sets I of V_1 require a one in $3.2^{(n-1)/2} - 1$ spaces of V_o. The zeros and dashes in the β community sets II of V_1 require a zero in $3.2^{(n-1)/2} - 1$ spaces of V_o. The rank V_o can have dashes in $2^n + 2 - 3.2^{(n+1)/2}$ of

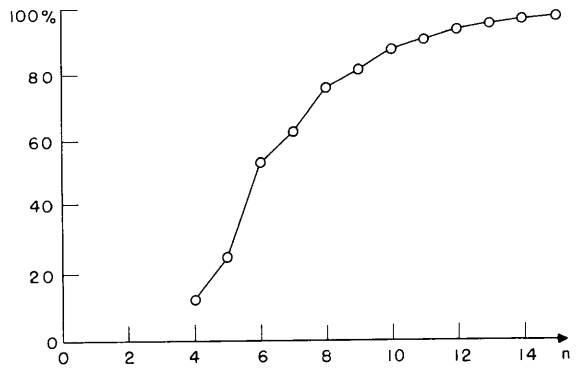


Fig. XIII-4. Graph of the ratio of the number of dashes to the number of spaces in V_o expressed as a percentage.

its spaces. Table XIII-1 gives the number of dashes and the ratio of this number to the total number of spaces in V_o. This ratio is represented in the graph of Fig. XIII-4. A particular example of the method for the construction of infallible nets of two ranks, V_1 and V_o, with fallible neurons is given in Fig. XIII-5.

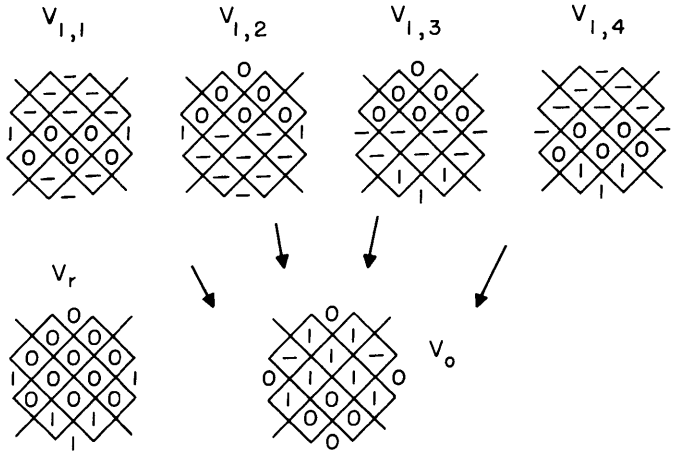


Fig. XIII-5. Example of the construction of an infallible net with four input lines, n = 4. The input-output function is given by V_r. The first rank consists of $V_{1,1}$, $V_{1,2}$, $V_{1,3}$, $V_{1,4}$; the second rank is V_o.

In general, the algorithm just given results in having the first rank half-filled with dashes. The second rank can have a number of dashes, as indicated in Table XIII-1 and Fig. XIII-4. The method does not depend on the input-output function V_r of the net. For some particular V_r, it is often possible to make a better scheme, that is, a scheme having more dashes in the Venn diagrams. The percentage of dashes in V_o increases with n.

It is possible to use the results of this and of similar constructions to obtain error-correcting networks.

L. A. M. Verbeek

D. TURING MACHINES, FINITE AUTOMATA, AND NEURAL NETS

[A paper of the same title, of which this report is essentially an abstract, has been submitted to the Journal of the Association for Computing Machinery. The terminology used in references 1, 2, and 4 will be used in this report without further explanation.]

"Equivalence" theorems have been given formal proofs for: (a) nets with receptors, and finite automata; and (b) nets with receptors and effectors, and Turing machines.

THEOREM 1: Given a net N and any output o of N whose firing depends only on the internal state of N, we can find a finite automaton A whose function is equivalent to that of the net, in that input sequences to N realized by o and tapes accepted by A may be regarded as equivalent; and vice versa.

With this theorem at our disposal, we may telescope large portions of the papers by Rabin and Scott (4) and by Copi, Elgot, and Wright (1). In fact, the analysis and synthesis theorems of the latter paper may now be regarded as corollaries of theorems in the former.

THEOREM 2: Given a net N with n inputs, if we choose any n + 3 outputs of N, then we can find a Turing machine Z whose function is equivalent to that of N, in that if we identify the inputs of N with the output wires of TS'_n, n of the output wires of N with the input wires of TP_n, two of the remaining output wires of N with the input wires of TM, and the last output wire of N with the input wire of CO (where TS'_n, TP_n, TM, and CO are a precisely defined tape-scanner, a tape-printer, a tape-mover, and a cutout, respectively), then the machine so obtained behaves precisely as Z; and vice versa.

Theorem 2 leads to the formal proof of the remarks on Turing machines and neural nets made by McCulloch and Pitts (3).

Furthermore, a new notation has been introduced to replace net diagrams. It has also been shown that the sets of positive integers 'accepted' by finite automata are recursive, and a theorem of Kleene has been given a strengthened form that shows that regular sets on an infinite alphabet are primitive recursive.

M. A. Arbib

(Department of Mathematics, M. I. T.)

References

1. I. M. Copi, C. Elgot, and J. B. Wright, Realization of events by logical nets, J. Assoc. Computing Machinery 5, 181-196 (1958).

2. M. Davis, Computability and Unsolvability (McGraw-Hill Book Company, New York, 1958).

3. W. S. McCulloch and W. H. Pitts, A logical calculus of the ideas immanent in nervous activity, Bull. Math. Biophys. 5, 115-133 (1943); see especially p. 129.

4. M. O. Rabin and D. Scott, Finite automata and their decision problems, IBM J. Res. Develop. 3, 114-125 (1959).

E. OLFACTION

Our investigation of the olfactory system shows that the action potentials from the receptors last longer than those from the larger cells in the nervous system. We also see a clear-cut discrimination between different stimuli. A stimulant may increase the resting rate of a cell, cause a cell to fire that is not spontaneously active, or decrease

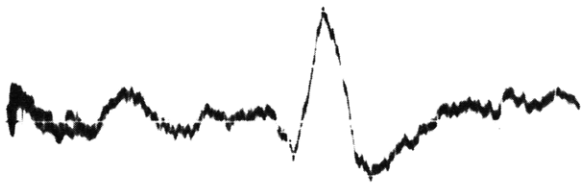


Fig. XIII-6. Shape of the action potential. The sweep length is 20 msec, and the amplitude of the spike is approximately 200 μv, peak to peak. The spike is initially negative and triphasic.

Fig. XIII-7. Recordings of single receptors from the frog olfactory mucosa. The top trace shows the resting discharge in ambient still air. The center trace, with the electrode in the same position, shows a previously quiet unit firing when cotton soaked in n-butanol is held near the nose. The lower trace, with pyridine used as a stimulus, shows no response from the preceding cell, but inhibition of other units. The total length of the sweep is 5 seconds, and the vertical sensitivity of the oscilloscope is 50 μv per centimeter.

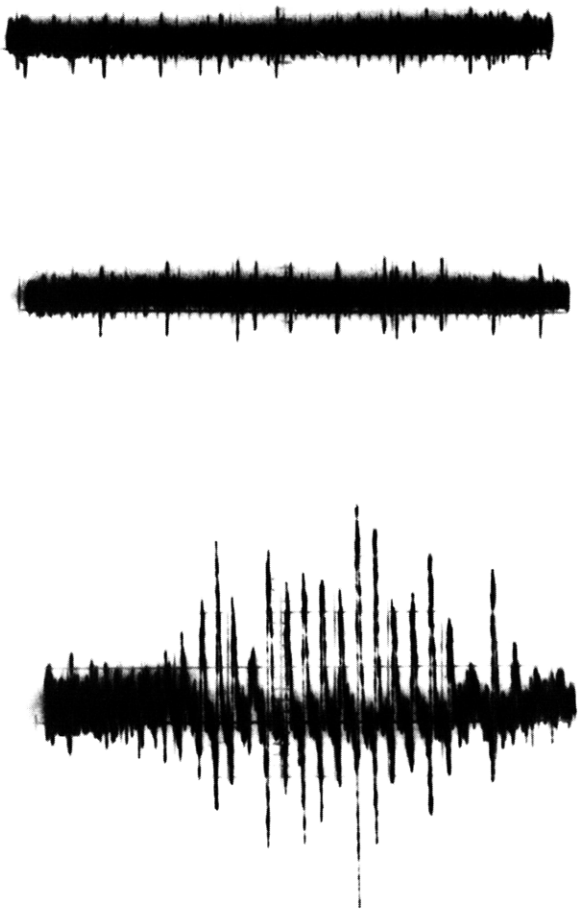


Fig. XIII-8. Recordings with the electrode in a different position from that in Fig. XIII-7. The top trace exhibits the resting activity; the center trace, the response to a small amount of cigarette smoke; and the lower trace, synchronous activity resulting from much higher concentrations of smoke, with an initial increase in the rate of the units, and then oscillations as the units synchronize. The scales of time and voltage are the same as in Fig. XIII-7.

the resting rate. Different cells, even if they are very close together, may respond differently to the same stimulus. The oscillograms shown in Figs. XIII-6, XIII-7, and XIII-8 demonstrate some of these effects.

R. C. Gesteland, J. Y. Lettvin, W. H. Pitts

F. OCTOPUS VISION

Our work in Naples at the Stazione Zoologica, June 1960 to January 1961, did not result in any one complete thesis; but we did set up a reasonable preparation, find the

most useful means of recording, invent criteria for judging results, and can now state the problems more interestingly than when we started – can say what we seek.

1. GENERAL COMMENTS

Why work on octopus at all? Octopodes (octopuses or octopus – never, according to O.E.D., octopi) are cephalopods, invertebrates, whose only common ancestor with vertebrates is at about the level of the medusa. They have a fine intelligence (they are the brightest of all invertebrates) and possess sense organs which are, in many ways, equivalent to ours. So profound are the similarities in purpose between their statocyst organ and our vestibule, their eyes and ours, that one does not feel embarrassed at all in discussing these organs teleologically, as we shall do in this report. The common ancestor between us vertebrates and the octopus has no eye at all. The development of a cameral rather than compound eye by the octopus will have occurred independently enough for us to suppose that the only common factors with vertebrates are, first, the propotoplasmic nature of animals, and specifically the nature of nerves; second, the nature of the environment – that there are prey and predators that must be recognized and handled. Otherwise, those genetic accidents that are sculptured to particular ends in evolution are very unlikely to have been the same between them and us, so that neither materials nor arrangement will be common. It is as if the octopus were a man from Mars. There are no other animals in nature besides the cephalopods where such parallel evolution of a cameral eye has happened. (From arguments on optical efficiency some physiologists and anatomists hold that below a certain size compound eyes are better than cameral, as with insects, and the reverse is true for large eyes. Since the cephalopods are the only invertebrates with large eyes, the argument would seem to hold.)

It might be useful here to describe the general anatomy and behavior of the octopus, since probably very few of the readers will have had any experience with such animals, but since our space is limited I refer the interested reader to Lane's "Kingdom of the Octopus" (1).

The body, or mantle, of the octopus consists of a sac wherein lie its vital organs. The sac has a tube protruding from it beneath the head end, the siphon. The sac can be closed by a pair of valves, and the siphon can be collapsed; thus the animal can respire, collapsing the siphon during inspiration and closing the valves during expiration. By a sudden, profound expiration it achieves a jet action with its siphon if it wants to move rapidly or squirt at a threat. The dorsal aspect of the sac is attached by a neck to the head, which possesses two eyes looking out to either side and a horny beak on the under surface. Between the eyes is a cartilaginous case, the only hard structure in the octopus beside the beak, and in it lies the brain. There is no other rigid or elastic structure used as skeleton. The head splays out, opposite to its attachment to the sac, into a broad foot whence spring the eight arms studded with suckers in single or double lines,

according to species. These arms are capable of extraordinary movement; not only can they coil and extend and show all sorts of sinuation, but the vascular system therein can be used for support – the vessels can be filled to that degree of pressure necessary to stiffen parts of the arm. The octopus has almost that control of its circulation that we have of our limbs and it can walk on stiffened out arms as we do on our legs. Much of the nervous system controlling an arm lies in the arm itself, and a severed arm shows quite remarkable autonomous movement, as does the body of a spinalized vertebrate.

2. VISUAL PROBLEM

The eye of the octopus is dioptrically like ours. It is a camera with lens, adjustable aperture by means of an eyelid-like pair of structures before the lens, and retina. There is no point in discussing the anatomy of the camera further except for the retina, which differs markedly from ours. Its photosensitive pigments are, chemically, kin to ours, but undergo a different set of reactions. The actual receptors face outward, toward the lens, rather than inward like our rods and cones, and they are packed in a square array. The anatomy of the individual receptor is still not entirely clear, despite that electron microscopy is being done by several people, notably J. Z. Young and his staff at University College, London. Each receptor is a long retinular cell with elliptical cross section of the body but convoluted by villi to the envelope of a rectangular prism. Seen end-on, the cell is an oval lacuna that is the diagonal of a brush with square envelope, and all of the fingers or villi of the brush lie normal to the long axis of the ellipse. Packed together in the retina, these receptors form a square array that looks like Cartesian graph paper made with dashed lines. This is quite a startling sight and departs too much and too systematically from simple dense packing to be considered accidental. The villated surface is the cell wall. Each receptor prism narrows at its base to a very fine neck, loses the pigment, and passes through the basement membrane of the retina. Then it expands again into a cell body whence issues the axon of the optic nerve. At least, this seems to be the current view of the anatomy, although neither Maturana nor I is convinced that this one-to-one correspondence of cell body and photoreceptor necessarily exists. By the way, the cell-bodies are equipped with small processes to which the efferent fibers to the retina attach.

The optic axons from the many cells coalesce to many bundles, which leave the eyeball through a great number of small holes in the sclera. These bundles then undergo a vertical decussation; that is, those from the bottom of the eye systematically cross those from the top so as to map the retina inverted and reflected on the optic lobe. These bundles are very odd to watch when they are cut, for each has a small musculature at the place where it leaves the eyeball, and the cut nerve wiggles and jerks about. An avulsed eyeball with long strands of nerve attached to it looks like the head of Medusa in a dish of sea water. The optic lobe to which the decussated bundles go floats free in

the orbital or retrobulbar sinus, which we discuss under the section on methods.

This optic lobe is the nervous equivalent of the second- and third-order neurons of the vertebrate retina and the cells of the colliculus (in the frog). The very surface of the lobe is made of a granular layer of amacrine cells – the outer amacrines – which is penetrated by the entering fibers. The amacrines do not seem to have axons, simply a cell body whence by a peduncle arises a dendritic tree projecting into the more central plexiform layer where the optic axons end. There are several varieties of amacrine, and many of them seem to have their trees organized in planar form locally in one of two mutually orthogonal directions. The plexiform layer itself seems to have several concentric zones suggested strongly both by depth of penetration of the optic axon and by level of arborization of dendritic trees. Even more central to the plexiform layer is another layer of cells, the inner amacrines, which also send trees into the plexiform, and a layer of cells with axons, the equivalent of our ganglion cells, with broad dendritic expanses in the plexiform. Young has pointed out very clearly that the arrangement of dendritic trees of the cells receiving from the plexiform layer is predominantly along mutually normal planes perpendicular to the surface of the lobe. Some of the ganglion cells, incidentally, send their axons back out through the plexiform layer, and presumably it is this group that makes up the efferent complement mentioned in our results. It is unnecessary to go further into this anatomy here.

It is evident, by now, to the anatomist that on organizational grounds there is a marked resemblance between the visual system of cephalopod and vertebrate if the plexiform layer of the octopus lobe is taken as equivalent to the inner plexiform layer of the vertebrate retina. In both instances there is a set of neurons, unique in nature save for these two places, the amacrines. There are broad connections of ganglion cells with several types of arbors to the many-leveled plexiform layer. The most profound differences that prevent the analogy from being stronger are the absence of bipolar cells and the axonal extensions of the primary receptors. It was at this point that Maturana and I made a rather violent assumption. Before we state it, however, let us first discuss octopus behavior that guides our view of the system.

Sutherland, of Oxford University, and Young have been studying visual discrimination in octopus for several years. What they have found appears to suggest a rather simple law. Since they have used only rigid silhouettes against plain background, all of the objections inherent to such a limitation apply. Nevertheless, the law accounts for the observed behavior and must, in some sense, reflect the nature of the system. Take the slit of the octopus pupil as the horizontal axis of ordinary graph paper. Consider a silhouette image drawn on the paper. Now project the silhouette onto the horizontal and vertical axes in this way: For every value of x enter the sum of the internal distances between the intercepts of the line at x with the figure; the same with every value of y. All of those figures that have the same x and y projections are

indiscriminable. Thus a horizontal line can be told from a vertical, but a diagonal cannot be told from the one orthogonal to it. So, also, with more complex figures, so devised that although to our eyes they are significantly different they do not differ in their projections. Sutherland has devised test figures to check this law and finds it uncommonly adequate.

To go back to the anatomy of the system, we find that the Cartesian form occurs throughout; not only are dendrites of amacrines and other cells arranged in a mutually orthogonal relation to each other locally, but the very receptors themselves lie in a square array. It is at this point that a frank metaphysical bias enters. Maturana and I firmly believe that such a structure as this retina cannot be adventitious to the visual functions, that the Cartesian layout must be required for the Cartesian projection method of recognizing shapes. That method may be wrongly inferred, and there may be an infinite variety of laws to account for the same psychological data, yet all of the laws must have in common the fact that diagonals have less weight in recognition, by far, than verticals and horizontals. It is possible to suggest, as Young does, that the dendritic orthogonality in the lobe is where this rectangular visual function begins; but if so, why this incredible coincidence of the rectangular arrangement of receptors? Were each receptor concerned only with total luminous flux over its small glebe, there would be no sense in making it one shape rather than another. Fortunately, we are assured of the importance of the array by Moody and Parriss' demonstration (2) that octopodes can be trained to discriminate uniform fields of light polarized orthogonally to each other. Since there are about equal numbers of both horizontally and vertically polarized receptors, this effect cannot be simply an intensity discrimination. Either the differently polarized receptors are connected to the lobe in different ways, or this effect could not come about unless there were an intrinsic difference between mutually orthogonal receptors beside the accident of position. However you interpret it, the experiment of Moody and Parriss shows the retinal arrangement to be significant.

But form discrimination is not done, in general, by polarized light. Thus the mechanism that allows the telling of one polarized light from another has virtually no effective use unless that same mechanism is somehow concerned with telling a boundary in one direction from that in another. From the description given above one can invent several ways in which verticals and horizontals are extracted, although none of them is worth anything until demonstration. For example, the brushiness of the villi arranged orthogonally to the oval column of the receptor makes, at these dimensions, an excellent polarizing mechanism, each villus being well below the wavelength of light in diameter, and there being hundreds to thousands of layers of them along the length of a receptor. Since each of the two triangular prisms adjoining the oval column has an altitude of only a few times the wavelength of light, many of the polarized light phenomena intimately associated with edges and diffraction but only a wavelength or so wide, can be used.

Ordinarily, in a nonpolarized system of small receptors one takes such effects into little account. Maturana and I conceive that it is not impossible – indeed, we both suppose and hope it very likely – that an individual receptor is capable of signaling a combination of total flux upon it with the difference across the central axis. Our present outrageous guess is that the cell bodies bear that functional relation to the two halves of the receptor prism that we have assumed some bipolar cells bear to rods and cones in the vertebrate retina. It is this assumption that we are preparing to test next year on the basis of the results given below.

3. METHODS

Octopus need a good supply of oxygen. Our first problem was to construct an adequate operating tank. It was made of Plexiglas, about 18 inches × 9 inches × 9 inches with two apertures at the bottom of one end. Through one we led a stream of sea water which was oxygenated by running it through an ordinary Venturi water pump but partially occluding the air input with an adjustable clamp; thus the bubbles were formed under very reduced pressure, promptly collapsed at tank pressure, and gave a fine mist of suspended air in the underwater jet in the tank. The other opening was the exhaust, so arranged that the water level in the tank could be kept constant at any height by a feedback. We used a collapsible section (for example, the finger of a surgical glove cut to make a tube) in the outflow pipe. The principle is quite simple; you can blow through such a tube but not suck through it. The column of water in the pipe below the flexible segment keeps the tube collapsed until the pressure in the column above the tube is great enough to open it. Thus by setting the height of the collapsible tube the height of water in the tank could be set more or less independently of small variations in input flow and without the noises of an aspiration system or the messiness of an overflow system.

Octopus can shut off circulation immediately in the stumps of amputated legs so that they do not bleed. They have to do this, naturally, or morays could wipe out the whole population. There is nothing more unhappy for an experimenter than an octopus arm wrapping itself around a manipulator or microelectrode; thus we severed all eight arms of the octopus from the foot with one pass of a sharp scalpel when first removing the animal from its home tank. The web of eight arms in search of a body would remain writhing on the table and the octopus removed to the operating tank. If the cutting were done quickly the animal would neither ink nor jet, and in about five minutes would regain the warty unblushing appearance that fisherman and biologists think is the sign of a happy octopus. The foot was then nailed to a board and the siphon immobilized by a small glass rod passed down it and tied to the underside of the board. (One must not underestimate the accuracy of the jets of a tethered octopus; it can hit you in the eye at ten paces if it does not like what is happening. This is not a joke; it can aim the siphon at you, and a vigorous animal can drench you in no time. The decision to immobilize the siphon came

after one beast shot some sea water at the Tektronix 536 and short-circuited the power rectifiers.) This procedure was done without anesthesia, for it seems that the foot and arms are not a source of pain to the octopus. I can say this, for we did find the sensitive regions as described later, and then there was no mistake about response. We are not denying sensitivity of touch and perception of texture to these arms, it is just that while they will make defensive reflexes they can be manipulated and cut without provoking the violent spasms of breathing, jetting, and inking that handling of certain portions of the head provoke. The procedure thus far is bloodless.

After the animal has been allowed to accomodate himself to the tank and is settled down and pleasantly warted, a matter of about another twenty minutes, we begin surgery on the eye. Here the whole set of surgical principles changes violently from those in vertebrates and ought be told in detail. Attached to the equator of the eye is a large capsule enclosing the back of the eye, the optic nerves, optic lobes, and a host of other structures. It lies between the eye and the cartilaginous brain case and is surrounded by muscles. It is filled with blood, for it is a vascular sinus, so that optic nerves and optic lobe lie in a bath of the blue blood of the octopus. In this bath the nerves and lobe are surrounded by a fatty collar, called the white body, a blood-forming organ at least. The wall of the capsule is the so-called white membrane, a tough, duralike sheet. This membrane is the most sensitive part of the octopus. Simply touching it provokes all the signs of discomfort or pain. After you have touched it several times, but without opening the capsule, you will notice that the blood within it has turned colorless (i.e., not oxygenated) and the eye reflexes vanish. The animal acts as if it has turned that eye off – committed ocular suicide. Sometimes, I think, if one should wait long enough, circulation might start again, but we did not try enough cases to establish this fact. If one has not removed most of the muscle around the eye and tries, cleverly, to work on the eye through a small aperture in the white membrane an astonishing thing happens: with a strong continuous push the octopus delivers its whole eye through that small hole complete with all nervous structures avulsed. It is a horrible sight.

Those men who have worked on cephalopod eye previously (for example, Wohlbarsht and MacNichol) find that the whole eye is dead in 5 minutes if they lay open the white membrane fully to expose the nervous structures. In talks with them, I found that they, too, discovered that one must not touch (I mean literally not touch) the dorsal artery leading into the eye, otherwise all circulation stops. This artery is quite visible under the insertion of the white membrane on the eyeball. Maturana and I, after watching eye after eye die in ocular suicide from not opening the white membrane more than a little, from not having a care for the artery, and so on, finally decided to bypass the sinus circulation of the octopus with aerated sea water. That is, opening the capsule very widely and rapidly, leaving the retinal artery intact, quickly removing the white body, and deflecting some of the aerated sea water into the cavity, we finally got eyes that

stayed alive and reflexive for several hours with the whole nervous apparatus exposed. The whole idea, you see, is that if the animal is going to shut off his own circulation to this part we will not let the eye die on that account. (Learning this one small technique occupied about two months of work.)

One might think that with the nerves and optic lobe exposed everything should now be simple. Not so. The bundles of nerves are there, but are impenetrable with a microelectrode. Before one can get through the surface, either the electrode breaks or the nerve is so stretched that it would be a wonder if anything stayed alive within it. The connective tissue sheaths, not only around the bundle but around the microbundles within, are cohesive and adhesive. We tried with every trick we could imagine, with every sort of electrode devised, and with little success. We even tried drilling the microelectrode slowly into a nerve bundle. Then Maturana, together with Pierre Tardent at the Stazione, suggested that changing the ionic composition of the sea water might change the composition of the connective tissue. We did not actually try the method immediately because I felt stubborn about manipulating the ions, but a lucky accident with the heart of Squilla Mantis proved that if the magnesium were reduced, the tough gel of the connective tissue becomes solated, the proteolytic enzymes are released, and these supporting strands become as soup. Thus we were led to playing a small jet of magnesium-free sea water on the nerve we planned to penetrate for about 20 minutes before the penetration. Very surprisingly the structure becomes very easy to enter with a microelectrode. As a general technique with all marine invertebrates I cannot recommend this too highly; it makes accessible many fibers that are ordinarily not easily entered. The method should not be used in junctional regions.

At the same time we found that the whole technique of making microelectrodes had to be changed, and this led to a useful new method of making tools for the embryologist. (Not that the electrodes were so successful in the octopus, although later they proved themselves in the olfactory mucosa of frog, as described in Section XIII-E by Gesteland and Pitts.) If one takes a thin rod of glass, say no more than a mil or two in diameter, it is possible to sharpen it to an incredibly fine point on ordinary double-zero carborundum paper (the finest grade just short of rouge) if the paper is moved at high speed. We simply plastered little discs of this paper on the top of a centrifuge rotor. The points so obtained are sharper under high power than any metal point we could get by electrolytic sharpening or grinding; and further, were stronger. Similarly, one could grind a microchisel, a knife edge, and the like, with very little difficulty, and manufacture sets of instruments of some sophistication at a microscopic level. So far as we were concerned, the technique was important in fashioning a sharp cutting edge of glass to precede by 2 or 3 μ the metal portion of a microelectrode. That is, our electrodes were beveled so that glass, rather than metal, led; and this seemed to make an appreciable difference in penetration of nerve and the partial success with single units.

Finally, as a last measure in dealing with these optic nerves, Maturana discovered that the bundles sometimes have small anastomotic branches with neighboring bundles. These could be severed and dissected for a distance of approximately 0.5 mm or 1 mm, and I set up a platinum hook within a glass tube through which either mineral oil or Silicone 550 could be made to flow continuously so as to insulate the branch from the sea water. With this method we were able to record 50-100 afferent fibers at a time, never much less, but 2-5 efferents at very good signal levels, as will be seen in the results.

We spent over five months on these methods; they developed slowly even though we ran one to two animals per day. Insofar as the methods may be useful to others in preventing such an expense in time we mention them in a little detail here.

4. RESULTS

If an octopus eye is illuminated, a change in potential occurs across the retina. This was one of the first electroretinograms measured, and in 1911 Fröhlich investigated it thoroughly. He discovered it to be logarithmically related to the intensity of the light, and his records also show that the return to base level after turnoff of light is a more or less logarithmic function of the time over a period of more than a minute. We thought we had verified this and spent only a day or two at it, doing no more than checking a few points on the curve. Recently, however, we have learned that a paper by Hagins will show that the amplitude of cephalopod retinogram is linearly related to incident light.

What is strange about the retinogram is its oscillatory character in the freshly prepared retina when the eye is taken out of the animal. This was remarked by Fröhlich and has since been occasionally confirmed. What happens is that to a sharp turnon or turnoff of light, and riding atop the change in potential, there is a long-lasting oscillation that gradually decays to a very low level. The frequency is not fixed but changes slowly in both directions; nevertheless, crudely, it looks like a damped ringing. We found this to be most probably due to the structures of the retina on the back, or outer, side of the limiting membrane. The experiment is this. Leaving the eye intact in the animal we insert one electrode into the eyeball on the receptor side of the retina and another through the sclera at the back of the eye and touch the nervous side of the retina. The whole animal is immersed in sea water that is grounded. Now the retinogram taken from inside the eye to ground shows, on sharp on or off of illumination, a smooth rise and fall with just the barest hint of oscillation. The retinogram taken from the back of the eye to ground shows an inverted sign, roughly the same time course of rise and fall, but with very large oscillations riding on it, and these are quickly damped out. If one records with a push-pull system from front to back of the eye, the retinogram becomes about double size, as you would expect, and also shows the oscillations. This is what you see in the avulsed eye, measuring the change across the retina. Now, were the oscillations generated across the basement membrane, one would expect a symmetry of the two records,

inside the eye to ground and outside the eye to ground. The asymmetry tells us that the oscillations are due mainly to the nervous elements (cell-bodies and axons) and not the receptor elements, and further, that whatever is producing the oscillation does not spread up into the receptors even though they may be continuous with the cell-bodies. Now, if one cuts the optic nerves these oscillations increase markedly. If one even cuts one nerve and records from it alone, the fluctuations seen there are larger and more prolonged, damped out much more slowly, than in the intact system. There are two possible geneses. On the one hand, the fibers in the nerves do not seem to braid extensively; they are dense-packed and seem parallel (although this awaits confirmation). Thus one would expect great interaction of adjacent elements, particularly with such procedures as cutting. This sort of oscillation can be seen, for example, in freshly prepared dorsal roots of cats, where interactions at the cut end set off fibers which, going to the spinal cord set off reflected impulses, which going back out to the cut end set off more fibers, and so on. That the oscillations in octopus eye increase sharply on severing the nerves may suggest such a mechanism as most likely. In this case the reflecting process, necessary for oscillation, is by means of the basement membrane, just as it is in the olfactory mucosa of vertebrates (in the so-called Adrian oscillations to tobacco smoke) where a similar geometry occurs. The provenance might possibly be this simple were it not that we have also discovered the efferent activity to the retina and suspect it to have a damping factor.

Let us now look at the output of the nervous elements themselves as seen in optic nerve. If one takes up on a fine electrode a small bundle (say about 25-50 μ in diameter) occurring as anastomosis between larger bundles, and, cutting it at its central end, insulates it by flowing oil, and records from it with respect to ground, then two very clear groups of fibers appear. Since we are dealing with a population that may be several hundred even in so small a bundle, the separation of the crude groups can only be made with respect to gross stimuli over an area of several degrees in visual angle. One group responds to the turning on of light with initially high activity, gradually dropping off to a lower maintained rate. Another, and much smaller group, responds, after a delay, to complete darkness. Figure XIII-9 shows what we mean. The noise level in the base line indicates in part a continuous background firing. From the pictures, the two separable groups are those neurons that fire only to light, and those with activity in the dark the activity of which is inhibited by low levels of illumination whether or not they are also stimulated by large levels. Only the first group was seen by MacNichol and Love (3) in their work on squid. Incidentally, it is apparent, from the form of these records, what it was that they were recording by their special electrodes which penetrate cartilage. They had impaled one of the small component bundles inside one of the nerves and were doing the equivalent in the closed animal of what, by dissection, we were doing in the open sinus. That this was the case we checked by building their kind of electrodes with a small collar of glass behind the exposed tip and recording in a similar fashion to theirs. We

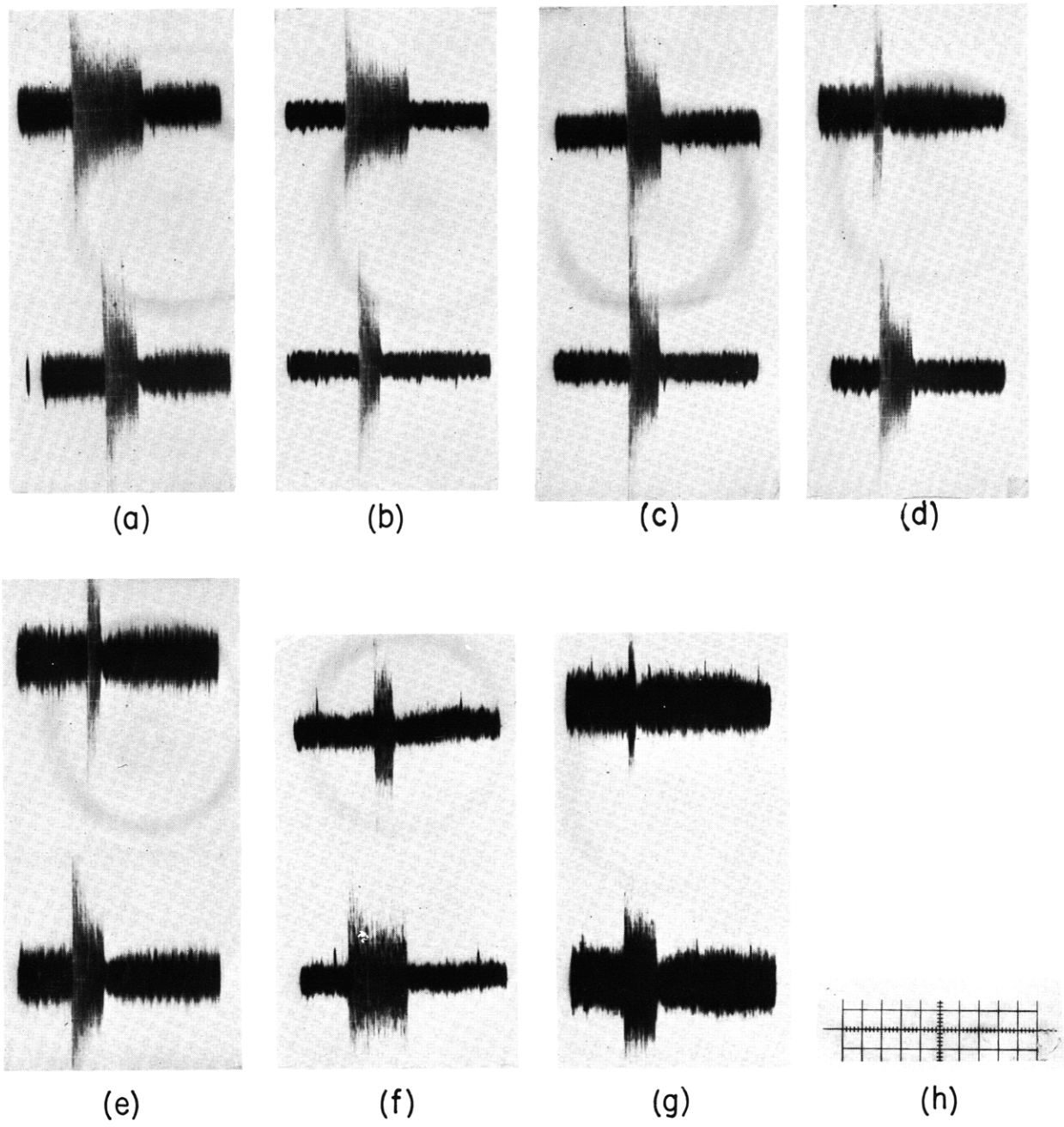


Fig. XIII-9.

(See page 205 for legend.)

Fig. XIII-9. Small astomotic branch between two optic nerve bundles was cut at its central end, and the 0.5-mm stump was drawn up on a platinum wire inside a glass pipette through which mineral oil was constantly flowing.

(a) Complete dark in the room. The sudden increase of signal is a response to a diffuse light turned on in the room; the sharp decrease signals the turning off of the light. Note the diminution of noise following the off, and a gradual return to the base line.

(b) Dim diffuse light in the room. The same light as in (a) was turned on and off. Note the relative absence of background activity.

(c) Immediately after turning off the background dim light. The room is again in darkness, but the animal has not yet adapted to darkness. Note that the background activity is not larger than in (b).

(d) After 10 minutes of dark adaptation. Note in the top part of the figure the increased background activity, as in (a) the reinstitution of the background activity after the short flash, but its diminution eventually back to base-line activity of (b), so that the second flash of light in the lower part of the figure does not appear significantly different in its effect on the base-line activity from that in (c).

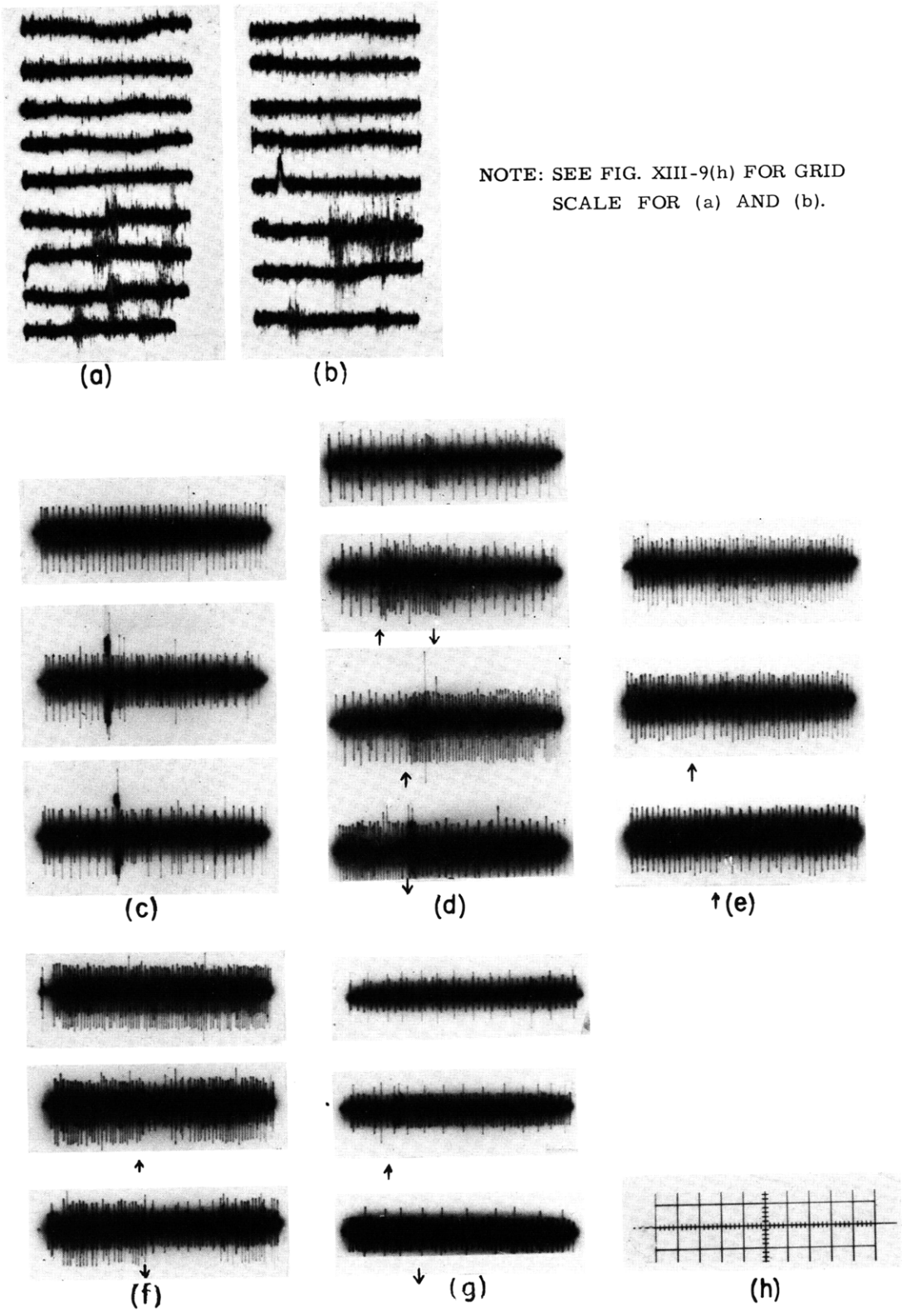
(e) After waiting 15 minutes in the dark we have the relation between background activity and response as in (a) and even more marked because the animal had not been completely adapted to darkness in (a). The activity represented is from about 100 fibers, and it is clear that some of these fire in absolute darkness and are inhibited by moderate light. We regard these pictures as proof of dark-sensitive receptors.

For traces in (a) to (e): Time, 2 sec per division; amplitude, 35 μv per division; bandpass, 80 cps-1 kc.

(f) On and off of light, with Bunsen burner illuminating the room at the far end.

(g) The Bunsen burner is turned off, and the same stimulus repeated. For traces in (f) and (g): Time, 1 sec per division; amplitude, 30 μv per division. These 2 figures show another astomotic branch.

(h) Grid scale.



NOTE: SEE FIG. XIII-9(h) FOR GRID SCALE FOR (a) AND (b).

Fig. XIII-10.

(See page 207 for legend.)

Fig. XIII-10. (a) Another anastomotic bundle was cut at its distal end and recorded from, as in Fig. XIII-9, under dim room illumination. Resting activity is shown in the first 5 lines. In the next 4 lines a brighter diffuse light was switched on and off in the room with every sweep. The transient increases in activity signal the on and off.

(b) The first 5 lines again show resting activity. In the sixth sweep the light was turned on and left on, and there is an increase of activity which lasts a short while. In the seventh sweep, the light was turned off and left off, and off is signaled by transient increase in activity. In the eighth sweep, made immediately after the seventh, the light was flashed on and off very quickly in the first one-quarter of the sweep, resulting in very small transients, indeed. These are efferents that respond to light.

For traces in (a) and (b): Time, 0.2 sec per division; amplitude, 30 μv per division; bandpass, 80 cps-1 kc.

(c) Another efferent group as in Fig. XIII-10a. Top, resting activity. Middle, stamping on the floor causes a transient increase of one spike and its subsequent erratic firing, with an increase in the firing rate of a smaller spike. Bottom, immediate repetition of the same stimulus after partial return to base-line activity. The transient to the stamp is almost as profound as in the previous sweep, but the subsequent pattern is different. These long-term effects are seen with most efferents.

(d) Same group. Top, resting activity. Second sweep, on and off of light followed by a period of increased base activity. Third sweep, light was turned on and left on. Fourth sweep, light was turned off and left off. (Arrows indicate on and off of light.)

(e) First sweep, resting activity under moderate diffuse light. Sac was touched at arrows in second and third sweeps.

(f) Bright light kept on in receptive field of efferent fiber. Resting activity shown in first sweep. Sac touched at arrows in second and third sweeps.

For traces in (a) to (f): Time, 0.2 sec per division; amplitude, 50 μv per division; bandpass, 80 cps-1 kc.

(g) Another bundle of efferents. Top sweep, resting activity under moderate illumination. Light was turned on at arrow in middle sweep, and off at arrow in bottom sweep. One of the fibers is not affected. Time, 0.5 sec per division.

(h) Grid scale for (c)-(g).

were rather more satisfied with the dissected fibers, for then we were well independent of any movement and could stay with one group for over an hour.

Now, suppose instead of cutting the central end of this small bundle we were to cut the distal end, and taking up the nerve in the same way, see whether or not we can record from the efferent fibers from lobe to retina which are known anatomically to be there. Since only the tiny bundle is cut and the rest of the system is intact, we hope that the activity is not too much perverted, if the central cells from which these fibers arise are connected over wide regions. There are very many efferents, perhaps about 1 per cent of the number of fibers in the optic nerve. They seem to be larger in size than the afferents. This we infer from the shape of the spike, for when we record single afferents the spike may be as long as 5 msec, or more (the same, incidentally, is true in the olfactory system of the frog), whereas it is only about 1.5 msec in the efferents. Besides, in the electron microscopy of the nerve, as done in Young's laboratory, there is a group of fibers, about 1 per cent or a bit less of the population, that are several times larger than the rest, which are uniformly of the same diameter.

Most of the efferents are continuously active at low rates. They respond to a bewildering variety of stimuli, and some require combinations of several modalities. When looking at such elements one realizes how silly it is to make a priori decisions about the workings of a nervous system, however shiny the computer is, and however seemingly rational is the program. We have seen an efferent that responds only to a light being turned on if beforehand we tapped our foot on the floor, or tapped the tank, although it would signal neither alone. Others respond to touching of the mantle or the foot. Still others signal lights in the opposite eye (checked by cutting the optic nerve on the one side). One very lovely efferent had a receptive field of several degrees in the same eye. That is, it would respond to objects moved into an area over a small visual angle. The trouble was that, in checking it, that angle dilated by an order of magnitude to the second movement – that is, the response would begin very much farther out. Some of the efferents are shown in Fig. XIII-10.

We were finally able to record a few single afferent units in the optic nerve by microelectrodes before we had to interrupt our work in Naples. We cannot characterize them now because we saw only a few. But two of them were more responsive to an edge than to light. Our worry is that these two may have been efferents rather than afferents, although the field over which the response occurred was incredibly small, much less than a degree. Until we have a greater population of single units we shall not discuss the matter except to say that we were not surprised by the occurrence of what seemed a primary unit showing an edging operation. (Incidentally, it is also possible to record single cells in the retina, but we do not advise it. The connective tissue is so strong that distortion is inevitable, and so characterization of function is impossible.)

Finally, we have thrust microelectrodes of various sorts into the optic lobe, not so

much to systematize findings as to discover difficulties. There are a variety of neurons visible. On the very surface of the lobe itself one discovers discharging units of somewhat complex behavior with respect to vision. These can only be the amacrines, and so it would seem that, whether or not they have an axon, these cells do exhibit spikes. As one thrusts deeper there is a layer in which no firing is found to visual stimulus and then another layer of cells. This, of course, is in accord with anatomy. However, it is disappointing to us that the terminal arborizations of the afferent fibers in the plexiform layer should not exhibit some distinguishable single-unit activity as in the frog colliculus, for then our work would have been much easier. We have found among the cell types of the lobe many different sorts. In fact, we are in the same position with them that we were with optic nerve of the frog two years ago – every unit seems to follow a law unto itself, and it is very hard to tell what the groups are. Certainly there are on, off, on-off units and suchlike all over the place, but neither of us considers that this characterization is right. I might add a word about the mechanical problems with the optic lobe; whatever microelectrode we used, however easily it seemed to penetrate, it exerted a serious drag on the tissue, and we both had the feeling that we were destroying local units and distorting nervous relations much more than would be allowed for a decent analysis of function.

J. Y. Lettvin, H. R. Maturana

References

1. F. Lane, Kingdom of the Octopus; the Life-history of the Cephalopoda (Sheridan House, New York, 1960).

2. M. F. Moody and J. R. Parriss, The discrimination of polarized light by Octopus: a behavioral and morphological study, Z. vergl. Physiol. 44, 268-291 (1961).

3. E. F. MacNichol, Jr. and W. E. Love, Science 132, 737 (1960).

G. HEART MUSCLE OF SQUILLA

In another project in which Maturana and I were engaged while in Naples we worked with Miss Hilary Maunsell, a Ph. D. candidate from Oxford, on the heart of the squilla mantis. This heart is a long tube extending the full length of the shrimp, and its two layers of muscles are spirally wound in opposite senses over the entire length. The heart exhibits a neurogenic beat, and the nerve cells that are responsible are fifteen in number and distributed along the length of the heart. Both cells and heart have been wonderfully described by Alexandrovicz; the nerve cells, in particular, are of a form that is most interesting for physiological study. I shall not describe them in detail here – our work was mainly on muscle – except to say that their axons make multiple connections with the muscle fibers, and each muscle fiber is densely innervated along

its length, in common with other crustacean heart. The muscle fibers in this species are large, about 40 μ in diameter, with an abundant sarcoplasm and a much-folded sarcolemma or membrane, and are invested with loosely packed bundles of myofibrils whose sarcomeres, at least in individual bundles, are lined up in the usual fashion of striated muscle. The folding of the sarcolemma in the stained section is related in the subharmonic fashion to the myomeric period, but not in any fixed ratio.

Behaviorally, when the heart is removed from the animal and placed in flowing sea water, little if any beat is visible, although the nerve fibers are firing in their typical bursty fashion which will be described a little later. When sea water is passed into the heart so that the sac inflates, a visible beat appears which varies in amplitude with the flow or pressure within the heart. The neurons fire several times in a short burst, which is repeated at intervals of a couple of seconds. This feature is common to crustacean heart in general.

When a single fiber is penetrated with a KCl pipette, there is a potential of about 15 mv across the membrane. During an actual beat of the heart the nervous signals are reflected in a set of confluent intrafibrillar transients of a much longer time constant than the nerve spike, and the total amplitude of the set never exceeds 30 mv. When the same transients are recorded directly outside the membrane before penetrating they have the same sign as inside the fiber – namely, positive with respect to distant ground – but much attenuated; and this is so wherever one goes on the muscle fiber. Thus, it seems that no part of this muscle membrane is electrically active during contraction. If one imposes through the intracellular microelectrode as anode sufficient current to cause a marked sustained local contraction, the transient that is due to the beat is little, if at all, attenuated. If measurements are made with two microelectrodes relatively close together inside the same fiber, with one microelectrode as a current source and the second measuring potential, then during a beat of the heart the transient rides unchanged on the potential shift due to the current source; that is, the lines are parallel, which suggests no resistance change during a beat. If one uses a current source with ac between 20 cps and 2 kc and balances out the signal in the recording electrode within the same fiber as current source, then measuring to the 1 per cent level, no change in impedance is seen in the muscle membrane during the beat. Lastly, if one pokes a hole locally in the muscle membrane in the healthy part, or flows a small amount of potassium chloride solution locally over a small patch of membrane, the muscle there does not contract until a beat occurs, but then it contracts more strongly than the surrounding tissue.

Because of these experiments, Miss Maunsell and we concluded that the membrane here is relatively passive and that the change in potential remarked during the beat transient was probably due to a resistive voltage drop across the membrane rather than to an endogenous shift in membrane potential as set up by the membrane and independent of current. We supposed, then, that it is possible for the myofibrillar elements to be

excited perhaps by longitudinal currents within the muscle fiber rather than by membrane potential, as is the current view, from the superb work of Huxley and Taylor, and Hodgkin and Horowicz. Recognizing fully the strength of their experiments and the caveats suggested by them, nevertheless, we thrust microelectrodes into single muscle fibers to see the figure of excitement by electric current. We came upon this phenomenon: with 0.05 μa through the microelectrode as anode, it was possible to excite a single bundle of myofibrils in such a way that that bundle contracted over a length greater than the transverse diameter of the muscle fiber itself, and none of the neighboring bundles of myofibrils were so contracted. This could be seen most clearly in the fibers that bifurcate. There, separate bundles of myofibrils run from the parent fiber into the two branches. As the current was increased locally in the parent fiber, we could see strong contractions passing up one branch of the fiber but not up the other. The greater the current, the farther out was the contraction visible, the myomeres contracting clearly. We took records to show that the contraction is smoothly and monotonically related in strength and distance to the amount of current passed. No such effect is visible with cathodal currents until one goes to very high strength. It might be suspected that this effect is due to shifting of membrane potential were it not that with an electrode poised external to the fiber, the anodal current again caused local contraction where the cathodal did not, and the amount of current needed to cause contraction of fibers in this way was about 10-20 times that required internally, or roughly the inverse of the attenuation factor of the beat transient between the inside and outside of the muscle membrane. This effect, incidentally, is independent of the composition of the solution in the pipette, whether K^+ or NA^+, CL^-, SO_4^{--}, HPO_3^{--}, $H_2PO_3^-$, NO_3^-, or indeed if the electrode itself were made out of platinum.

After we left Naples, Miss Maunsell did two additional experiments which she has just sent on. First, on thrusting two microelectrodes a short distance apart into the same muscle fiber and connecting them to a current source of 10-megohm resistance, she found not only that the myofibrils contracted markedly around the anode but extended beyond their resting length at the cathode; that is, they showed not merely relaxation but superrelaxation. This, of course, might be expected on any muscle, but here the membrane is relatively inactive, and it is hard to see how the phenomenon can be coupled to membrane process. Second, she severed the muscle fibers on both sides of the nerve cord, the rope of 15 ganglion cells; that is to say, only a small segment of each muscle fiber was left attached to the nerve, and each segment was open at both ends. Nevertheless the segments kept contracting with each beat over a very long time. We take it from Miss Maunsell's letter that it was at least one half-hour. It is very hard to understand how this could occur if membrane potential were the important factor. In fairness, we must also say that the muscle opened up by a longitudinal cut severing the nervous supply ceases to be excitable electrically in its myofibrils. Miss Maunsell

considers this significant. We do not, yet; but we shall all resolve the point during the coming summer.

Now, it is perfectly true that if we replace the sodium in sea water by potassium, the whole heart goes into contraction. I do not see how this proves anything in the light of multiple innervation. Miss Maunsell and we are of the opinion that it is quite possible that the nerves are connected to the muscle in a fashion similar to that discussed by Furshban and Potter for other crustacean synapses; namely, as if the junctional membrane were a diode whose forward direction is to the inside of the muscle fiber. Under such circumstances the depolarizing of the terminal nerve membrane distributed widely over the surface of the muscle would serve as a distributed source of current both to excite myofibrils and cause the beat transient. Under conditions of continued and profound depolarization by a bath of external potassium-rich solution, the muscle would then go into contracture. On the other hand, because of diodic connections through such a conjunction, one could never excite a nerve backward from the inside of the muscle fiber with anodal current, however much current one used, and this certainly seems to be the case.

I must in fairness admit to making here an enormous jump which I am not prepared to defend completely. Nevertheless, it is not easy to think of another mechanism that explains all the observations given above.

Miss Maunsell and we plan to conclude this work this coming summer.

J. Y. Lettvin

H. STATOCYST OF OCTOPUS

The other project in which we engaged, with Dr. Helga Schiff of Stazione Zoologica and a young American student, Miss Suzanne Sperling, was the examination of the statocyst system of the octopus. Again, we are faced with a remarkable convergent evolution between vertebrates and cephalopods. The statocyst organ of the octopus lies in a cavity within the cartilage below the orbit and lateral to the brain cavity on either side. It consists of a sac held suspended in a perilymph by numerous trabeculae and connected nervously to the brain by a nerve exiting medially and passing through a hole in the cartilage. The anatomy of this system has been well described by Young in a monograph, and I shall attempt to excerpt it very crudely here.

The sac itself has a body of fluid in it, the endolymph, of much the same composition as the perilymph, and both are very much like sea water. The inner fluid is thixotropic, at least in our experience. The wall of the sac is invested with numerous ganglion cells and hair cells, some of which are organized along three mutually orthogonal arcs of great circles, in a manner reminiscent of our own semicircular canals. There is, in addition, a special structure – a small yellowish-white pebble – the otolith, that rests on what seems to be jelly and hair cells. The nerve fibers from various scattered

nervous elements over unspecialized and specialized portions of the organ course along the outside in different bundles, to coalesce finally into several major bundles that together exit to the brain.

Thus, if we penetrate the perilymph with a microelectrode and touch the surface of the sac, we meet with nervous elements whose triphasic spike assures us that they are not cell bodies. Some of these fibers are firing continuously, and some are silent save for a vibrational stimulus. Some are exceedingly sensitive to barely perceptible seismic disturbances such as someone walking around on a stone floor, and some have a high threshold and require a tap on the tank. In any case, there are no slower transients than those of nerve fibers themselves observable on the outer surface of the sac, or in the nerve where it is gathered in a hole through the cartilage. As the sac is penetrated slowly with a fluid electrode, cell bodies appear and can be penetrated so as to record internally, or identified externally by this diphasic character with an initial negative phase. At the same time as vibrational stimuli are applied, a slow transient appears, entirely negative in sign, whose fluctuations show as an integrated full-wave rectification of applied stimulus. This transient slowly grows in size to the same stimulus as we penetrate deeper into the sac, and then smoothly comes to a maximum as we come completely into the endolymph. There this "microphonic" – fully-developed and entirely negative in sign – can be seen, and it looks extremely much like that recorded from lateral line organs in vertebrates.

What is very elegant in this system, outside of the nervous elements that in their final make-up will not differ markedly from lateral line neurons, is the character of this microphonic, which here shows no external sign of its presence anywhere around the sac. We have not, of course, been fortunate enough to penetrate exactly where a large nerve bundle exits from a crista or the otolith base, so what is said here may not apply completely. Nevertheless, the hypothesis put forth by Dr. Schiff and Miss Sperling is this: Suppose that all sensitive endings lay organized radially to the inner surface of the organ, were fairly dense and almost uniform in distribution, and the intervening tissue were of high impedance. The inside of the sac would then be topologically unique in looking at the ends of all sensitive elements, and therefore if all of these sensitive elements showed excitement by depolarizing, the endolymph should display a continuous potential with respect to the outside that represented the integral of all the activity of all the elements. At the same time, since by symmetry there is very little external path for such depolarizing, there will be no sign of such activity on the outside of the sac. If one pokes a hole in the sac, then there will be a small attenuation of microphonic in the endolymph and an appearance of an attenuated version of the microphonic externally of the same sign and same time course, because of the geometry of nervous elements. This seems to be the case.

Doctor Schiff and Miss Sperling are planning to continue their studies on this system

during the coming summer under Dr. Maturana's and my guidance. They are also planning to publish preliminary notes on the system.

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References

1. J. Z. Young, The statocysts of Octopus vulgaris, Proc. Roy. Soc. (London) B152, 3-29 (April 1960).