XVI. NEUROPHYSIOLOGY

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A. OPTIC PROJECTIONS OF THE TOAD (Bufo marinus) AS SEEN WITH AUTORADIOGRAPHY

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In this report we look at the distribution of optic fibers in the brain of the toad Bufo marinus. In Section XVI-B some of the anatomical results obtained here are used to study the optic nerve neurotransmitter in the tectum.

Toads (Bufo marinus), obtained from Mogul-Ed (approximately 13-cm body length and 400 g), were cold-anesthetized in crushed ice. A hole was made in the sclera with a #22 hypodermic needle immediately behind the iris on the dorsal surface of the eye. Inserted into the hole, making a tight fit, was a capillary tube (OD 1 mm) that had been pulled and tapered in a heated wire, broken off to give a tip of approximately 0.2 mm, and filled with 8 μl amphibian Ringer's solution containing 30 μC ^{3}H L-Proline (New England Nuclear Co.). The proline was injected into the eye over a 40-min period. The tube was left in place for an additional 5 min before removal.

The animals were then maintained at approximately 22° C. The brains were removed after 12, 26, and 72 hours and fixed in buffered, neutral 4% formaldehyde. Serial sections 10-μ thick were cut from the paraffin-embedded brains, coated with Kodak N.T.B. 2 emulsion and exposed for times up to 16 days. The sections were then developed in Kodak D-19 and counterstained lightly with cresyl violet.

Counts were seen over the tectum 12 hours after ocular injection, and more counts were found after 26 hours. At that time relatively few counts were seen in fibers of passage, specifically in the basal optic tract and the fascicles emerging from the optic chiasm. The optic nerve, however, did have some counts. At 72 hours there seemed to be fewer counts in terminal areas.

The method of injection yielded an even incorporation of the labeled proline into retinal ganglion cells throughout the retina. This was determined by finding a uniform

distribution of counts in rostral or caudal, lateral or medial sections of the tectum. Background was quite low, and boundaries of projections were sharp. Six distinct projections were found:

1. Contralateral tectum. The largest projection of the optic nerve is to the tectum. Counts were found in the outer one-third of the tectum (Fig. XVI-1, E-J and Fig. XVI-2I). Two layers of counts could be distinguished: a thin lamina, the stratum zonale immediately beneath the surface, and, after a small separation, a much larger layer encompassing the remainder of the outer third of the tectum. In the latter layer 3 inchoate subdivisions could be sensed.

2. The contralateral basal optic projection. This is a small and compact area located in the rostral end of the ventral tegmentum (Fig. XVI-1G, H and Fig. XVI-2G).

3. The contralateral pretectal projection is well represented and separate from the adjacent tectal projection. It runs diagonally and is medial to the extreme rostral end of the tectum (Fig. XVI-1E, F and Fig. XVI-2E).

4. The ipsilateral pretectal projection mirrors the contralateral projection, but the counts are 20 times less numerous (Fig. XVI-1F).

5. The contralateral anterior thalamus has two incompletely separate parts (see Fig. XVI-1B) corresponding to the dorsal nucleus Bellonci and the more ventral lateral geniculate body (Fig. XVI-1, A-D).

6. The ipsilateral anterior thalamus, like the ipsilateral pretectum, has many fewer counts than its contralateral counterpart. Counts can be seen in the nucleus Bellonci and in the periphery of the lateral geniculate (Fig. XVI-1, B-D). The ipsilateral projection does not appear to extend into the central area of the geniculate.

The distribution of optic fiber terminals that we have described in the toad closely follows the pattern described in other anurans. Using a degeneration stain, Scalia et al.[1] showed terminal degeneration in the superficial zone of the tectum that did not include the stratum zonale (lamina A of Potter[2]) of Rana pipiens. In a later autoradiographic paper using intraocular injection of tritiated proline, Scalia[3] found that the stratum zonale also shows significant counts as is the case in the toad. He also showed a separate, very thin deeper lamina (lamina G of Potter) which also has counts. In the toad lamina G (or its spatial equivalent) is cold.

We found that the distribution of optic fibers in the toad thalamus is similar to the distribution in the European frog Rana esculenta.[4] Using a degeneration stain, they showed a greater separation between optic fiber terminals of the nucleus Bellonci and the lateral geniculate than in the toad. There is also in the frog a restriction of ipsilateral lateral geniculate optic terminals to the dorsal medial area.

Our use of an autoradiographic method was precipitated by the difficulty we had in trying to adapt modifications of Fink/Heimer degeneration staining for the toad. We had successfully and routinely used these stains in previous work on frogs and salamanders.

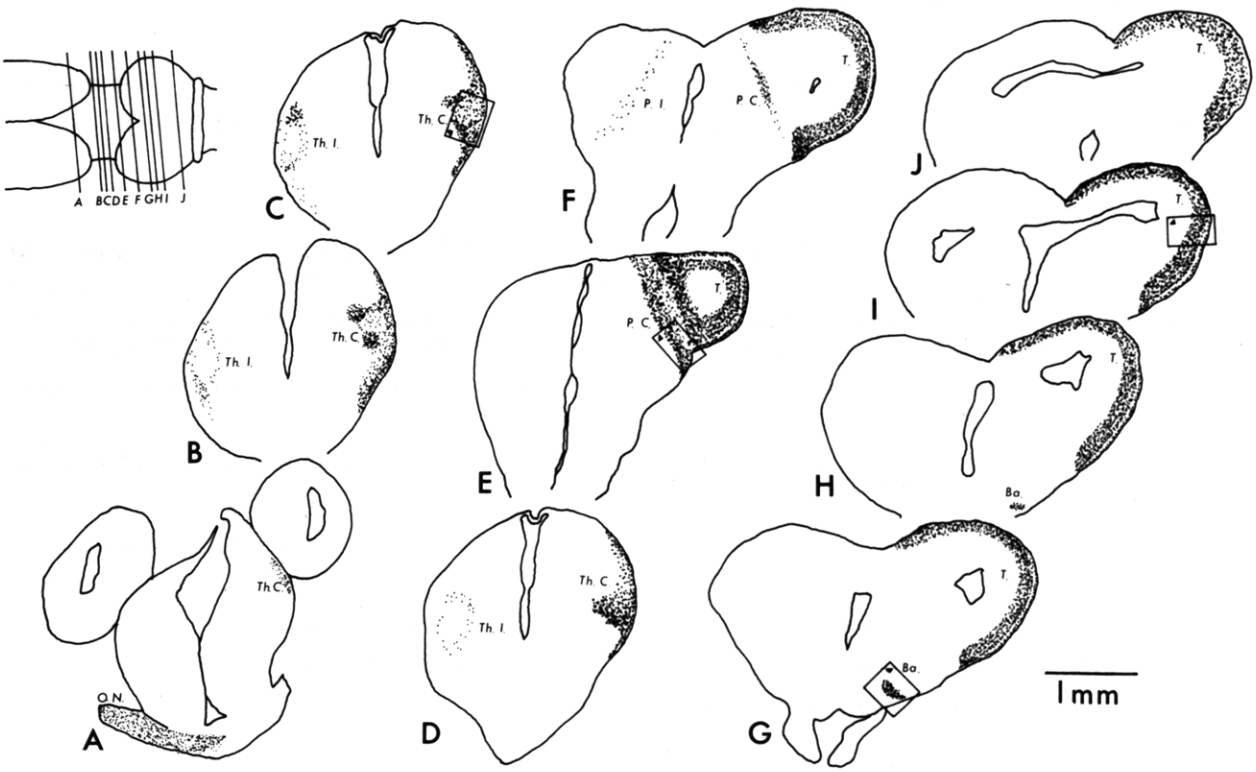

Fig. XVI-1. Camera lucida drawings of almost transverse sections of toad brain showing distribution of optic fibers. Intraocular injection of 30 μC ^{3}H L-Proline was followed by excision of brain 26 hours later. Brain sections were covered with photographic emulsion and exposed for 16 days. Section A, most rostral, section J, most caudal. Abbreviations: T, Tectum; Ba, basal optic projection; P. C., contralateral pretectal projection; P. I., ipsilateral pretectal projection; Th. C., contralateral anterior thalamic projection; Th. I., ipsilateral anterior thalamic projection; O. N., optic nerve. Inset shows dorsal aspect of brain. Lettered lines correspond to rostral/caudal locations of the planes of the sections.

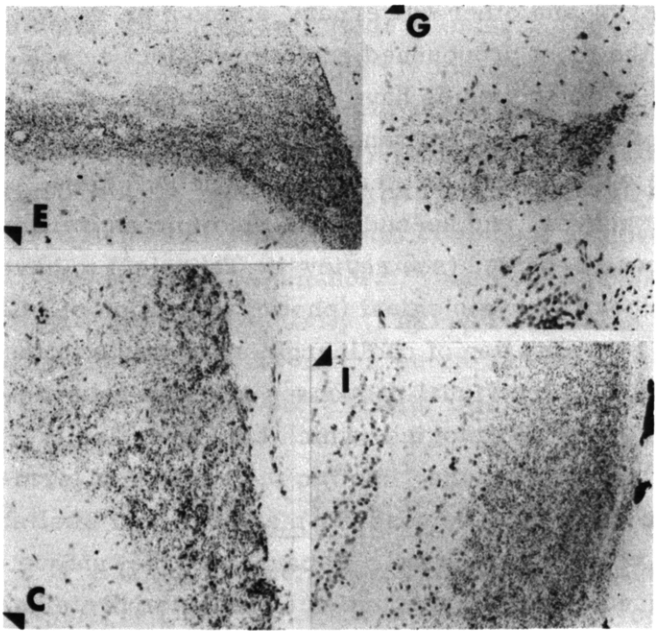

Fig. XVI-2.

Photomicrographs of some representative areas of Fig. XVI-1. Letters correspond to lettered sections of corresponding boxed areas in Fig. XVI-1. Black triangles in the corners and the boxed areas in the drawings give the orientation.

We were able to stain toad degenerating fibers of passage (9 days at 23° C). Terminal degeneration (fine particles) was spotty and surprisingly late (14 days at 23° C).

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B. AChE ACTIVITY AND ACh SYNTHESIS IN THE OPTIC TECTUM OF THE TOAD Bufo marinus: PROBABLE IDENTIFICATION OF AN OPTIC NERVE NEUROTRANSMITTER

National Institutes of Health (Grant 5 RO1 EY01149-02)
Bell Telephone Laboratories, Inc. (Grant)

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The identity of the optic nerve neurotransmitter, while long a subject of interest, has remained elusive.[1] Curtis and Davis reported[2] that of a variety of putative transmitters studied iontophoretically, acetylcholine (ACh) and other quaternary ammonium derivatives, particularly carbamylcholine, had the most pronounced excitatory effect on activating LGN neurons in the cat. A variety of other studies have confirmed this. ACh, however, is now thought not to be an optic nerve neurotransmitter for two principal reasons: First, the levels of ChAc in the optic nerve, as measured by Hebb,[3] Ross,[4] and others, are much lower than in motor nerves, and second, both nicotinic as well as muscarinic cholinergic antagonists have been reported (see review by Tabecis[1]) to have little or at best an inconsistent effect in blocking physiological (photic) activation of LGN neurons. Stevens[5] and George[6] report that application of cholinergic antagonists to the frog optic tectum produce profound alteration of the visual responses of tectal neurons. We have found[7, 8] that a variety of labeled snake neurotoxins, which bind specifically to nicotinic cholinergic receptor protein in other systems,[9] bind to the regions of termination of optic nerve fibers in the optic tectum of the toad B. marinus, and abolish synaptic activation of tectal neurons by visual stimulation. Because of the unique possibilities that these toxins provide in studying the properties of receptor proteins,

especially during development and during natural regeneration, we have undertaken a reexamination of the possible role of ACh as an optic nerve neurotransmitter in the optic tectum of the toad, Bufo marinus. This animal is particularly well suited to combined anatomical, biochemical, and electrophysiological studies because of the highly organized, laminar structure of its optic tectum, in which the optic nerve terminals are distributed in compact, largely cell-free laminae and in which the neuron somata are rather larger than in some other amphibia, thereby facilitating intracellular recording. In this report we show that specific ACh-Esterase (AChE) is distributed in all major projection areas of the optic nerve terminals, including especially the laminae of optic nerve terminals in the optic tectum. We also show that ACh synthesis occurs in significant amounts in the same regions, despite the relatively low concentration of choline acetylase (ChAc) in the optic nerve trunk. With reference to other work, we suggest that the optic nerve fibers are cholinergic.

1. Acetylcholinesterase Distribution

Toads were anesthetized by cooling over crushed ice. The brains were removed and frozen in a bath of 2 methyl-butane cooled by dry ice. The brains were then mounted in a chuck and sectioned transversely at 15 μm in a cryostat set at -15° C. The sections were mounted on uncoated slides and fixed for 30 min in phosphate-buffered 4% neutral formalin. They were washed in several rinses of distilled water and incubated, using the method of Karnovsky and Roots[10] with acetylthiocholine iodide (Sigma) as substrate and pH 6.0 sodium acetate/acetic acid buffer. Specificity of the reaction was confirmed by substitution of butyrylthiocholine iodide as substrate or complete absence of substrate. Neither case yields staining. The positive reaction obtained with acetylthiocholine iodide was completely inhibited by 10^{-4} M BW284C51, a selective inhibitor of AChE. Other sections were put aside for staining with cresyl violet. Tissues were incubated at approximately 32° C until the stain was visible (~30 min). They were post-fixed in 2.5% glutaraldehyde for 10 min, dehydrated through a graded set of alcohols and xylene and mounted with Permount on coverslips.

As with the salamander[11] each optic projection and the optic nerve fibers showed significant AChE activity (Fig. XVI-3C). Midbrain sections revealed three dark bands of activity over the entire outer half of the tectum alternating with two lighter bands (Fig. XVI-3a and 3b). Within this area are the retinotectal terminals described in Section XVI-A.

2. Acetylcholine Synthesis

The brain was removed from a toad anesthetized by cooling on crushed ice. It was transferred to a culture dish that was kept cool (but not frozen) by dry ice. When it

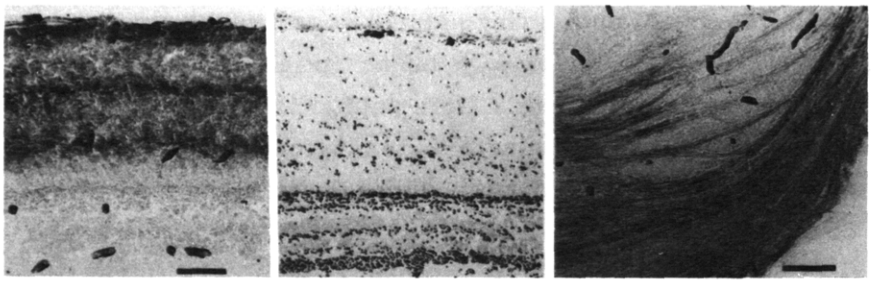

Fig. XVI-3. (a) Histochemical localization of AChE in the toad optic tectum.
(b) Section adjacent to (a) stained with cresyl violet.
(c) Histochemical localization of AchE showing stained fascicles of optic nerve fibers leaving optic chiasm on left.

was reasonably firm, after one or two minutes, the brain was dissected under a microscope. The dura was removed from the dorsal midbrain and with care a part of the tectum could be excised and separated into an outer portion which extended more than halfway (to include all dark staining areas found from the AChE incubation) and a complementary inner portion. We saved the optic nerve and divided it into two parts, one with the dura intact and one with the dura removed. We also separated a region of the telencephalon (dura removed) which showed no apparent AChE activity.

Tissues were incubated individually at room temperature in a medium of 8 parts L-15 (GIBCO) deficient in choline, tyrosine, tryptophan, and glutamic acid; 2 parts water containing 1000 units/cc pen/strep; 1 part fetal calf serum; and 20 μC/ml choline-methyl ^{3}H chloride (New England Nuclear Co.). The tissue was incubated overnight, approximately 16 hours at 22° C. Tissue samples were then transferred to homogenizers and each was homogenized in 50 μl of pH 1.9 solution (2% formic acid, 8% glacial acetic acid). Five μl aliquots were removed to 1 cc test tubes for Lowry protein microassay. The remainder was spotted on Whatman 3MM paper along with a sample of the labeled choline, the medium in which the outer tectum had just been incubated, and unlabeled AChCl and ChCl crystal markers. The samples were electrophoresed (origin positive) at 4500 V for 1.5 h during which time the current increased from 100 mA to 110 mA. The paper was dried for one hour and sprayed with freshly mixed 1% iodine in acetone solution so that the ACh and Ch markers could be localized.

The paper was cut in 1 cm × 2.54 cm strips and transferred to individual scintillation vials containing 5 ml Aquasol (New England Nuclear Co.). Samples were counted for 10 min on a Beckman LS-250 liquid scintillation counter system.

The Lowry procedure is the Oyama/Eagle modification using folin-ciocalteau as the color developer.[12] To the 5 lambda aliquots of the homogenized tissue were added 15 μl 1N NaOH, the latter to raise the pH to approximately 7. In addition, controls were

made with a graded set of known quantities of protein to which a 1 mg/ml solution of bovine serum albumen was added to test tubes containing 0, 3.75 μl, 7.5 μl, 15 μl, and 30 μl BSA, respectively. Freshly prepared solution C [A: Na_2CO_3, 200 g; NaOH, 40g; NaK Tartrate, 2 g q.s. to 10 liters; B: $CuSO_4 \cdot 5H_2O$, 5 g; q.s. to 1 liter (50 parts A to 1 part B forms Lowry's Sol. C)] was added to all of the tubes containing either unknowns or controls to bring the total volume to 0.75 ml. Water (0.075 ml) was also added, followed by the addition of 0.075 ml folin-ciocalteau reagent and was mixed rapidly with a vortex stirrer. After waiting 20 min for the solution to become stable, the optical densities were measured on a Zeiss spectrophotometer at 750 mμm incandescent illumination.

ACh synthesis is clearly seen in the outer tectum (Fig. XVI-4), little ACh is synthesized in the inner tectum (dotted line). On a normalized protein basis, synthesis of ACh is at least 5 times greater in the outer tectum (Table XVI-1) than in the inner tectum, the optic nerve, or the telencephalon.

The high rate of synthesis of ACh found in the outer tectum cannot at this point be directly linked to optic fiber terminals. But coupled with the distribution of AChE activity, two necessary conditions for cholinergicity are satisfied.

3. Discussion

All of the cholinesterase found in this study was of the specific type. This confirms previous findings of Shen et al.[13] on the distribution of AChE in the frog tectum, and of Minelli and Quaglia[14, 15] in the tectum of Triturus, and of Gruberg and Greenhouse[16] in the tectum of Ambystoma tigrinum. Brightman and Albers[17] found only specific cholinesterase in the spinal cord of Bufo marinus. AChE was present in all retinal projection areas examined, including the tectum, the nucleus of Bellonci, the corpus geniculatum thalamicus, and the posterior thalamic nucleus. From this we conclude that the AChE is associated with retinal ganglion cell terminals. A similar association of AChE with areas of ganglion cell termination has been found in other vertebrates, including the leopard frog,[13] pigeon,[18] newt,[15] salamander,[11] rhesus monkey,[19] and albino rat.[20, 21] The preponderant evidence, from this and from earlier studies (reviewed by Gruberg and Greenhouse[11]) suggests that AChE is associated with ganglion cell terminals.

Our results demonstrate that the regions of optic nerve termination in the optic tectum have a high ACh synthesis as compared with the optic nerve, the telencephalon, and areas of optic nerve devoid of terminals. Similarly, Feldberg and Vogt[22] demonstrated high values of ACh synthesis in the canine LGN and superior colliculus, and in screening the optic tectum of the tiger salamander for a number of different putative transmitters (using a similar technique to the one employed in this study), we also

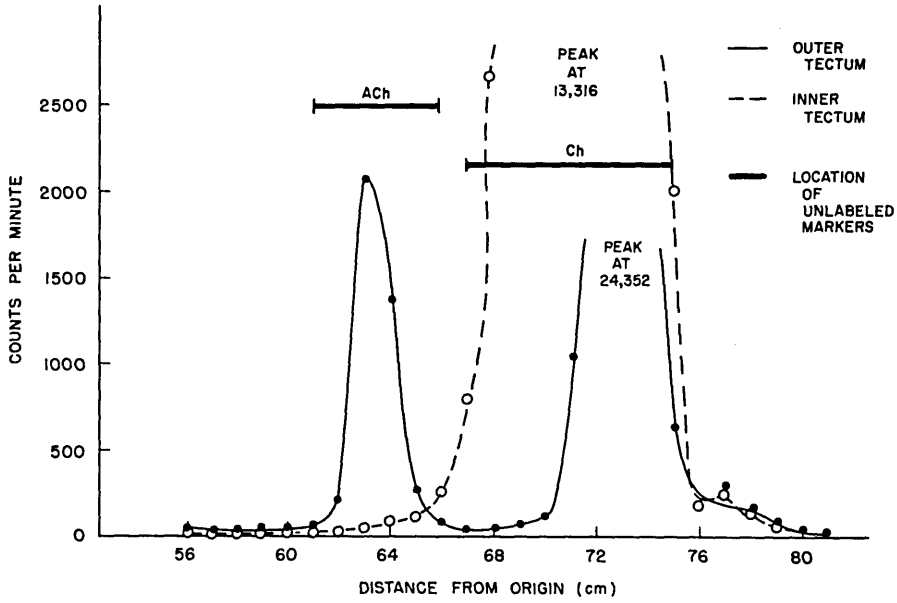

Fig. XVI-4. Comparison of ACh synthesis in outer tectum (containing optic nerve fiber terminals) vs inner tectum. Radiochemical analysis of electrophoretograms with unnormalized scintillation counts shown as a function of distance of labeled Ch and newly synthesized labeled ACh from origin. From Lowry procedure, total inner tectum protein = 12.5 μg, total outer tectum protein = 13.9 μg.

Table XVI-1. Comparison of ACh synthesis in different parts of the toad brain.

	Brain #1		Brain #2		
	Forebrain	Outer Tectum	Unsheathed Optic Nerve	Inner Tectum	Outer Tectum
ACh counts/min/10 μg protein	423	2168	580	512	2940
Ratio ACh synthesis/unit protein of tissue to outer tectum	0.195		0.198	0.175	

found significant amounts of ACh synthesis in the optic tectum.[16] On the other hand, previous measurements[3,4] of the levels of ChAc in the optic nerve as compared with motor nerves have shown that this enzyme is present in very low amounts. Several possibilities might explain these findings: (i) the levels of ChAc might be normally low in the optic nerve but concentrated in the terminals. This is suggested by the studies of DeRobertis[23] and by Marchbanks[24] that demonstrated ACh synthesis by synaptosomal fragments (terminal axonal endings isolated from their preterminal fibers), (ii) the high

levels of ACh synthesis that we and others have observed in regions of optic nerve termination are produced by nonretinal fibers also ending there. Lazar and Szekely[25] describe in the frog optic tectum a system of ascending axons in the superficial tectal neuropil that might provide such a source of cholinergic fibers, but they do not show them arborizing specifically in the regions of termination of each class of retinal fiber, nor have we seen such a system of fibers in our Golgi material on the optic tectum of Bufo marinus.

Further evidence implicating ACh as an excitatory optic nerve neurotransmitter has been obtained from two related studies now in progress:

1. We have found[7, 8] that tectal neurons, identified by intracellular injection of procion yellow and monosynaptically driven by the optic nerve, are depolarized and generate propagated action potentials in response to iontophoretic application of ACh, and this excitatory response is blocked by d-tubo-curarine and by α-bungarotoxin, but not by atropine. Similarly, Curtis and Davis[2] and Tabecis[1] have found that ACh is an extremely potent excitant of LGN neurons, although, whereas the excitatory response of LGN neurons to iontophoresis of either ACh or carbamylcholine is reported to be blocked by nicotinic cholinergic antagonists in low concentration, the response to photic stimulation is either not blocked, or blocked only by much higher concentrations. In a related study, Masland has found[26] that some nicotinic cholinergic antagonists (e.g., d-tubocurarine, dihydro-β-eythopoidin) may have either an inhibitory or an excitatory effect on cholinergic transmission on retinal ganglion cells of isolated rabbit retina, depending on concentration, pH and ionic strength, which points out the difficulties inherent in the attempt to identify neurotransmitters by purely pharmacological means.

2. Furthermore we have found[7, 8] (also manuscript in preparation) that elapidae snake neurotoxins labeled with either ^{3}H-, FITC or horseradish peroxidase, bind specifically to the same regions of tectal neuropil occupied by optic nerve terminals, which suggests that the receptor protein is cholinergic. Preliminary studies have shown that this binding is saturable and specific, that it can be displaced by unlabeled toxin, d-tubocurarine, or hexamethonium but not by atropine, and thus that it satisfies the operational definition of a nicotinic cholinergic receptor protein. Using a modification of the Folch-Pi procedure,[27] we have extracted a proteolipid from a membrane fraction; it behaves similarly to nicotinic cholinergic receptor protein extracted from muscle, brain, and Torpedo electric organ[28] with respect to specificity of binding of nicotinic cholinergic agents in vitro. We are now attempting to purify the receptor protein by using a detergent rather than an organic solvent extraction procedure. We have found that the receptor protein, assayed radiometrically in a membrane pellet, is present in high concentration in the tectal optic neuropil (specific activity in the tectum being 8 times higher than in telencephalon and 4 times higher than in muscle). The pharmacological distinction between nicotinic vs muscarinic cholinergic receptor has

yet to be definitively clarified in the central nervous system,[23] which again points out that the results obtained by iontophoretic studies should be viewed with caution.

Although it is not yet possible to measure directly or to simulate experimentally the actual identity and quantities of neurotransmitters liberated by the optic nerve terminals, our histochemical, biochemical and physiological studies taken together provide what we believe to be reasonably compelling evidence for concluding that in the toad, ACh serves as an optic nerve neurotransmitter.

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C. EXPLANATION OF INTERMITTENT CONDUCTION BASED ON ACTIVITY-DEPENDENT CHANGES IN NERVE THRESHOLD

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Stephen A. Raymond, Paul Pangaro

Intermittent conduction has been noticed several times. The term originated with Barron and Matthews,[1] who recorded intermittent trains on the central ends of dorsal root fibers in cats. Because these trains were continuous at their source in muscle

spindles, Barron and Matthews concluded that there was some sort of periodic failure of conduction in the spinal cord. Since then, very similar intermittent conduction has been seen in the terminal branches of rat phrenic nerve,[2] in crayfish motor nerve terminals,[3] in dorsal column fibers of cat[4] and frog,[5] and in frog sciatic nerve fibers given trains of near threshold stimuli.[6-8] In 1971, Newman and Raymond[8] gave an explanation for the periodicity of intermittent conduction in terms of experimentally measured threshold shifts. We became convinced that subthreshold, long-term processes exert a strong influence on impulse conduction wherever it is probabilistic. Several such places, called regions of low-conduction safety, have been found and studied.[9-11] Because intermittent conduction seems to be an example of the way in which mild influences can mediate conduction at regions of low-conduction safety, we have expanded our explanation and tested it on a model axon. The sequential oscillation of the threshold shows up vividly in motion, so we are making a film of nerve threshold during intermittent conduction in an effort to create a clear visual image of some of the consequences of nervous activity in axons.

1. Method

We present here results of stimulating a model axon that has the following properties. The axon fires probabilistically to stimuli having an "effectiveness" of 0 to 1, provided that successive stimuli are more than 3 sec apart. Otherwise, the results of one stimulus measurably alter the probability of responding to the next.[7] A stimulus of 0 is barely strong enough to fire the axon once in 100 tries. A stimulus of 1 will result in only one failure in 100 tries. We refer to the region of stimulus effectiveness between 0 and 1 as the gray region. Outside of this gray region the firing of the nerve depends deterministically on the relation between the stimulus and the threshold.

Once the model axon fires, initially it is refractory, then superexcitable, then depressed, and finally recovered. The time course and magnitude of these effects are specified by a system of equations drawn up from experimental work in frog sciatic nerve.[12] The periodicity in response to near-threshold stimuli shown by the axon as we present it here, remains to be tested against the periodicity of real sciatic nerve axons subjected to the same conditions. The threshold curves match well. Intermittent responsiveness of sciatic axons was not used as a criterion in developing the model axon equations for the computer.

2. Results

Figure XVI-5 shows the threshold curve following a response of a single axon to a stimulus. What is most significant is the transient phase of superexcitability. If a second stimulation at a strength of 0.5 occurs in this phase (as in the second pair,

75 msec apart), the second response is guaranteed. Furthermore, such a response adds its own superexcitability to that left over from the first. The enhancement of superexcitability can be seen in the threshold curves following two spikes at 250 msec and following five spikes at 500 msec. This property suggests why a run of successful stimuli should follow a first response. It does not show why that run ceases or why, once the run has ceased, it does not start again immediately.

Figure XVI-6 shows how the periods of conduction are stopped. Each firing of the fiber contributes an increment of depression. As depression builds up, the superexcitability phase begins to shorten and its magnitude is reduced. This is reflected in a progressive migration of successive peaks of superexcitability toward higher and higher threshold. Note also the gradual increase in rate of return from peak superexcitability during the period of continuous response before the eventual failure. Soon the threshold is in the gray region, and response to the next stimulus is no longer guaranteed. After the first failure, the threshold continues to climb until it moves past the gray region into a deterministic zone in which no response is possible even though stimulation is continuous.

The axon remains depressed until recovery processes return the threshold to the gray region. Figure XVI-7 shows this sequence. Once there is a firing, the superexcitable phase following it guarantees that the next stimulus, which happens to be 150 msec after the first response, will also be successful. A new period of conduction, with its attendant buildup of depression, is initiated. This period also culminates in failure. The threshold during two such oscillations is shown.

We have explained intermittent responsiveness in terms of threshold curves. We believe intermittent conduction is analogous, except that the near-threshold electrical stimulus must be replaced by nerve-impulse currents that are not quite strong enough to stimulate the membrane distal to the zone of low-conduction safety. In intermittent conduction, factors besides activity-dependent threshold change may be important. Such factors are impulse size changes, or variation of coupling cable parameters. Threshold changes, however, are sufficient to account for the experimental observations that have been made thus far. A separate experimental test has not yet been made but available records of periodicities for intermittent conduction agree with the computer projections based solely on threshold curves.

The equations also predict the following behavior.

a. For continuous trains of stimuli there will be a relation between the frequency of stimulation and the period of intermittence. The higher the rate of stimulation, the slower should be the alternation between conduction and failure periods (Fig. XVI-8) (also see Fig. 9, in Raymond[4]).

b. The duration of the first period of continuous conduction remains approximately the same (18-30 sec) from frequencies of 10 per second to 70 or 80 per second. This

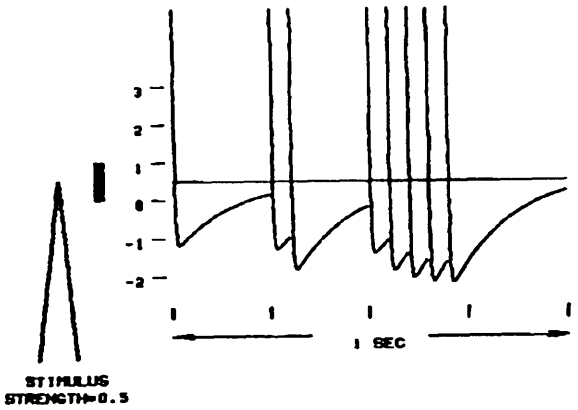

Fig. XVI-5.

Response of a single axon to stimulus. The y axis is calibrated in "threshold units" equal to the variation in stimulus required to span the gray region (denoted by the vertical bar) where response is probabilistic. Horizontal line shows the 50% response threshold for rested nerve. Note that peak superexcitability is well outside the gray region for stimuli that are only 50% effective at rest. Firing is thus ensured for the second stimulus of the pair, since it occurs 50 msec after the first when threshold is approximately one threshold unit below the gray region. Subsequent pulses further lower the threshold.

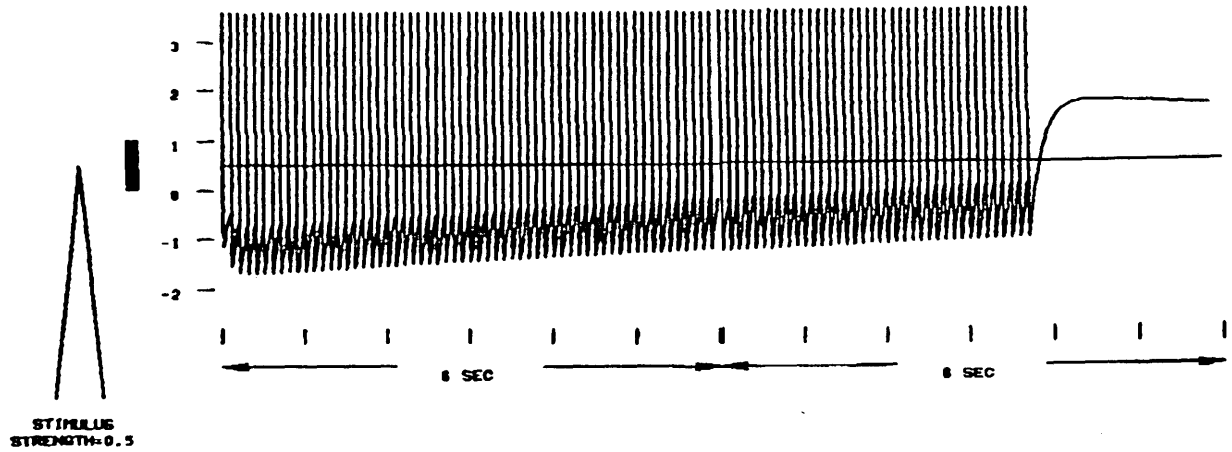

Fig. XVI-6. Threshold during 12 sec of stimulation at 100-msec intervals at an effectiveness of 0.5. One successful response leads to a run that ends 10 sec later. The superexcitable peaks first add and then are reduced slowly as depression grows. Approximately 100 msec after the first failure, the threshold moves beyond the gray region to depression. The gradual increase in rate of return from the superexcitable peaks can be gauged by the threshold level reached before the refractory period of the next response.

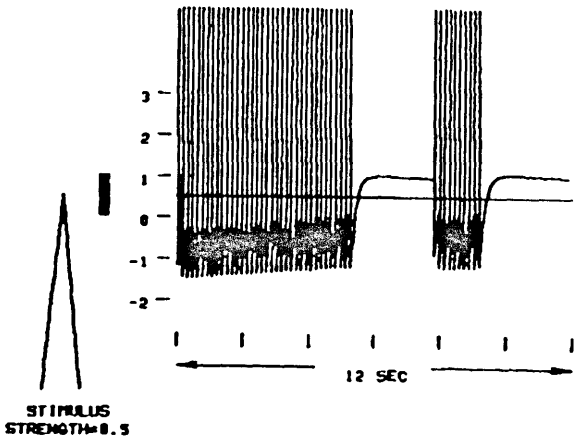

Fig. XVI-7.

Threshold during 2 cycles of intermittent responsiveness. With the expanded time axis the fast threshold shifts after each spike are superimposed, so we see the envelope of the supernormal peaks. Threshold during periods of nonresponse is depressed, and recovers to reenter the gray region. With these time scales and at these frequency rates the alternation is rather strict.

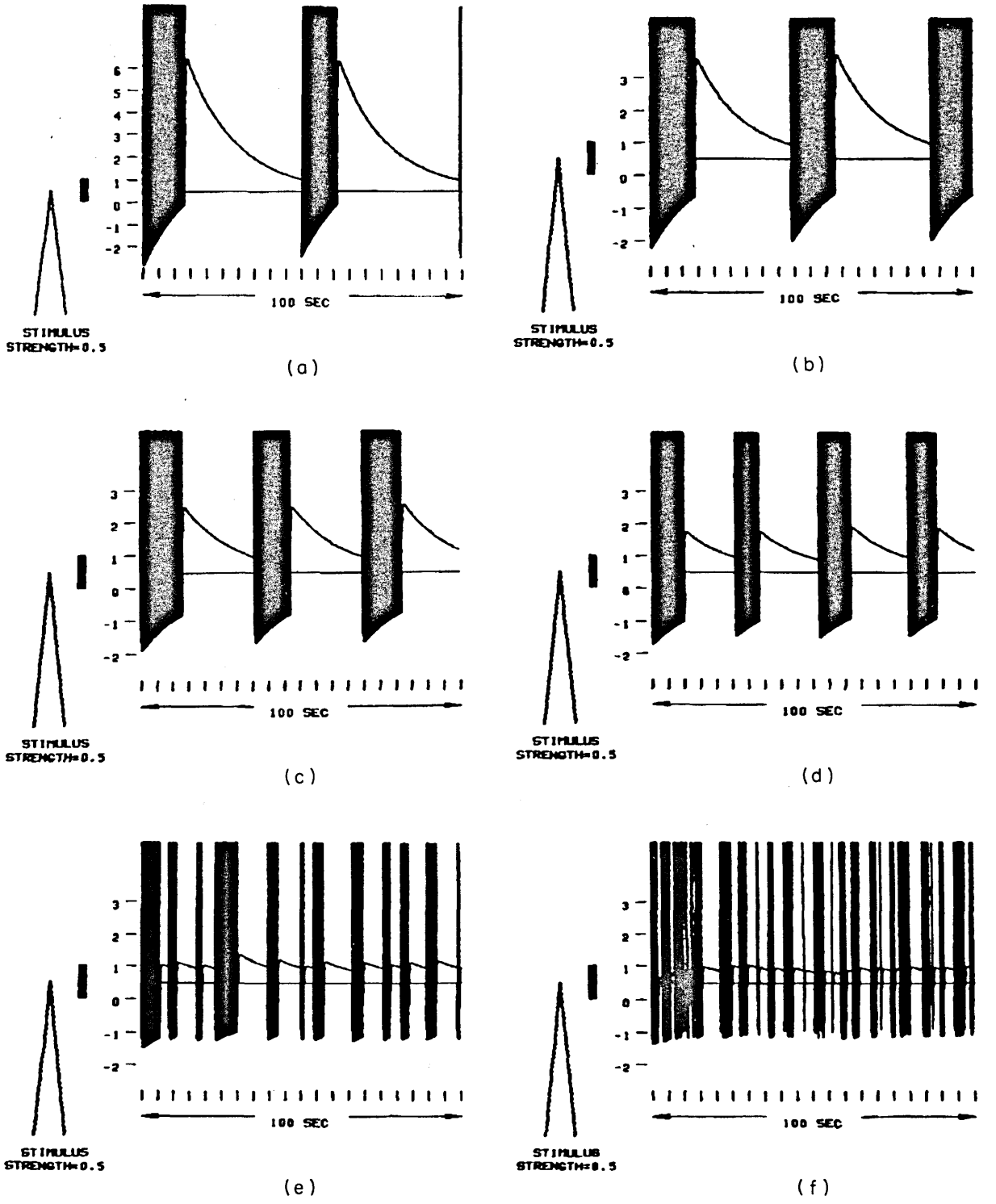

Fig. XVI-8. Relation between stimulation rate and periodicity of response. Thresholds during 100 sec of continuous stimulation are shown for stimulation rates: (a) 40/sec, (b) 20/sec, (c) 13/sec, (d) 10/sec, (e) 7/sec, (f) 5/sec. At higher rates the increased depression prolongs the nonresponse period. Increased superexcitability at high frequency prolongs the response period as well. Note change of threshold scale in (a).

feature reflects the tradeoff between two opposing effects. As stimulation becomes faster, the depression accumulates more quickly. Within the rates mentioned, however, faster stimulation also means that each stimulus is closer to the peak superexcitability following the last response. Thus the same sized stimulus will remain effective even if depression is very large. Consequently, the on periods are about the same, but the recovery periods vary widely, since a good deal more depression builds up at higher frequencies of stimulation than at lower rates (Fig. XVI-8).

c. Given similar conditioning activity, fibers having the larger threshold variations will have slower periods of oscillation, since they spend more time outside the gray region than fibers with lesser magnitude threshold curves (Fig. XVI-9). Since the proportion of conducting vs failing time depends on the threshold curves, a complete relation between stimulus frequency and periodicity of conduction in an intermittent fiber would allow us to infer the threshold curves for that fiber. Those curves are dominated by approximately five variables. With sufficient study of their interaction with drugs, temperature, and activity, it may eventually be possible to infer with confidence membrane properties that cannot be measured directly at the zone of low-conduction safety.

3. Discussion

These tests show that equations intended to represent only the relation between activity and threshold also predict intermittent responsiveness for constant amplitude stimuli near resting threshold. The periodicity of that intermittent responsiveness matches the limited experimental observations made thus far on frog fibers. With appropriate changes in rate constants associated with recovery from depression and return from superexcitability, threshold curves can also be made to generate the faster alternation between conduction and failure that is observed in mammalian nerve at a zone of low-conduction safety. By using only the refractory period, a theoretical accounting for brief blocks in trains has been made.[13] Theoretical pursuit of the consequences of activity-dependent changes is not well advanced.

In the frog axon, zones of low-conduction safety produced experimentally by partial crushing or by local cooling can show intermittent conduction. It is extremely difficult, however, to hold constant the degree of reduced conduction safety. Therefore tests of this sort are impractical, and we have turned to a branched peripheral nerve axon[14] to examine the relation between frequency of firing and intermittent conduction.

4. Significance

The problem of intermittent conduction is a curious one without widely accepted general importance. We have, however, been intrigued by the implications of the following notions. First, every axon tapers as it forms its terminal arborizations. The

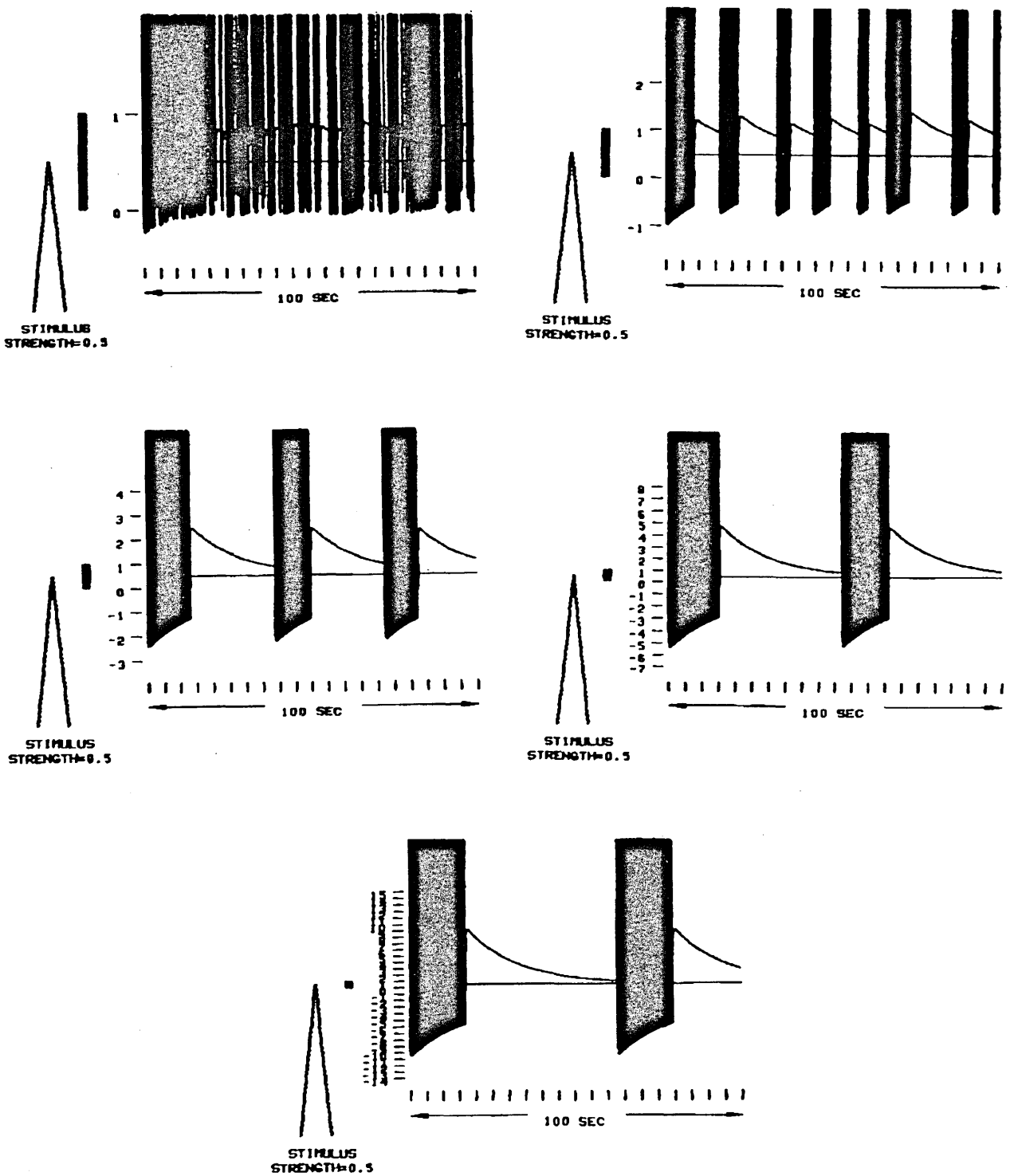

Fig. XVI-9. Effect of variation of magnitude of threshold shifts at 10/sec activity rate. If some fibers show probabilistic firing in only a narrow range and others in a wide range with respect to the amplitude of their threshold shifts, then the expected effect on periodicity of conduction is as shown in Fig. XVI-8. The relative size of the gray region is shown on each y axis. As the threshold curves dominate the gray region, the periodicity of intermittence gets longer.

fine terminals cannot fire repetitively as rapidly as the trunk axon does. Therefore, some of the impulses in the parent fiber do not reach all of the daughter terminals. This argument indicates that zones of low-conduction safety are common and widely distributed in the central nervous system. Second, activity-dependent threshold changes are one of the influences mediating which pathways of any particular tree are invaded. Observation of how such threshold changes transform a continuous train into widely spaced bursts indicates that even small long-term aftereffects of impulses can mediate conduction. We have been studying the ways that aftereffects help determine the invadability of an axon tree subjected to patterns of impulses carrying information.[12] It is possible that activity-dependent changes in functional connectivity of the CNS represent one of its basic strategies of operation. We believe intermittence is a case in point.

There are many other possible examples. Adrian[15] stressed the notion that supernormality overcomes the effects of a region of decrement so that "a single impulse will have no effect on the tissue beyond the region of decrement, but a series of impulses will succeed in passing through." Decremental conduction[16] will be affected by threshold changes. It is easy to imagine that decremental conduction could be offset by a barrage of impulses, all adding to superexcitability. Decremental conduction, however, is usually found in cathodally depressed fibers. Superexcitability seems to be caused in part by a depolarization. One might thus expect a barrage of impulses to exacerbate cathodal block and to delay resumption of successful conduction. Similarly, the aftereffects of impulse travel in axons can be used to invent explanations for posttetanic potentiation, presynaptic inhibition[17] and the origins of slow potentials[4] without invoking changes in the synapses themselves. Experimental discrimination between these alternative points of view on such phenomena has not yet been made. What we intend here is simply to exhibit a vivid instance of one situation that can be understood clearly in terms of threshold curves – intermittent conduction. Given the difficulties associated with measuring threshold near a zone of low-conduction safety, we cannot prove that our explanation is true. We merely wish to state it clearly.

We are indebted to the Speech Communication Group of the Research Laboratory of Electronics for use of their computer facility.

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D. SOME EFFECTS OF TEMPERATURE CHANGES ON THE THRESHOLD OF SCIATIC-NERVE FIBERS

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

Michael J. Binder, Stephen A. Raymond

There have been many experimental attempts to determine the way in which temperature affects the threshold of nerve fibers. Investigators have found that there are temperature effects, but they disagree on the magnitude and even on the direction of the threshold changes. Given the effects of temperature on rate constants of the Hodgkin-Huxley formulation, we expect temperature to have a threshold lowering or raising

effect, depending on which variables are affected most.[1] Furthermore, widely different effects of temperature on threshold can be obtained, depending on the duration of the stimulus used to measure it. A wide variety of types of preparation have been used.[2] Voltage-clamped squid axon[3-5] and sucrose-gapped myelinated nerve[6-8] have been the most prevalent preparations, and neither is noted for stability or "naturalness." There is so much disagreement among authors that we are not prepared to give a sense of the literature regarding temperature effects on threshold.[1]

Our interest in threshold stems from previous concern with the problem of how nervous activity alters conduction through axons. Threshold proved to be rather sensitive to impulse activity, and we are trying to form a more complete description. We shall show some observations on the effects of temperature on rested nerve. Our chief purpose, however, has been to discover how temperature changes affect the oscillatory threshold shifts that follow impulse activity. It appears that the activity required to test for threshold can be great enough to alter it significantly, especially if the nerve is cold. Some of the long-standing inconsistencies among the findings of investigators appear to be resolved if the activity-dependent shifts in threshold are subtracted from the temperature-dependent shifts. Previous studies have not been concerned with temperature-induced changes in activity-dependent threshold shifts.

1. Experimental Procedure

We measured threshold in terms of stimulus duration changes required to hold the probability of response at 50%.[9] The section of the nerve that is stimulated is bathed by circulated Ringer's solution flowing through a hole 3-cm long drilled in a silver block. The fine branches of the nerve lie in a plastic dish with its own separately circulated Ringer's solution where they can be recorded by using suction electrodes. The block is cooled and heated by a Peltier-type junction heat pump. One side of the Peltier device is on the block; the other is pressed against an aluminum block containing channels of running tap water. The temperature of the nerve is controlled by using a thermistor bead mounted so that it is bathed by the Ringer's solution a few millimeters upstream of the stimulating electrode. There was continuous monitoring of pH.[10]

2. Results

a. Rested Nerve

A "rested" nerve is defined as a nerve with a stable threshold that is unaffected by testing. In practice, sciatic nerves gave stable "resting" threshold readings at test rates of 1 stimulus every 3 seconds. Since stimuli were 50% successful, this works out to an average acivity of 1 response every 6 seconds. For some cooled nerves the test rates had to be even slower to avoid measurable depression.

When the temperature is changed slowly enough in the range 12-24°C, the resting threshold remains essentially the same throughout. This is a striking observation. Even though variables such as sodium and potassium activation and inactivation, axoplasm and membrane resistance, metabolism, and Nernst potential are all changed by temperature,[11-13, 4] the threshold is not. In some way threshold, which is a function of all complex processes that interact to determine what strength stimulus will be needed to generate an impulse, seems to be temperature compensated. Heating or cooling produces changes in the amplitude and duration of the spike, and changes in conduction velocity,[14, 7, 11] but the threshold for square current pulses ~150 μs long is remarkably constant. This observation appears not to be artefactual, since rapid changes in temperature produce transients in threshold curves that die back to this same level.

In Fig. XVI-10 the temperature is changed rapidly (maximum rate, 4°C/min). First, the rested nerve is cooled 5°C from 18°C, then warmed back to 18°C. At each step in temperature, there is a transient shift in threshold. At the onset of cooling the nerve became more excitable for a few minutes. The threshold shift on heating made it temporarily harder to fire the nerve. In both cases the threshold returned to the original level. This took approximately 1 1/2 times as long when the nerve was cold as it did at 18°C, which suggested that a metabolic process was involved. The system at rest behaves exactly as though threshold were homeostatically and actively controlled for variation in temperature. In an active nerve, however, large steady-state effects of temperature on threshold emerge.

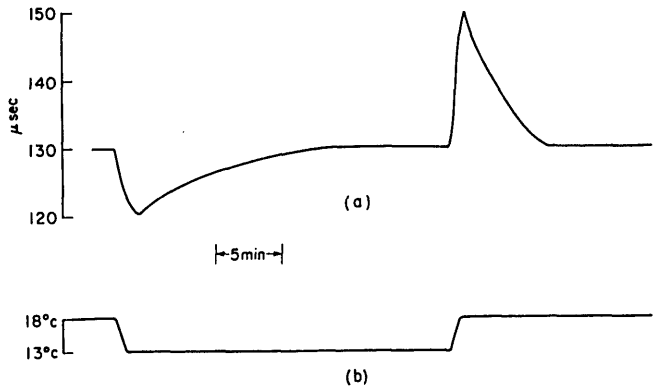

Fig. XVI-10. Rapid temperature change in rested nerve. (a) Threshold of resting axon vs time. (b) Temperature of same axon vs time.

b. Active Nerve

After each impulse in an axon that has been active for a few minutes, the threshold undergoes a sequence of swings. First, the axon is refractory, then superexcitable, then depressed, and finally recovered.[9] The timing and magnitude of each of these phases is affected by temperature. This has been demonstrated by using the following strategy. We subjected the axon to tetanic bursts of 10 impulses 30 ms apart. Every five seconds the burst was given, followed by a test stimulus at a particular delay after the burst. As the temperature changed, the threshold measured at the particular delay would also change. After a number of experiments at a variety of delays, we could form a picture of the temperature effect on the entire threshold curve of active nerve.

c. Refractory Period

The relative refractory period is prolonged by cooling. This is true regardless of the level of depression or the extent of the conditioning activity. For a typical A fiber at 18°C, the threshold crosses the resting level ~4-5 ms after a spike; at 13°C it may require 7.5-10 ms.

d. Superexcitable Period

If a nerve is not depressed, the superexcitable phase is larger after a burst than after a solitary impulse. Peak excitability occurs ~15 ms after the last spike of a burst, and it takes approximately 1 second before threshold returns to resting level. In such a rested nerve, the superexcitable phase is very slightly diminished by cooling to 13°C. In nerves depressed by activity the superexcitable phase becomes briefer and smaller as temperature falls. This does not necessarily imply a strong effect of temperature on the processes underlying superexcitability because depression is strongly affected by temperature, and even within the superexcitable period there is antagonism between depression and superexcitability. Thus the shortening and shrinking of superexcitability may be due chiefly to the increase of depression with cold. Figure XVI-11 shows plots of threshold during superexcitability in an active nerve at two different temperatures. If the transition from one temperature to the other is rapid, transient threshold changes occur similar to those shown in Fig. XVI-10. These die away to the new steady-state values plotted in Fig. XVI-11.

e. Depression

We have not yet gathered sufficient data showing how temperature affects the rates of accumulation and of decay of depression. Our evidence indicates that cooling increases the rate of accumulation of depression and slows the rate at which it decays when activity ceases. We are confident that the magnitude of depression is augmented

by cooling and reduced by warming. That is, at our activity rates (2 impulses/second) the same fiber at the same activity rate showed a much larger equilibrium value of depression when cooled. Figure XVI-12 shows an example of this change in equilibrium levels. It also shows the transient threshold shifts accompanying the rather rapid temperature changes.

Some cold nerves (13°C) were depressed by activity rates as low as 1 pulse per 6 seconds.

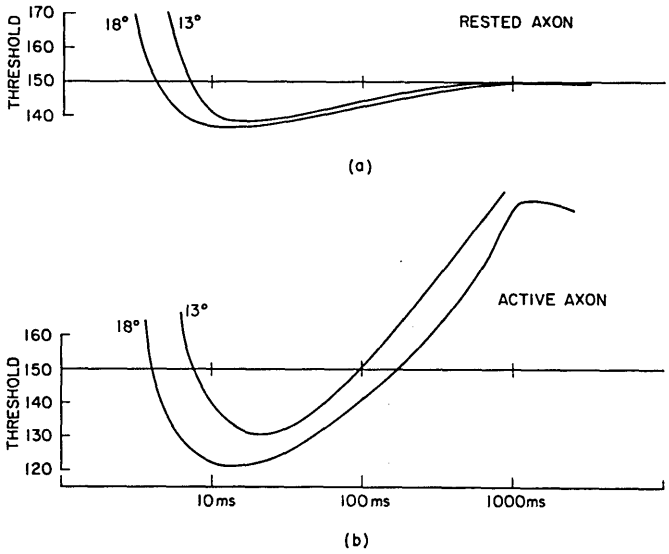

Fig. XVI-11. (a) Rested axon. Delay (in ms) after conditioning impulse vs threshold.
(b) Active axon. Delay (in ms) after conditioning volley vs threshold.

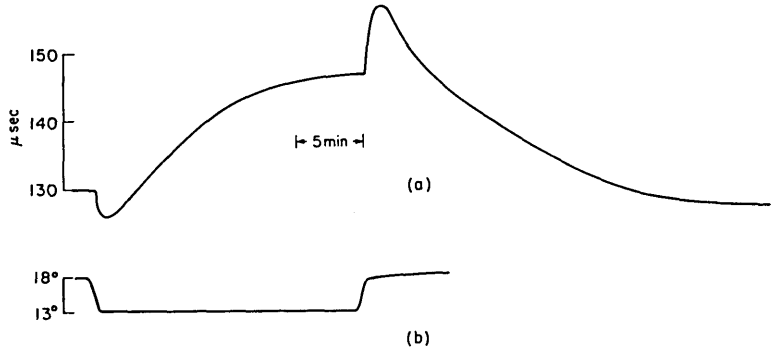

Fig. XVI-12. Rapid temperature change in depressed nerve.
(a) Threshold 1.5 s after intermittent tetanus vs time.
(b) Temperature of same nerve vs time.

3. Discussion

Figures XVI-10 through XVI-12 are from experiments in which it was possible to begin at one level of threshold, change to another as a consequence of cooling or warming, and then return to exactly the first level. Since some of the experiments required approximately 6 hours, not all of our data met this test. In our experience with a variety of nerves and a variety of depression levels, we have found that the threshold curve retains its normal character over the range 13-24°C, although the level of threshold, particularly during depression, changes quite a bit. In other words, the whole curve moves. It is displaced toward depression by cooling and away from depression by warming. There is more variation with temperature in the depression phase than in the others.

Working with summer frog, Gasser and Erlanger[15] found that the superexcitable phase disappeared completely at ~10°C. Most of our results were obtained on winter frog and indicate that superexcitability persisted at 10°C. The discrepency can probably be laid to the differences in test rate and consequent depression between our nerves. In some nerves, well depressed by activity, the threshold during the superexcitable phase did not drop very far below resting threshold even at 10°C. Otherwise, we agree with their results on refractory and supernormal phases. They did not study depression.

Our current experiments show that depression cannot accumulate or persist in a nerve fiber poisoned with Ouabain. This suggests that an electrogenic ion pump lies behind the depression. If the principal pumped ion is sodium, then the larger spikes associated with cold could account for the increased depression as follows: (i) more Na^+ remains inside at the same activity rates; (ii) normally, the pump activity is controlled principally by the amount of intracellular Na^+; (iii) more Na^+ means increased net positive ion extrusion resulting in a hyperpolarization type of depression. This would account for the effects of cooling and warming on depression level and possibly also on the superexcitability phase.

It is still difficult to account for the transients. It appears that the effect of temperature might be on the pump itself rather than on the impulse. Cooling would slow the pump, and result in less resting hyperpolarization and an increased excitability. These features could not persist, since more Na^+ would be in the axon, and hence there would be a tendency to speed up the pump.

4. Conclusion

In active nerve, cooling prolongs the refractory phase, diminishes and shortens the superexcitable phase, and increases and prolongs the depressed phase. Warming has opposite effects. In rested nerve temperature changes do not affect resting threshold except transiently, when such changes are faster than a few degrees C per minute.

Temperature effects on refractory and superexcitable phases are somewhat smaller than in active nerve.

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E. EVOLUTIONARY CONSTRAINTS ON THE DIMENSIONALITY OF COLOR SPACE

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

Lynette L. Linden, Jerome Y. Lettvin

Our perception of color can be described in terms of three primary action spectra or in terms of the three variables, brightness, saturation, and hue. The geometrical statement is that color space is three-dimensional.

Many animals have a more limited capability for color vision, but no animals studied thus far have shown ability to discriminate more than is given by three independent color variables. We are fooled by the same coloration that protects prey from their predators, and therefore it is reasonable to assume that we treat colors in much the same way as birds, monkeys, bees, etc. who have three-dimensional color systems do.

Newton[1] showed that color space is three-dimensional. He described the visible spectrum along a weightless wire bent into an open plane (convex) curve. Weights proportional to the flux at each point of the spectrum for a given color are placed on the wire, and the color then changes as the center of gravity of the weights changes. This became the basis for the color triangle in colorimetry, with intensity or brightness being the third dimension. Grassman[2] formalized Newton's description of color space, showing that addition and subtraction of colors is equivalent to addition and subtraction of vectors in three-space. He described a system of two holes in a gray background with lights behind it. Any visible color of light in the hole on one side can be matched by varying the amounts of three lights on the other side, or by matching two lights on one side to two lights on the other. This became the basis for trichromatic theories of color vision.

In 1802, Thomas Young[3] established the first known psychophysical trichromatic theory of color vision. He theorized that there are three types of color receptors in the eye. His theory made no impact until 1852 when Helmholtz[4] enthusiastically endorsed his scheme. Helmholtz established a wealth of evidence through psychophysical experiments and provided tremendous insight into color theory. He also showed that Hering's opponent color theory was mathematically equivalent to Young's theory.

Hering[5] proposed on subjective grounds that all colors are composed by varying the ratios of blue to yellow, red to green, and black to white. He calls these color pairs opponent or antagonistic. Between each pair there must exist a perceptually neutral point. The three pairs, blue-yellow, red-green, and black-white, are the three degrees of freedom in Hering's perception space. A point in this space represents the perception of a particular color. The origin of Hering's color space is the perceptually neutral or achromatic point.

Hering's color space has its origin within a color solid that can be conceived in terms of a semipolar coordinate system with the origin within the solid (see Fig. XVI-13). Brightness varies along the achromatic axis which passes through the origin. Orthogonal to this axis and going through the origin is the hue plane bounded by the hues. With one hue taken as a reference, all other hues are expressed in terms of angle with respect to this hue. In this plane (which at one time was called the isolychnic plane) the ratio of the distance of a color along a hue radius to the maximum distance is called saturation. For colors outside (above and below) this plane, the saturation is achieved by projecting the color locus onto the plane along a line parallel to the achromatic axis. Thus pure spectral colors outside this plane (which is set by adaptation) are desaturated.

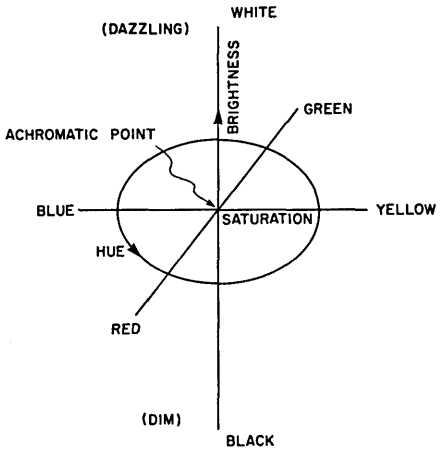

Fig. XVI-13. Hering's color space.

Many theorists in attempting to explain psychophysical results have proposed more than three perceptual variables. But, as Peddie remarks:

> "In the fact that, by means of no more than three fundamental colours suitably chosen, it is possible to form a match to any spectrum colour, we have evidence, sure and unescapable of a fundamental triplicity in the nature of colour perception. We may ignore that tripleness, substituting for it a more complicated character and that quite successfully for particular purposes perhaps; but in so doing we cannot fail to miss, in our inquiry into the mechanism and processes of vision, the most basic aspect of the whole matter . . . . But this fundamental tripleness corresponds to something real. The reality can only be one of structure or function and difference of function involves difference of structure somewhere."[6]

We shall propose a geometrical argument for establishing the upper limit of three on the dimensionality of color space. Our arguments will be based on evolutionary considerations of the purpose of vision, anatomical and physiological considerations of the visual system, and the character of the visual world as stimulus to the visual system.

An animal moves about in a three-dimensional world defined by the relations among the objects in it from the animal's point of view. This world has voids and solid objects, and the objects are arranged accidentally (in the sense of nonessential) and are of irregular shapes relative to one another. An animal must represent this three-dimensional world by its projection onto a two-dimensional image cast on a rigid, sensitive surface such as the retina. Other views of the world such as touch and smell have different projections into the world image of the animal.

Our first hypothesis is that the purpose of vision is to identify and track things in the visible world. Because objects rather than light itself seem to be most consequential to an animal, the strategy expected of an eye is to extract invariances about objects themselves rather than invariances in the accidents of the light they reflect.

Our second hypothesis is that an image on a rigid sensitive surface is necessary for vision. The method of formation of the image can vary in different eye structures. For simplification, we shall deal specifically with the anatomy of the human eye, but the ideas presented here apply to corresponding anatomies in other animals.

The third hypothesis is that there is no source of visual information other than light and its distribution as stimulus to the eye.

Finally, we shall deal with vision only at photopic levels, so that only cones are operational.

Stiles[7] was the first to abstract from psychophysical experiments the actual pigment processes of the human eye, insofar as their operative action spectra were concerned. Later microspectrophotometric studies by Marks[8] and others showed that goldfish and primates have three types of cones and, to a good approximation, the pigments of these three types of cones absorb light maximally as 445, 535, and 570 nm. The three photopigments in the cones behave as three broadband, overlapping, well-behaved, unimodal filters spanning the finite spectrum of visible wavelengths of light.

A color-blind person has one or more of the cone systems missing from the retina. Thus, instead of three responses associated with each stimulus, there are only two responses or one response. Since each photopigment response is independent of the others, we shall refer to a retina containing N different photopigments as an N-dimensional cone system.

A stimulus light presented to the eye will elicit a response from each of these filters. The response for each filter is given by multiplying the energy of the stimulus with the energy of the photopigment at a given wavelength and summing for all wavelengths. A given stimulus will have associated with it three responses (in terms of quantum catches), one from each of the three types of filters. These equations show such response computations:

$$B = \int_{\lambda=400}^{800} B(\lambda)\, L_R(\lambda)\, d\lambda$$

$$G = \int_{\lambda=400}^{800} G(\lambda)\, L_R(\lambda)\, d\lambda$$

$$R = \int_{\lambda=400}^{800} R(\lambda)\, L_R(\lambda)\, d\lambda$$

where $B(\lambda)$, $G(\lambda)$, and $R(\lambda)$ are functions of the photopigment filters depicted in Fig. XVI-14, and $L_R(\lambda)$ is a function of the reflectance spectrum of the stimulus under illumination, as in Fig. XVI-16.

Figure XVI-15 shows the normalized spectrum of daylight under varying conditions. It is interesting to note the flatness and smoothness of these curves. They are normalized

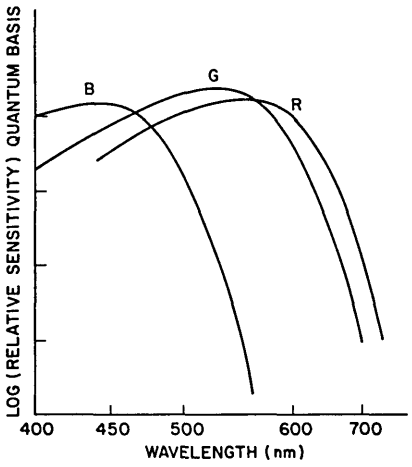

Fig. XVI-14.
Cone pigment curves.[9]

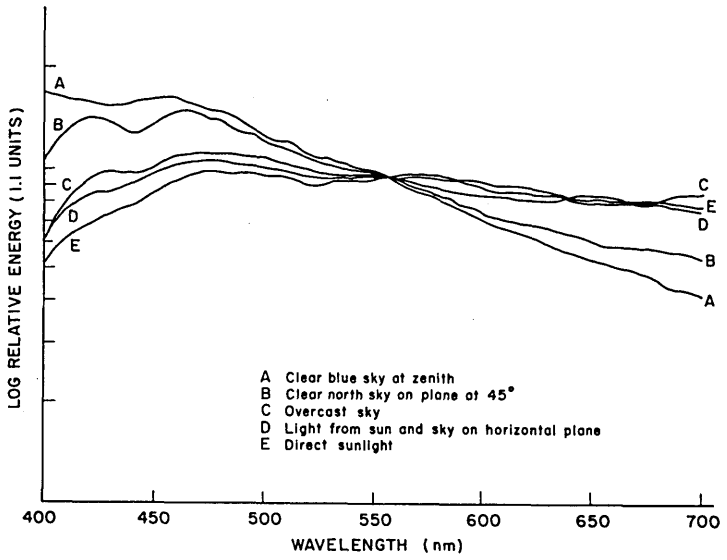

Fig. XVI-15.
Spectral distribution of daylight. Data taken from LeGrand[10] and Henderson[11] replotted on log energy scale.

so as to cross at approximately 555 nm, arbitrarily chosen to correspond to the most sensitive region in human vision. The greatest variation in distribution occurs in the blue region of the spectrum. (Westheimer and Campbell[12] following Maxwell[13] have found a severe falloff in the transmittance of the ocular media in the blue region of the spectrum, because of the yellow pigmentation of the lens and the macula lutea.)

Figure XVI-16a shows the reflectance of grass under daylight. Sommerfeld, in a

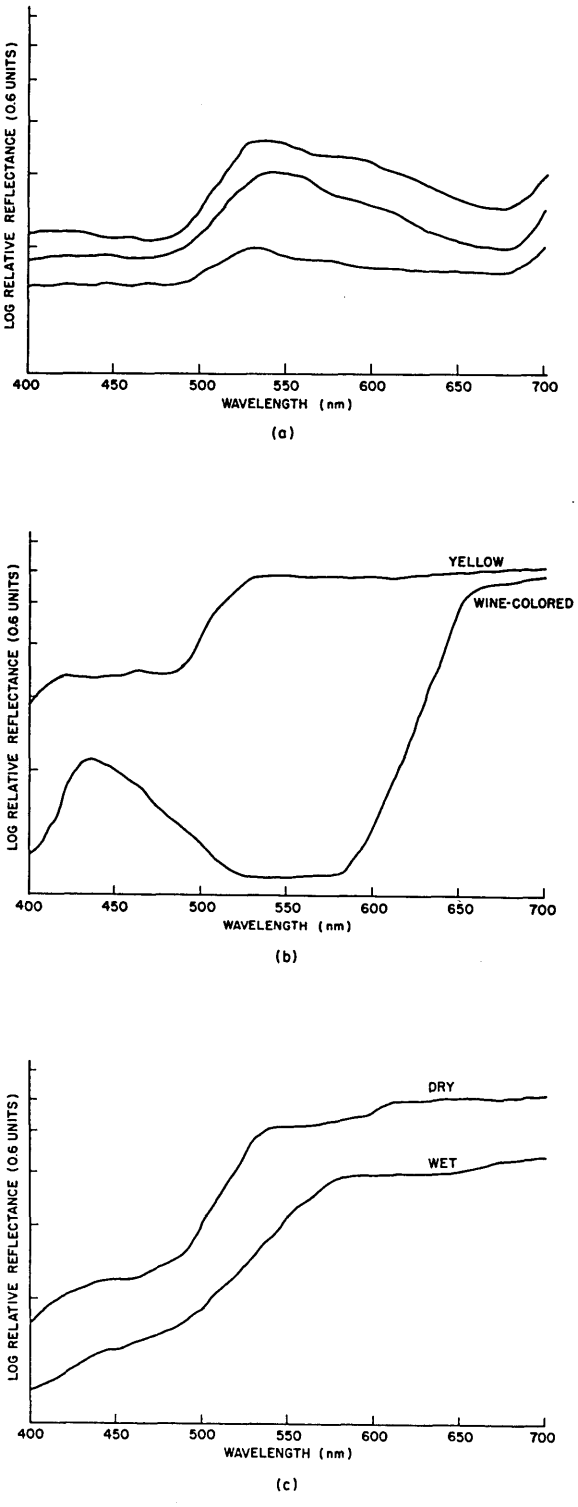

Fig. XVI-16. Reflectance curves: (a) grass and leaves (three materials); (b) desaturated yellow gladiolus and wine-colored gladiolus; (c) wet and dry soil. Data taken from Evans[15] replotted on log energy scale.

discussion of the properties of surfaces,[14] notes that except for metals and for interference colors found on some insects and birds, the reflectances of surfaces are unselective. In fact, in order for foliage to appear light green its surface must have sufficient inhomogeneities between the grains of chlorophyll to reflect other spectra. The same principle is true of textiles, which would appear dark and colorless if the inner fibers were not able to reflect a wide range of spectra from various depths.

Consider an image of the physical world. This two-dimensional projection into the retina can be described as a graph. The retina is a two-dimensional array of photoreceptors containing the three types of photopigments which are bleached by stimulus light. Responses of the photopigments are in terms of quantum catches of stimulus light.

The graph or image contains areas, boundaries, and vertices. An area is a homogeneous region such that a photoreceptor will receive an identical input at any point within that region. A boundary is defined, then, as a change in input to a receptor. This changing input can be provided spatially, by considering identical receptors between two adjacent areas of the image, or temporally, by moving a receptor between adjacent areas. A vertex is simply the point of intersection of boundaries. The degree of a vertex is given by the number of boundaries intersecting at the vertex. We shall consider first- and second-degree vertices to be degenerate cases (i.e., a second-degree vertex is just a point along a boundary).

This image of physical space with its areas, spatial boundaries, and vertices has many notable characteristics. Spatial boundaries can be of two varieties in terms of the objects themselves. An object boundary occurs between the edges of objects or between objects and voids. A surface boundary defines the microstructure of the object such as texture, or the macrostructure such as shadows. These microstructure patterns are discussed by Thompson[16] and Stevens.[17]

The two types of boundaries imply two types of vertices — object vertices, made up at least in part of object boundaries, and surface vertices which are the intersections of surface boundaries.

Most object boundaries are a consequence of collapsing the third (depth) dimension of space in the image. Our remarkable perception of depth must rely on interpretation of the image by binocular disparity, motion parallax, figure-ground distinctions, and so forth. A monocular depth cue of unique importance is termed interposition by Helmholtz.[18] It is defined as a cue for the perception of relative distances of two objects that results when one object partially obscures or overlaps the boundary of another object. This is the phenomenon referred to as "T junctions" by researchers in pattern recognition.

Most object vertices and surface vertices are of degree three. Consider first a random array of separated and smoothly bounded opaque objects in three-dimensional

physical space and imagine various points of view. No interpositional vertex of degree higher than three is stable under small perturbations of any point of view — i.e., a fourth-order and even higher order object vertex that one can contrive dissolves instantly under a small change of the point of view. Certainly these singularities are accidental and of no consequence in passing through the world. Shadows in the natural world arise only as a consequence of direct sunlight. The object interposed between sun and the illumined surface, as well as the light from other reflecting surfaces in the vicinity, determines a more or less homogeneous light in the shadow. But a shadow can only cross a boundary; rarely, transiently, and accidentally will it cross at a vertex. The fourth-order vertex of a shadow crossing a boundary reduces to two kinds of light illuminating two kinds of surfaces, rather than being a fourth-degree vertex surrounded by four independent areas.

Therefore, we shall consider all vertices in an image to be of degree three.

Because the eyes are in constant motion, photoreceptors are continuously moving over an image.

The involuntary tremor of the human eye has a frequency between 50-100 oscillations per second with amplitudes in the range of a few seconds up to one minute of visual angle. This is approximately a few cones width in the fovea.

Involuntary microsaccades occur every 1.5-2.0 seconds with amplitudes in the range of a few minutes to a few degrees of visual angle. In addition to the tremor and microsaccades there are also large voluntary eye, head, and body movements which have a large range of amplitudes and occur at a variety of time intervals.

Except in an experimental situation, then, the photoreceptors are continuously being stimulated by different parts of the visual scene (image). Ditchburn,[19] Riggs,[20] Yarbus,[21] and others have shown that stabilization of the retinal image causes vision to fade quickly (within a few seconds) into a colorless, featureless, changeless neutral field which was termed by Hering "intrinsic gray," or eigengrau.[22] Eigengrau is also experienced by an observer after a few seconds when his entire visual field is illuminated by a homogeneous light (ganzfeld). The same indifferent sensation is reported by a person after becoming blind.

Because eigengrau describes the subjective effect of a stabilized image, as well as the effect of a ganzfeld on the retina, the effect is local as well as global (as in Yarbus' experiments). This indicates that the photoreceptors must receive a changing input in order to produce a sensation other than eigengrau. In other words, visual sensations require the presence of temporal or spatial boundaries.[23] The tremor and microsaccades of the eye insure that the retinal array of photoreceptors is jittering so that even when gaze is fixated the cones near the projected boundaries of the image are receiving changing input. Troxler,[24] Helmholtz, Hering, and others report a degradation in

perception (but not completely sensationless) under concentrated, voluntary fixation.

The results of these experiments led researchers to propose the "filling-in hypothesis" based on Walls' theory[25] in order to explain our perception of homogeneous areas of color inside boundaries.

If a subject fixates on a point in the center of a red spot on a gray wall the spot does not disappear. If the same image is stabilized on the retina, the spot disappears within a few seconds. The only difference in the two kinds of fixation is due to the jitter of the eye at the boundary in the voluntary fixation.

What about the color judgment itself? In a color-matching experiment, the subject observes a gray background field from which a circle a few degrees in diameter has been cut out. Behind the circular hole are two white screens dividing the hole down its center. A stimulus light plays on one screen. The observer controls three independent colored light stimuli on the other screen, or he may control one of the lights on the stimulus screen and the other two lights on the other screen. The observer is told to match the lights on the two screens without regard to the "absolute" color of the lights.[26] His eyes are allowed to wander over the background and the stimulus lights. The two fields of lights, then, intersect down the center of the circle. As the observer approaches a match, the boundary down the middle of the two screens disappears so that there is a uniform circular light field surrounded by the background, and the exact color of the circular field begins to vary and become indefinite.[26] The color of the field changes, as does that of the background. The only change that has taken place in the setup is that the circular field becomes a uniform color instead of being divided into two different colors separated by a boundary. In terms of the image, this means that a field with two third-degree vertices is reduced to a field with a single boundary.

There is a principle of diversity[27] in color vision that has been regarded since the time of Helmholtz. The effects mentioned in the previous experiment do not occur in situations where the colors are more numerous. The more diverse the colors in a scene in terms of brightness, saturation, and hue, the more definite the colors seem to be, given that the areas are relatively small in visual angle. (This diversity is necessary in order to produce the gamut of colors in Land's pictures.)

There is one more experiment of particular note that exemplifies the phenomenon of simultaneous contrast. In a room with diffuse white light, if a filtered light is played upon an object to produce a shadow against a white background, the shadow will appear as the complementary color of the filtered light. For example, if the filtered light is blue, the shadow will be yellow. This has been utilized by Land who has taken simultaneous contrast to a beautiful extreme.

In general, areas bounding one another tend to accentuate differences in one another in brightness, saturation, and hue. A bright area and a dark area, when juxtaposed,

will appear even brighter and darker respectively, and so forth. This suggests that the position of an area of color in relation to other colors will cause changes in its appearance (as the Renaissance painters discovered).

If a double colored shadow is made where the shadow is a strip with nonparallel boundaries, and there is red light on one boundary and green light on the other boundary, the shadow appears yellow. This is because the red light induces a green shadow, the green light induces a red shadow, and these two induced colors add to produce yellow. If an observer pays attention to the green boundary, the shadow appears more orange; if he pays attention to the red boundary, the shadow appears greenish-yellow. This experiment suggests that both bounding areas contribute to the color of the shadow.

The perception of color is dependent on the adaptation level of the observer. There is a neutral point of view from which to judge whether a stimulus is brighter or dimmer, more blue or more yellow, more red or more green, etc. This level is dependent on the illumination of the retina. As stimulus conditions change, this adaptation level changes. This is what Hering proposed.[5]

The phenomenon of simultaneous contrast is closely tied to this adaptation level. Since the adaptation level is an average level for some condition in the environment, making judgments relative to an average value rather than some absolute scale can change the perceived qualities of the differences in stimuli.

Perhaps it would be useful to offer a paradigm of the adaptation level as eigengrau. By adaptation we do not mean the bleached pigment level alone but rather what Rushton[28] and Baker[29] measured as having approximately a two-second time constant and Yarbus[30] as having a one-to-three-second time constant. It is the adaptation to incremental threshold found after change of illumination and is a combination of pigmental adaptation and another faster kind.

Suppose a particular sharply demarcated zone of the visual field is brought to eigengrau by stabilizing, and suppose the light to which it is stabilized is a fairly saturated red. The light in the stabilized sector is then changed to less pure red light. It will not appear pink, however, but greenish. If it is changed to a more pure red light, it turns red. Thus from eigengrau, the hue that we see is given by the direction of change of light.

Consider a voluntarily fixated image on the retina. For the duration of the fixation each photoreceptor will jitter over a small region. These local regions can be characterized as homogeneous regions, boundaries, or third-degree vertices and will be treated independently of other local regions. We shall assume that each homogeneous region has N numbers associated with it, corresponding to the responses of the N photopigments (N cone systems) to that reflectance.

From the results of psychophysical experiments we know that boundaries are necessary for sensations, and we can claim that the type of information measures available

are not absolute but relative. Thus we are dealing with a system that responds to differential stimuli and normalizes out common values such as illumination.

We can consider the available information in terms of these local areas and the types of measurements that can be made by an N-dimensional cone system. If we consider each local retinal region to be a conservative system, each reflectance will have N degrees of freedom given in an N-dimensional cone system.

The relative nature of our perception is such that average intensity or brightness is normalized; hence, this acts as a constraint on the system. Constraints on a conservative system reduce the number of degrees of freedom of that system.

A local homogeneous region is normalized to its average value. Since there can be no comparisons when there is only a single color, we may say that the response of a homogeneous region is normalized to its local eigengrau.

A boundary has two regions, each with N degrees of freedom. Normalization to the average level of intensity imposes one constraint, giving a total of 2N-1 degrees of freedom. We see that this is the number of relative differences between the two colors.

At a vertex there are three areas, each with N degrees of freedom. Normalization to the local average intensity level leaves 3N-1 degrees of freedom to specify relatively the three local colors.

A cone that is presented either spatially or temporally with varying stimuli at a boundary will measure a difference between the bounding areas and normalize to the average response value. There is one independent measure for each cone system for a bourndary; therefore, N independent measures.

In a local homogeneous region there are no relative differences between regional stimuli. The response of the receptor will be equal to the adaptation level and will normalize to zero sensation.

For a vertex, the average adaptation level is an average response of three stimulus values for a given cone system. There are two independent measures of the differences among the three response values for each of the N cone systems, or 2N responses. Each of these measures is normalized to the local average level.

In addition to these difference measures, for $N > 1$ there is an additional measure of the intensity (or brightness) that is normalized to the average intensity value. For a local homogeneous region this normalizes to zero (eigengrau), since there is no difference. For a boundary there is one brightness difference measure, and for a vertex there are two independent brightness measures.

Thus for a local homogeneous region there are no difference measures, for a boundary there are N+1 difference measures, and for a local vertex there are 2N+2 difference measures.

If we compare the number of degrees of freedom for each of the three types of local regions with the number of difference measures at the local regions, we see that a

homogeneous region always tends to normalize to eigengrau, a boundary is uniquely specified by a one- or two-dimensional cone system (since intensity difference is the same as the cone response differences in the one-dimensional case), and a vertex is uniquely determined by a three-dimensional cone system and overspecified by a cone system of two dimensions and uniquely determined by a one-dimensional cone system.

The model predicts that boundaries are underspecified (therefore judgmentally unstable) by three-dimensional systems. Psychophysical evidence supports this notion of instability or drift in an image where only boundaries and no vertices are present. This is the situation as the observer approaches a match in the previously described color-matching experiment.

Another example of instability of sensation when only boundaries are present is the effect of "vibrating" boundaries in op art paintings of vertical stripes, annuli, etc. There are no vertices in these paintings. A painting with no vertices represents a two-dimensional world since there are no depth cues, no objects partially obscured by other objects. Albers describes this effect:

> "As a deception, this effect is related to our earlier experience in which two colors appeared as three or four colors.
>
> However, the additional illusionary colors often are hard to define as to their hue.
>
> They often appear as a shadow on one side of the boundary and as light reflected on the other side.
>
> Or sometimes this vibration presents just a duplication or triplication of the boundary line ...
>
> This initially exciting effect also feels aggressive and often even uncomfortable to our eyes. One finds it rarely used except for a screaming effect in advertising, and as a result it is unpleasant disliked, and avoided."[31]

On the basis of this dimensionality argument, which receives support from psychophysical observations, we claim that apperceptive color judgments are made at vertices, since for trichromats decisions made at boundaries are subject to instability or drift. This claim is consistent with the desire to allow the greatest perceptual diversity between different regions. This also allows color judgments to be made quickly at various points in the image, since no global information about the image is required. If judgments were made at boundaries there would be a tendency to judge bounding areas as complementary to one another.

Not all paintings that appear to contain only boundaries vibrate. A painting with texture contains many other boundaries and vertices in order to define the texture itself.

Lettvin has discussed a "virtual vertex"[32] where the boundary itself is another color.

For example, if the boundary of one sheet of paper overlaps another sheet of paper, the boundary color generated by fine shadowing and diffuse reflection at the overlapping edge will be the subtraction of the colors of the two papers, thereby creating a third color. This situation behaves as a vertex in the foregoing analysis, since the local adaptation level is made up of the two areas of color and the boundary color. For this reason not all op art paintings vibrate. In order to produce color vibration great care must be taken not to introduce a third color at the boundary, creating a "virtual vertex."

Another important prediction of this model is that if two areas at a vertex are of equal brightness this reduces the number of independent difference measures to 2N+1. This would imply instability of judgment in the three-dimensional case.

Parsons quotes Helmholtz from the second edition of the Physiological Optics:

> "I scarcely trust my judgment upon equivalence of the heterochromatic brightnesses, at any rate upon greater and smaller in extreme cases. I admit, however, that one can gradually so darken one of two coloured fields that no doubt remains as to the other being now the brighter... ."
>
> "As far as my own senses are concerned I have the impression that in heterochromatic luminosity equations it is not a question of the comparison of one magnitude, but of the combination of two, brightness and colour-glow (Farbengluth) for which I do not know how to form any simple sum, and which too I cannot further define in scientific terms."[33]

A heterochromatic brightness match is very difficult to make. Evans notes that "such a match is 'confusing' or that brightness matches are 'obscured' by the strong presence of other variables."[34] Such problems forced colorimetrists to seek different observation methods of brightness matching, using the cascade and flicker method. These statements suggest an instability as the observer approaches a brightness match which is not present when there is a brightness difference among all three areas in a heterochromatic brightness match.

This "confusion" of brightness matching led Evans to define his fourth perceptual variable in order to explain these effects. In terms of our model, however, he has confused the idea of a perceptual variable with the disappearance of an important difference measurement, creating instability of sensation because not enough independent measures are available to specify color judgments uniquely.

But what is meant by specified, underspecified, and overspecified? What are these judgments to which we refer? They are what Hermann Weyl called the "subjective-absolute" rather than the "objective-relative":

> " Thus the objective state of affairs contains all that is necessary to account for the subjective appearances. There is no difference in our experiences to which there does not correspond a difference in the underlying objective

> situation (a difference, moreoever, which is invariant under arbitrary coordinate transformations). It comprises as a matter of course the body of the ego as a physical object. The immediate experience is subjective and absolute. However hazy it may be, it is given in its very haziness thus and not otherwise. The objective world, on the other hand, with which we reckon continually in our daily lives and which the natural sciences attempt to crystallize by methods representing the consistent development of those criteria by which we experience reality in our natural everyday attitude – this objective world is of necessity relative; it can be represented by definite things (numbers or other symbols) only after a system of coordinates has been arbitrarily carried into the world. It seems to me that this pair of opposites, subjective-absolute and objective-relative, contains one of the most fundamental epistemological insights which can be gleaned from science. Whoever desires the absolute must take the subjectivity and egocentricity into the bargain; whoever feels drawn toward the objective faces the problem of relativity."[35]

The fundamental principle which must underly perception was stated by Helmholtz: "a difference in the perceptions offering themselves to us is always founded on a difference in the real conditions."[36] If our judgments are underspecified, therefore unstable, then this principle is violated; the same stimulus conditions will have a locus of judgments so that observations when the eye is affected in the same way at different times may result in different judgments. What is specified or overspecified, therefore stable, will always result in the same judgment. Thus it follows from the hypotheses that stability of judgment is a necessary principle in perception.

In addition to stability of judgment, we also seek to distinguish objects from one another. The more characteristics we have available to distinguish, the greater our ability to separate objects. This suggests that ideally we would want as many dimensions as possible in color vision without violating the stability criterion.

If there were four or more dimensions in color space, a mutual comparison of differences in more than three areas would be required to produce stable perceptions. In this three-dimensional physical world, however, only three mutual comparisons between areas at given points in the image are guaranteed; thus, by analogy with the boundary in three-dimensional color space, sensations would fluctuate even at vertices.

Therefore, from the standpoint of stability and separability, a three-dimensional color space is optimal for the purposes set forth in our hypotheses.

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F. SYNAPTIC ORGANIZATION OF THE Sternarchus ELECTROMOTOR SYSTEM

Bell Telephone Laboratories, Inc. (Grant)

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We have been interested for some time in the neurogenic electrocytes of the gymnotid Sternarchus albifrons. In this species, the electric organ is composed of specialized axons that exhibit a unique degree of regional differentiation. The structure of the nodes of Ranvier changes markedly along the course of single fibers, the nonmyelinated segments varying in length in a regular manner from 1 μm in some regions to more than 50 μm in other regions. There are corresponding changes in physiological properties; spike electrogenesis does not occur at the enlarged nodes. It is hoped that this system may provide a model for the study of regional differentiation of nerve fibers and specificity in neuroglial interactions. Previous electron microscopic studies of this system were concerned with the structure of the peripheral axons.[1-3] The analysis of this neural system is now being extended to the spinal circuitry controlling the electric organ discharge. The high frequency of organ discharge (700 Hz to 1500 Hz) suggested that transmission might be electrotonic, as in most other electromotor systems. Spinal electromotor neurons were studied by light and electron microscopy. The cells are round to ellipsoid in outline and dendrites are not present. The initial segment of myelin often

extends partially over the cell body. Fine glial lamellae are interposed between closely adjacent cells, and somatosomatic gap junctions are not observed. The large majority of axosomatic synapses are characterized by gap junctions. Single axons were commonly found to establish gap junctions with two adjacent neurons. Such junctions provide a morphological correlate for electrotonic synaptic transmission.[4] Only a few synapses have the characteristics associated with chemically mediated transmission. The morphological data thus provide evidence of electrotonic coupling between electromotor neurons by way of presynaptic fibers. A further finding concerns the absence of dendrites in these cells. This finding, which stands in marked contradistinction to the elaboration of the dendritic component of most other vertebrate motoneurons, may provide a morphological correlate for the simple relay function of these cells.

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G. REGIONAL MORPHOLOGY OF CENTRAL MYELINATED AXONS

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As part of more extensive studies on myelin formation and its relation to reparative mechanisms in demyelinating diseases, we have been studying the regional morphology of myelinated fibers in the vertebrate central nervous system. Earlier studies showed that the geometry and specific morphology of the central nodes of Ranvier and of the myelinated internodal segments may differ from that in peripheral nerve, and in fact may differ from region to region in the central nervous system.[1, 2] The earlier studies on the dimensional analysis of central fibers, which were confined to inframammalian species, have led to a number of predictions about conduction properties of central

fibers.[3-5] This approach will be extended to the mammalian central nervous system. A secondary goal of the mammalian studies derives from the need for normative morphological data against which to compare the geometry of central fibers at presumptive sites of demyelination and remyelination. Studies carried out this year have concentrated on the relationship between fiber diameter and myelin thickness in cat oculomotor nucleus.

Electron microscopy of myelinated fibers in main lateral cell groups of cat oculomotor nuclei reveals that the majority have diameters of less than 3 μm, with more than one-half less than 1 μm in diameter. Myelinated fibers as small as 0.2 μm are present. Myelin sheaths of preterminal fibers extend to within several microns of the synaptic area. The values of the ratio g (axon diam/total fiber diam) range between 0.58 and 0.88 with a mean of 0.74 (Fig. XVI-17). The value of g was nearly constant and did

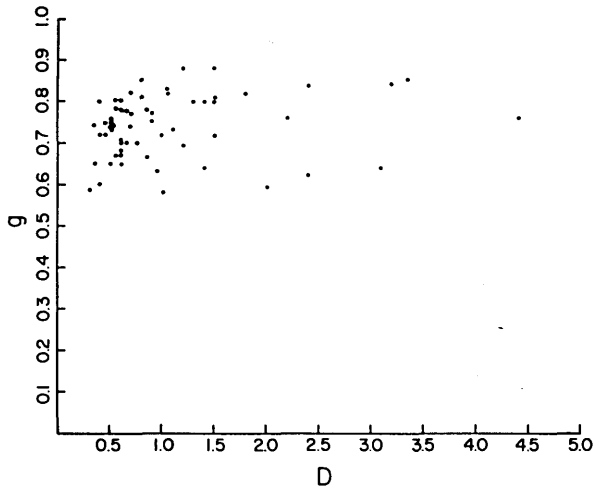

Fig. XVI-17. Values of g (axon diameter/overall fiber diameter) for myelinated axons in cat oculomotor nucleus. Values of g range between 0.58 and 0.88, close to the value at which conduction velocity should be maximal.

not vary significantly with fiber diameter. Dimensional arguments suggest a variation in conduction velocity of less than 20% for values of g in the range observed. For fibers that exhibit structural similarity, conduction velocity should be 5.5 m sec^{-1} μm^{-1} if specific membrane properties are constant.[4] Diameter spectra and observations on g are consistent with physiological data[6] on preterminal fibers in this nucleus.

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