XXIII. NEUROPHYSIOLOGY

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1. B-WAVE SENSITIVITY DURING LONG-TERM DARK ADAPTATION IN THE FROG'S EYE

National Institutes of Health (Grants 5 TO1 EY00090-03 and 3 RO1 EY01149-03S1)

Eric Newman, Jerome Y. Lettvin

A preliminary study on long-term, dark adaptation of the frog retina has been completed, using the threshold of the B-wave of the electroretinogram (ERG) as a measure of the sensitivity of the eye. In order to study the entire span of dark adaptation, care was taken to maintain the eye in a condition that was as "physiologically normal" as possible. To this end, a preparation was developed that allowed the recording of the B-wave of the ERG from the intact, normally circulated eye of a frog that was restrained by a few pins but allowed to breathe continuously in a normal fashion. It was not necessary to treat the frogs with either anesthetics or paralyzing agents to achieve satisfactory recordings.

The threshold of the B-wave of the ERG was determined in two series of experiments. In the first series, the light intensity (a 10-ms flash of white light) needed to produce a criterion B-wave response, which ranged from 15-80 μV, was determined during the entire course of dark adaptation. It was found that following an intense bleaching flash of light, the threshold of the B-wave fell steadily and did not reach a constant value for an average of 9 hr (range: 6-11 hr). This long, dark-adaptation period was seen in all preparations (7 frogs) which remained healthy for at least 12 hr.

It was observed that the test flashes used in producing the criterion B-wave responses were sufficiently dim so as not to affect the adaptation state of the eye. In order to rule out completely the possibility that the testing light flashes were in some way affecting retinal threshold so as to produce a seemingly lengthened adaptation period, a second series of experiments was conducted. In these experiments, the threshold of the B-wave was assessed indirectly by measuring the amplitude of the B-wave response (60-μV maximum) to identical test flashes presented at regular intervals, either 0.5 or 1 hr. It was found that following a bleaching adaptation light, the

amplitude of the B-wave did not reach a maximum value until 5 to 10 hours of dark adaptation had elapsed, depending on the intensity of the bleaching light. In one case, it was possible to maintain a preparation for 3 days. In this case, the B-wave, having reached a stable value after 10 hr in the dark, maintained that amplitude for the duration of the experiment.

These experiments indicate that the sensitivity of the B-wave process of the ERG requires up to 9 hr of dark adaptation to attain a maximal value and thereafter maintains that value in the dark for at least 3 days.

2. TRANSRETINAL CURRENT AND THE ACTIVITY OF FROG RETINAL GANGLION CELLS

National Institutes of Health (Grants 5 TO1 EY00090-03 and 3 RO1 EY01149-03S1)
Bell Laboratories (Grant)

Eric Newman, Jerome Y. Lettvin

The existence of a nonsynaptic mechanism of information transfer within the retina has been suggested by the work of Dr. Mark Lurie,[1] who demonstrated a correlation between the activity of type-four ganglion cells of the frog retina and the C-wave of the electroretinogram (ERG). Lurie has proposed that current produced by the pigment epithelium, which generates the C-wave, might modulate the activity of the retina.

Experiments have been conducted on the intact, circulated eyes of curarized frogs in order to confirm Lurie's results and to test the hypothesis of retinal sensitivity to pigment epithelium currents. The activity of the pigment epithelium was monitored with intraretinal electrodes by measuring the voltage across the proximal retina (between the receptor region of the retina and the vitreous). Ganglion cell activity was monitored simultaneously with Wood's metal electrodes positioned in the ganglion cell layer of a nearby portion of the retina. In this manner the activity of a ganglion cell could be compared with the local transretinal current from the same region of the retina.

Simultaneous recordings of type-four ganglion cells and retinal voltages following flashes of light presented to dark-adapted eyes have shown that there is a good correspondence between the peak of the transretinal voltage and the onset of periods of ganglion cell activity. This has been seen for a range of flash intensities producing delays of 3 to 50 seconds. Recordings from some type-three ganglion cells have shown a similar correlation between the onset of a prolonged burst of ganglion cell activity and the peak of the transretinal voltage.

Unit recordings were made from the optic tectum of the frog in order to monitor the activity of type-two ganglion cells. It was found that this type of cell, although it does

not respond to a diffuse flash of light, will give a delayed burst of activity following a flash if a black spot of the proper size is placed within the receptive field of the cell. The duration of the delay preceding the burst is roughly proportional to the log of the flash intensity. Thus, although this is difficult to show directly, it is possible that type-two ganglion cells, as well as type-three and type-four, display a time course of activity coincident with the transretinal component of the C-wave of the ERG.

Externally generated current was passed across the dark-adapted retina while recording from type-four ganglion cells in order to test whether the transretinal current previously seen to be correlated with ganglion cell activity was sufficient to account for the modulation of activity. It was found that externally generated current (applied between the vitreous and the choroid) of the same magnitude as the currents seen during the C-wave (measured by the IR drop across the proximal retina) produced significant modulation of ganglion cell activity. The polarity of the effect, however, is roughly opposite in the two cases. When transretinal current is generated by the eye in response to light, ganglion cell activity begins at the peak of the transretinal current and continues as the current decreases. When current is applied externally, activity is greatest as the current increases and stops when peak positive current is reached.

These experiments have demonstrated that the retina is sensitive to currents of the magnitude generated during the C-wave of the ERG as measured by ganglion-cell response. Because currents of internal and external origin seem to affect the retina with opposite polarities, however, it seems likely that those retinal elements sensitive to transretinal current do not lie in the proximal retina.

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3. THRESHOLD OF NERVE MEMBRANE

National Institutes of Health (Grant 3 RO1 EY01149-03S1)
Bell Laboratories (Grant)

Stephen A. Raymond

We have begun an experimental program to investigate the nature of the processes that interact to determine the threshold for generation of the nerve impulse. We have also worked on interpreting and presenting our past work on the relationship of activity to threshold changes. We continue to propose that the aftereffects of activity are important in determining which branches of an axon (or dendrite) will be invaded.

Activity-dependent connectivity appears to be a basic form of information handling by the nervous system. The experiments summarized here are part of a larger effort to elucidate the rules for the transformation of temporal patterns of impulses into spatio-temporal patterns of active subsets of terminal branches of a nerve. For the moment, we are concentrating our attention on the influence of the ion pumps and extracellular concentration changes. Temperature has some interesting effects, and we have made a new observation in that area.

a. Intermittent Conduction and Nerve Threshold

Stephen A. Raymond, Paul A. Pangaro

A 16-mm color film on intermittent conduction and nerve threshold was presented at the 6th Annual Meeting of the Society for Neuroscience, Toronto, Canada, November 7-11, 1976. The film makes the relationship between threshold and conduction more vivid and understandable than has been achieved through static plots and logic. The ideas that threshold affects conduction, and that activity affects threshold, are fundamental for our notions concerning information handling. Investigators have made curious observations for which these ideas lead to clear hypotheses. One of our purposes in making the film was to induce investigators working on a variety of systems to tell us of cases where activity-dependent threshold shifts provide an explanation of their observations. The film has been well received and two new cases of activity-dependent threshold shifts in invertebrates have been reported to us. Our notions about generation of repetitive firing, presynaptic inhibition, and uninvadable branches have all received more experimental support.

b. Effects of Nerve Impulses on Threshold of Frog Sciatic-Nerve Fibers

Stephen A. Raymond

A new series of experiments was undertaken to determine the shape of threshold curves with activity. The main improvement was to develop a scheme for quantifying the threshold axis. These experiments have been arranged in a logical progression that leads to a description of the threshold curve as a continuous function of activity. A paper covering observations and experiments from 1971 to the present will be submitted for publication. It will be the first published account of the relations that we have described.[1]

c. Activity-Induced Changes in Nerve Threshold Cause Intermittent Conduction

Stephen A. Raymond, Paul A. Pangaro

Based on the results of our studies of nerve fibers, equations have been written for each fiber's threshold curve. Intermittent responsiveness was observed during repetitive stimulation with a near-threshold stimulus magnitude. The period of the intermittence changed with the rate of stimulation. It also changed with the magnitude of stimulation. The equations for threshold showed the same rate-period and magnitude-period relations observed in the nerve. Our conclusion is that this evidence shows that res-paribus conduction depends on threshold changes; in fact, in this experiment conduction or block could be predicted well by threshold curves taken alone.

d. Effect of Ion Pumps

Stephen A. Raymond

Ouabain and strophanthidin counter the buildup of depression of threshold. These pump poisons completely eliminate depression that has been developed by maintained impulse activity in the nerve. The threshold drops below resting threshold within 10 minutes following the administration of the poison. It reaches the level associated with maximum superexcitability. At 10-30 ms intervals after a conditioning impulse, when the membrane in an unpoisoned axon is near peak superexcitability, the threshold of poisoned axons shows a slight transient depression. In other words, the superexcitable phase reverses: Processes that make an unpoisoned axon superexcitable make a poisoned axon depressed. This work suggests that depression is entirely due to metabolic action, presumably ion pumping, and that the nerve at rest is held away from its point of maximum excitability by the action of the pump. This work was reported at the 6th Annual Meeting of the Society for Neuroscience.[2]

e. Justification for the Hunter Circuit

Louis L. Odette, Stephen A. Raymond

We have examined our method of measuring thresholds by using the success or failure of each trial to modify the stimulus for the next trial. We had noticed that the output of the hunter circuit was tracking in a very narrow band near the center of the range of stimulus magnitudes between 0% and 100% probability of firing (the gray region). Outside the gray region large stimuli will always produce a response, and that will reduce the next stimulus; the opposite is true for deterministic stimuli that are too small. Thus the hunter paradigm converges. Within the probabilistic range two features operate.

First, the probability of success is greater with large stimuli, and hence the probability of a return is larger the farther the stimulus is from the center of the distribution. Second, the probability of a string many jumps in a row is very small as the successively smaller probabilities of continuing a jump in the same direction are multiplied. A short note on the general problem of measuring thresholds is in preparation.

f. Conduction Velocity Variation with Threshold

Stephen A. Raymond

Variations in latency have been observed during our experiments. An instrument has been built to allow the systematic determination of the relationship by plotting the latency of each trial with time.

g. Temperature Effects

Stephen A. Raymond, Michael J. Binder

[The work of M. J. Binder was supported by the National Institutes of Health (Grant 5 TO1 EY00090-03).]

We have previously[3] reported a curious compensation of the nerve for temperature changes in a broad range from 10°C to 30°C. Our evidence that the threshold was regulated actively and not merely carefully balanced is that there were transients in the threshold accompanying fast changes in the temperature. In a few minutes these would die away leaving the nerve at its resting threshold. During the summer we noticed that nerve fibers consistently did not show the transients. Even at rest, extremely rapid changes of temperature evoked no variation of threshold at all. In the autumn the transients reappeared. We suspect that what underlies this observation is the difference between summer and winter frogs. In any case, some active temperature-compensating mechanism seems to exist for threshold.

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4. THRESHOLD HUNTER DEVICE

National Institutes of Health (Grant 3 RO1 EY01149-03S1)
Bell Laboratories (Grant)

Stephen A. Raymond, Louis L. Odette

We have invented this device to serve as a general system for automatically measuring and tracking the threshold of nerve cells. It will work equally well for measuring thresholds of other mechanical, chemical or electronic bistable systems. The usual method of determining threshold is to use a series of trial stimuli that are analyzed to obtain the probability of response for each level of stimulus. The threshold hunter is designed to vary the stimulus so as to home in on the threshold. The outcome of each trial conditions the next stimulus as the threshold of the system varies, which it does with temperature and other variables. The output of the threshold hunter, which is a voltage proportional to the stimulus, follows the variations. The extent of trial-to-trial variation of the hunter contains information about the threshold "noise" of the system under study. We have proved the conceptual validity of the convergence of the threshold hunter to the system threshold, and have investigated optimal hunting strategies for a variety of situations. With some analysis, the hunter output will yield the probability of response to the stimulus magnitude ogive. Changes in this distribution, if they occur, can be read from the threshold hunter.

5. NERVE THRESHOLD CHEMOGRAPH

National Institutes of Health (Grant 3 RO1 EY01149-03S1)
Bell Laboratories (Grant)

Stephen A. Raymond

This device is an application of the threshold hunter device. A living axon membrane is used as a detector for neuropharmaceuticals. The threshold hunter monitors the threshold curves characteristic of the axon, and chemicals are circulated past the membrane. Those chemicals having an effect on the nerve membrane produce changes in the threshold that can be discerned easily by connecting the threshold hunter to a chart recorder. Our experience thus far indicates that any compound affecting the nervous system (strychnine, ACh, choline, pH, ethanol, pentobarbitol, digitalis, N_2O) has its own characteristic effect on nerve threshold. We are eager to find out whether these characteristic effects, once they are compiled for a wide sample of chemical agents, will be sufficiently indicative of the kind of drug and its effect so as to be useful in detecting novel neuropharmaceuticals of clinical importance.

6. NERVE MEMBRANE MODELS

Bell Laboratories (Grant)

Louis L. Odette

We are analyzing and synthesizing electronic analog models of nerve membrane. These models are monostables based on the equivalent circuit for the membrane, with the relation between the steady-state values of the variable conductances and the "trans-membrane" voltage derived from a single functional.

We have constructed a circuit that demonstrates many electrical properties of the squid giant axon: the form of the membrane action potential, as well as subthreshold response; anode break excitation; and the current-voltage-time relations revealed through voltage-clamp experiments.

With this background we shall explore the relations between the models and physical representations of the nerve membrane.

7. PROPERTIES OF THE CHOLINERGIC SYSTEM IN THE OPTIC NERVE AND OPTIC TECTUM

Bell Laboratories (Grant)

Edward R. Gruberg, Jerome Y. Lettvin

Optic nerve fibers were studied as a potential cholinergic system. Using ^{14}C-choline as substrate, we found active uptake of choline in the nerve. The uptake was higher per unit protein than in the tectum, the striatum, the pallium, the ventral root fibers, and the dorsal root fibers. The Q_{10} of uptake was 2.3 and was highly sodium-dependent. Optic nerve acetylcholine (ACh) synthesis from ^{14}C-choline is approximately the same as in the ventral root and an order of magnitude greater than in the dorsal root. High acetylcholinesterase activity is also associated with each of the optic nerve terminal projections. These results imply that the optic fibers are cholinergic.

The optic fibers are not sufficient to account for all the cholinergic activity of the tectum. Cutting the optic nerve leads to an increase of ACh synthesis in the contralateral tectal lobe. Isolating the tectum from lateral inputs leads to a decrease in ACh synthesis. The origin of these fibers was determined by iontophoresis of horseradish peroxidase (N. R. P.) into the tectum. N. R. P. is transported retrograde in axons to their cell bodies. The only cells stained were a discrete group in the nucleus isthmi. Electrolytic lesion of the nucleus isthmi reduces ACh synthesis in the ipsilateral tectal lobe to the same extent as lateral tectal lesion.

By iontophoresis of ^{3}H-proline into the nucleus isthmi and subsequent autoradiography, we have traced a bilateral projection of fibers to the tectum. The ipsilateral

projection ends diffusely in a pattern coincident with optic nerve fibers but restricted only to the medial aspect. The contralateral projection is rostral for the most part and in two thin layers of the superficial tectum. The projections have been confirmed by Fink-Heimer degeneration studies.

We are now engaged in two related studies. In the first, we fill the optic fibers with an electron-dense material while simultaneously labeling cholinergic endings with labeled α-bungarotoxin and doing electron microscope autoradiography. In the second, we attempt to see anatomically whether we can find sprouting of nucleus isthmi fibers in response to optic nerve lesions.

8. DESIGN AND CONSTRUCTION OF AN ARTIFICIAL LARYNX

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Bell Laboratories (Grant)

Donald W. Schoendorfer, Stephen A. Raymond

The major thrust in the work of the past few months has been the development and optimization of an internal artificial larynx for laryngectomized patients. We have been working with Dr. Donald P. Shedd of Roswell Park Memorial Institute, Buffalo, New York. He has developed a reed fistula technique of speech rehabilitation.[1,2] His technique has advantages over alternative rehabilitation techniques.

a. An artificial vocal source is used which in principle can be designed to produce an accurate reproduction of the sound made by the normal larynx at physiological pressures and airflows. It is, therefore, easier for the patient to relearn speech. Other techniques require that the patient first learn to produce an acoustic excitation for his vocal tract; for example, esophageal speech is done by burping air from the stomach.

b. The patient is able to use exhaled air from the lungs to power and control the artificial larynx, as in normal speech.

c. The artificial sound source is introduced low in the vocal tract so that the transfer functions of the patient's tract are similar to those prior to the operation.

In the past, Dr. Shedd's patients have used large external artificial devices that fit over the tracheostoma and rest on the lower neck. The sound output of these external devices was routed into the vocal tract by way of a skin fistula constructed during the laryngectomy. These skin fistulas vary in length from 3 cm to 20 cm and are 3/8" in diameter.

The external larynx is acoustically undesirable for two important reasons. First, it permits a large amount of sound to radiate from its thin walls and interfere with the voiced sounds from the mouth. Second, the downstream tube connecting the external larynx to the vocal tract introduces its own formants on the source spectrum. These

formants are relatively stationary but drastically decrease the normality of the patient's speech.

These two problems could be diminished by making an artificial larynx small enough to be placed at the end of the fistula. There would then be no downstream tube between it and the vocal tract, and its direct sound radiation would be damped by the tissues of the neck. A device of 3/8" or less in diameter was needed to turn the steady air flow from the lungs into pulsitile air flow with a sound spectrum of the shape generated by the normal larynx. We used a relaxation oscillator modeled after the common duck call, with a vibrating cantilever reed to interrupt the driving air flow. The spectrum of the device was tuned by mass-loading the reed at specific locations and by varying its nonlinear stiffness. The frequency of oscillation of the device could be varied by changing the driving pressure, which allows inflection in the patient's speech.

Two patients are now testing the new internal larynxes. Intelligibility tests will soon be conducted. We are of the opinion that this internal system improves the patient's speech.

A problem associated with the internal larynx technique is leakage of fluids from the pharynx through the fistula tube. A pressure-inflated cuff surrounding the internal larynx is used to impede leakage from around the device. The pressure of the cuff must be regulated to prevent overinflation, which will stretch the skin fistula. Leakage through the artificial larynx has been stopped by a tiny, one-way flap valve. The coordination of these two methods should enable the patient to eat and drink without removing the system from his fistula and plugging the hole with his thumb. The technique is now being tested clinically.

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9. AN APPLICATION OF FLOW-THROUGH COLLAPSIBLE TUBES: WILL THEY FUNCTION AS PROSTHETIC VOCAL CORDS?

National Institutes of Health (Grant 3 RO1 EY01149-03S1 and 5 TO1 EY00090-03)
Bell Laboratories (Grant)

Donald W. Schoendorfer, Stephen A. Raymond

Collapsible tube flow has been studied by numerous investigators.[1-3] We have become interested in the possibility of using one of the many relaxation oscillators, the

Starling resistor (named after E. H. Starling who, in 1912, used collapsible tubes as hydraulic analogs for flow in veins) as a possible vocal-cord prosthesis. We have constructed a model of the Starling resistor, and investigated its flow characteristics. We found that the system could operate at pressures and air flows produced by the lungs, and that the frequency of oscillation could be in the 100-400 Hz range. The resulting output sound spectrum was remarkably close in shape and intensity to that of normal vocal cords.

This model was tested as an external larynx by two laryngectomized patients of Dr. Donald P. Shedd, Roswell Park Memorial Institute, Buffalo, New York. The output sound was directed to the vocal tract by a fistula tube, and the resulting speech was satisfying.

A simplified analysis of the pertinent physical laws of air flow through the Starling resistor has furthered our understanding of the mechanism of oscillation. The analysis indicated also that it would be difficult to miniaturize the Starling resistor to the point where it could be used as an internal artificial larynx. A paper describing this work will soon be submitted for publication.

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10. TERRITORIAL BEHAVIOR OF Macrozoarces americanus

National Institutes of Health (Grant 5 TO1 EY00090-03)
Bell Laboratories (Grant)

William M. Saidel

The ocean pout, Macrozoarces americanus, exhibits a territorial behavior in an aquarium that is similar to the response directed at a scuba diver in the ocean. The aquarium behavior described in this report was studied during the winter of 1973 in the National Oceanic and Atmospheric Administration aquarium at Wood's Hole, Massachusetts; scuba observations were made during the summer of 1976 along the coast of Massachusetts. In the aquarium the territorial display had three sequential movements: an alert, or head-up position (Fig. XXIII-1), an oral display (Fig. XXIII-2), and a nipping motion. For an incident to occur, one of the fish had to be within its territory on the aquarium bottom: two pout meeting outside either pout's territory never

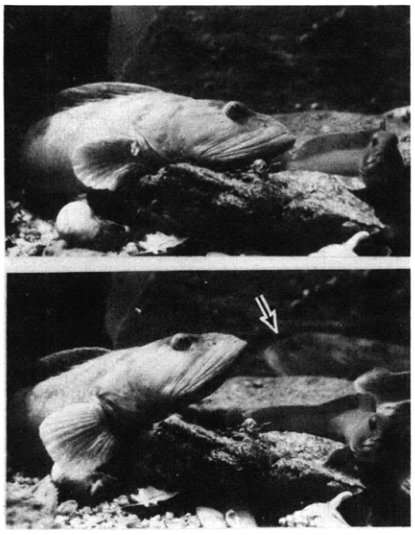

Fig. XXIII-1.
Head-up position initiated by the presence of a second pout (arrow).

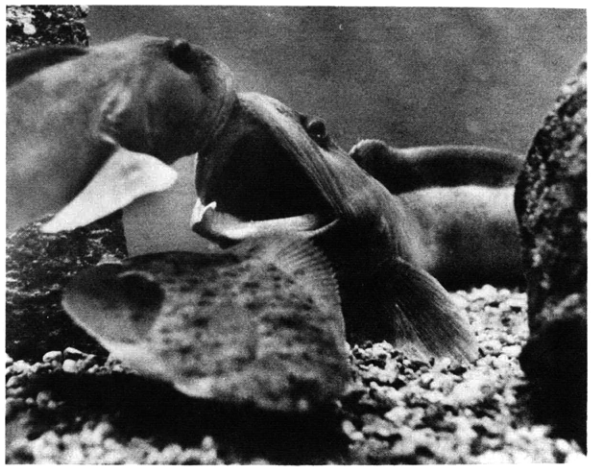

Fig. XXIII-2.
Oral display.

displayed this behavior.

Thirty-seven percent of all incidents in which a display was utilized by one or both fish were resolved by an alert posture; 49% by oral display; and 14% by the nipping motion. A resident pout initiated a display three times as often as an intruder. An intruder responded to a resident-initiated display with a display of its own only 30% of the time, while a resident responded to an intruder-initiated display more than 70% of the time. The nipping motion, always following the head-up and oral displays, was utilized only during provocative situations, such as when the fish were fed, when the fish were establishing their territories, and when a new pout was added to the tank. A resident pout always retained its territory when challenged regardless of the physical characteristics of the intruder such as size.

The territorial behavior was predominantly intraspecific. A small fraction (<14%) of the total incidents (not included in the percentage calculations) were with other genera such as Pseudopleuronectes and Gadus.

Five of the sixteen pout I encountered while diving responded to my presence with a territorial display. The maximal position used by three of them was the alert position; the two others followed the alert position with an oral display. All of these fish inhabited a protected area, e. g., at the juncture of three boulders, much like the territories defined by the pout in the aquarium. Ten of the eleven nondisplaying pout that immediately swam away were lying either on open sand or in seaweed. The eleventh fish retreated into a hole.

A territorial behavior, similar in form to that described for Macrozoarces has been

reported previously for members of the genera Blennius[1,2] and Hypsoblennius,[3,4] but this report is the first to deal with this behavior in the genus Zoarces. Despite the ten-fold difference in size between members of the family Zoarcidae and of the family Blenniidae, all three genera exist in what could be described as ecologically similar niches. The similarity of the territorial behaviors reflects this fact.

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11. ENERGY REQUIREMENTS DURING PIGMENT GRANULE MIGRATION

National Institutes of Health (Grant 5 TO1 EY00090-03)

William M. Saidel

Migration of pigment granules within the melanophore is bidirectional. The actual motion that an individual melanosome makes during migration in each direction is not identical. The movement of a granule during the inward migration, induced by catecholamines,[1] is a smooth, continuous motion, whereas the outwardly directed movement occurs in discontinuous "jumps."[2] As has been observed since the early 1900's, the inwardly directed movement is 1 to 4 times as fast as the distally directed migration.

Recent experiments have been performed that shed light upon the energetic requirements of these movements. DNP (2, 4-dinitrophenol) uncouples cellular oxidative metabolism from phosphorylation of ATP.[3] Normal flatfish saline[4] containing a 10^{-3} M concentration of DNP reversibly induces pigment aggregation in melanophores of the flatfish Pseudopleuronectes americanus. This aggregation is not due to the stimulation of the α-adrenergic site on the melanophore membrane because tolazoline hydrochloride, an α-site blocker,[5] does not affect the DNP-induced inward migration. This evidence strongly suggests that the outward pigment granule migration requires the breakdown of ATP, whereas the inward migration does not.

Two other pieces of information support this contention. The time course of

aggregation induced by DNP is approximately one fifth of the time of aggregation induced by a just maximal concentration of adrenalin. This suggests the presence of an endogenous ATP pool that must be used prior to the onset of aggregation. Second, in the absence of Ca^{2+}, a melanophore aggregates rapidly. This suggests, as in actin-myosin utilization of ATP in muscle,[6] that Ca^{2+} is a cofactor in the ATP breakdown, inducing distally directed pigment migration.

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12. BINOCULAR EFFECTS IN CHROMATIC ADAPTATION

National Institutes of Health (Grant 5 TO1 EY00090-03)
Bell Laboratories (Grant)

Michael H. Brill

Investigators have long sought to quantify the change in appearance of test lights arising from changes in an observer's state of chromatic adaptation. This is generally done by changing the spectral composition of a light presented to an observer in one adaptation state until this light matches a comparison light presented to the observer in another adaptation state.

In order to avoid the impreciseness inherent in performing color comparisons from memory, attempts have been made to place the observer in two adaptation states at the same time, e.g., by adapting the observer's eyes separately.[1-3] If one is to be able to infer properties of monocular chromatic adaptation from binocular comparisons, the lights presented to one eye must not affect the colors perceived by the other. Previous studies have indicated that the transfer of conditioning effects from one eye to the other is not significant.[4] Those experiments, however, used a single test patch on a spatially homogeneous background. Following a suggestion by J. Y. Lettvin, we have shown that this transfer is far more pronounced when the test field is a set of three differently

colored patches that are mutually contiguous in space.

The experimental arrangement is shown in Fig. XXIII-3. A piece of white matte board with 3 attached Color Aid papers hangs over an assembly of 4 mirrors. The display is illuminated by a tungsten lamp placed sufficiently far away that the white matte

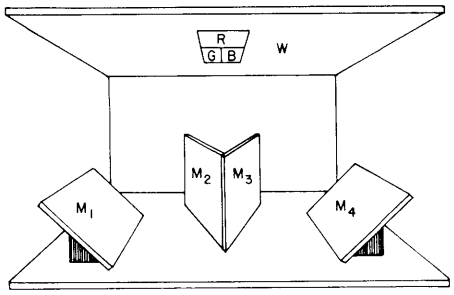

Fig. XXIII-3.

Apparatus for observing binocular effects of chromatic adaptation. M_1-M_4, mirrors; W, white matte board; R,G,B, Color Aid papers.

board appears uniformly lit. An observer preadapts one eye by looking through half a ping-pong ball at a projected colored light. Throughout the 20 seconds or so of chromatic adaptation, the other eye is open to ambient room illumination. When the observer then looks with one eye at mirror M_2 and with the other at mirror M_3 (Fig. XXIII-3), he will see two images of the triple patch, twofold rotated and displaced from each other on the white background.

If the right eye is adapted to red light, the colors seen by this eye are greener than those seen by the left eye, as expected. When the right eye is closed, however, the colors seen by the left eye become less red than they were. Reopening the right eye increases the redness of the colors seen by the left eye.

If the right eye is adapted to green light, there is a similar effect. The colors seen by the right eye are redder than those seen by the left. Closing the right eye makes the colors seen by the left appear less green; reopening the right eye makes the colors seen by the left appear more green.

After a few seconds, the effects of chromatic adaptation will weaken. If the left and right eyes are closed alternately, the colors seen by the two eyes will soon appear identical. But if both eyes are opened simultaneously, the color differences reappear, although by this time they will be quite attenuated.

This phenomenon is reminiscent of simultaneous contrast in monocular vision, and shows that the stimulation of one eye can influence color perception by the other. This is an interesting corollary to the results of dichoptic increment threshold experiments with achromatic lights.[5] We know[6] that the space of perceived colors is determined by spatial and temporal juxtaposition of lights presented to the eye: now it seems that color space can also be determined by binocular assessments.

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13. CONDUCTION VELOCITY AND SPIKE CONFIGURATION IN MYELINATED FIBERS: COMPUTED DEPENDENCE ON INTERNODE DISTANCE

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Bell Laboratories (Grant)

Michael H. Brill, Stephen G. Waxman, John W. Moore, Ronald W. Joyner

[Dr. J. W. Moore and Dr. R. W. Joyner are at Duke University School of Medicine.]

In previous anatomical studies[1,2] we showed that some central nerve fibers are characterized by closely spaced nodes of Ranvier. We have now begun to simulate impulse conduction in these fibers.

Huxley and Stämpfli[3] suggested that conduction velocity in myelinated nerve fibers should have a maximum at a particular internode length, and that the maximum should be relatively flat. They also predicted that the internodal distances of normal peripheral nerve fibers should fall close to the value for maximum conduction velocity. Other studies[4,5] have tended to confirm this prediction but failed to cover other "similarity classes" because internode lengths that were used were not short enough (less than one-half normal). Therefore, we have used computer simulations of conduction in myelinated fibers to examine the dependence of conduction velocity and spike duration on internode length. Throughout these simulations the nodal length (NL) and area are fixed and only the internode length (L) is varied (see Table XXIII-1).

We used a modification of the Fitzhugh model.[6] The equations were numerically integrated by the Crank-Nicholson method implemented in FORTRAN on a PDP-9 computer. This method had been used for unmyelinated fibers[7] and was adapted for the

Table XXIII-1. Parameters.

Symbol	Meaning	Value	Units
$\bar{g}_{Na}$	sodium conductance	1.2	mho/cm^2
$\bar{g}_K$	potassium conductance	0.09	mho/cm^2
g_L	leakage conductance	0.02	mho/cm^2
V_r	resting potential	0	mV
V_{Na}	sodium equilibrium potential[1]	115	mV
V_K	potassium equilibrium potential	-12	mV
V_L	leakage equilibrium potential	-0.05	mV
d	axon diameter (inner diameter of myelin sheath)	10	μm
NL	nodal length[2]	3.183	μm
r_a	axoplasmic resistance per unit axon length[3]	1.26×10^8	ohm/cm
g_M	myelin conductance per unit length	5.60×10^{-9}	mho/cm
c_M	myelin capacitance per unit axon length	1.87×10^{-11}	F/cm
c_N	nodal capacitance per unit axon length[4]	3.14×10^{-9}	F/cm
L	internodal length	variable	

1. All voltage signs are reversed from those of the original Hodgkin-Huxley formulation.
2. Calculated from nodal area of 100 μm^2.
3. Calculated from specific axoplasmic resistance of 100 ohm-cm.
4. Calculated from capacitance per unit area of 10^{-6} F/cm^2.

myelinated fiber by R. W. Joyner. This modified Crank-Nicholson method was found to give fast and accurate computation of impulse propagation and will be described in detail by Moore, Joyner, Brill, Waxman, and Najar (in preparation for publication). Extensive investigations into the variety of mathematical models for myelinated fibers have shown that the impulse propagation velocity is sensitive to the relative values of nodal-to-internodal characteristics but rather insensitive to changes in the description of the nodal membrane ionic currents.

Because of the insensitivity of propagation velocity to nodal ionic descriptions, we chose to describe the nodal membrane by the most convenient expression for excitable membranes, the Hodgkin-Huxley equations. The parameters used to describe our standard myelinated fiber are listed in Table XXIII-1. The numbers of sodium and potassium channels were increased by factors of 10 and 2.5, respectively, to match the nodal

conductances measured by voltage-clamp methods.[8] The nodal resting resistance was made compatible with the 55 MΩ resistance measured by Tasaki[9] by increasing g_L from 0.003 to 0.02 mho/cm^2. Then, to restore the resting potential to 0 mV, we changed V_L from +10.6 mV to -0.05 mV. We adjusted the rate constants to 20°C by multiplying all rate constants by $3^{(20-6.3)/10}$. We used a value of 5.60×10^{-9} mho/cm for g_M, the myelin conductance, and a value of 1.87×10^{-11} F/cm for c_M, the myelin capacitance.

Our results indicate that, for a 10 μm fiber, the internodal conduction time is a monotonically increasing function of internodal length L. For small L, the relationship is linear, but it departs from linearity as it goes above 2000 μm. Figure XXIII-4 presents these data in the form of impulse velocity as a function of L. There is a broad

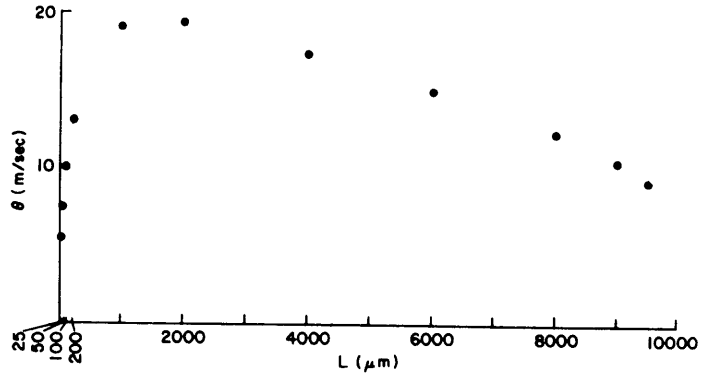

Fig. XXIII-4. Plot of the velocity θ of a steadily propagating action potential vs internodal length L.

maximum between 1000 μm and 2000 μm, which corresponds to observations on frog sciatic nerve[6] and agrees with the predictions of Huxley and Stämpfli.[3] Velocity decreases steadily for L above 2000 μm, and there is conduction failure before L reaches 10,000 μm. For internodal lengths less than 1000 μm, the velocity decreases dramatically. It is clear from Fig. XXIII-4 that, for short internode lengths, the velocity is very sensitive to L. For small values of L, the travel time (per unit distance) depends almost linearly on equal relative changes in L. The travel time is least sensitive to L in the 1000-2000 μm region.

Having carried out these computations for only one value of d (10 μm, which henceforth we call d_o), we can take any point on the curve to represent a different "similarity class." By using Rushton's correspondence principle,[10] we can interpret Fig. XXIII-4 more generally for different axon diameters. Rushton postulated that peripheral nerve fibers fall into an equivalence class in which fibers exhibit "dimensional similarity." Dimensional similarity requires that internode length, myelin thickness, and nodal area vary directly with fiber diameter. Given a class of fibers that exhibits dimensional

similarity and in which the intrinsic membrane properties are all the same, Rushton showed that internodal conduction time should be the same for all fibers of the class; i.e., conduction velocity varies linearly with fiber diameter.

Therefore, given a fiber with certain internode length L and impulse velocity θ, we can generalize Fig. XXIII-4 to fibers of other diameters by scaling θ and L by d/d_o.

From the maximum in Fig. XXIII-4, it is clear that fibers with L/d = 200 do not suffer large changes in θ when L/d is changed modestly. This is consistent with the observation that in remyelinated peripheral axons, as compared with control axons, conduction velocity is reduced but to a statistically insignificant degree.[11] On the other hand, the simulations predict that fibers with small L/d would be quite sensitive to variations in L/d. This sensitivity might provide insight into the possible physiological significance of the fact that some CNS fibers have an L/d ratio that is much less than that for peripheral nerve fibers.[1,2,12] Because the peripheral nerve impulse velocity is insensitive to small changes in L/d, there would not seem to be any signal-processing significance to minor local changes in L. On the other hand, for CNS fibers with small L/d ratios, we cannot ignore the effects on signal processing of small local changes in L because the velocity of propagation depends so dramatically on L. Local changes in L may allow fine tuning of the times of arrival of impulses at synapses or provide a convenient way of presetting route-dependent travel times in the central nervous system.[2,13]

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14. CYTOCHEMISTRY OF THE AXON SURFACE

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Bell Laboratories (Grant)

Stephen G. Waxman, Donald C. Quick

As part of our studies on the morphophysiology of the axon surface, we have studied the differential staining of the axon membrane at nodes of Ranvier and in internodal regions of normal peripheral nerve fibers, and at several types of nodes of Ranvier along the highly differentiated axons that comprise the electric organ in the gymnotid fish, Sternarchus albifrons.

Using a modified ferric ion-ferrocyanide method, we have noted a specific staining of the cytoplasmic surface of the nodal axon membrane. Our results indicate a high degree of local differentiation of the axon membrane with respect to staining properties with ferric ion-ferrocyanide, and demonstrate that nodal and internodal membrane exhibit structural differences.[1]

In the present study, we studied myelinated axons in rat sciatic nerve and in the electric organs of Sternarchus albifrons. The first site was chosen as an example of normal peripheral nerve. The second was chosen because the Sternarchus electrocyte axons exhibit two types of nodes of Ranvier, which are differentiated in terms of electrical properties as well as of morphology. In particular, the electrocyte axons have nodes with a normal morphology, which exhibit spike electrogenesis, and larger nodes, which do not generate spikes but rather function as a series capacity.[2-4] These axons thus provide an opportunity for comparison of membrane staining properties at active and inactive regions of single axons.

In rat sciatic nerve the nodes of Ranvier are intensely stained by the ferric ion-ferrocyanide method. Similar staining occurs at the narrow (1-2 μm) nodes of the Sternarchus electrocyte axons. By light microscopy, the stain appears as a dense ring roughly coincident with the unmyelinated gap at the node. In 3-5 μm sections examined

by light microscopy, other parts of the nerve fibers, including axoplasm, compact myelin, and myelin terminal loops, are also stained in light blue, but the color is much fainter.

In ultrathin sections examined by electron microscopy, the heaviest deposits of stain are found as dense aggregates on the inner aspects of nodal axolemmae. At the most densely stained nodes, the stain is deposited in a layer 20-100 nm thick, immediately subjacent to the nodal axon membrane. The dense aggregation of stain in all cases is confined to the nodal (i.e., unmyelinated) axon membrane, and dense aggregates do not appear subjacent to the terminating myelin loops on either side of the nodal gap.

In contrast to the axolemmae at the nodes of Ranvier, internodal regions of the axon membrane are not stained. Absence of staining of the internodal axolemmae is also observed near the cut ends of axons that have been severed after fixation and before exposure to the staining solutions. Fine electron-dense deposits, but no aggregates of stain, are seen in compact myelin, in terminating myelin loops near the nodes, in Schmidt-Lantermann clefts, and along axoplasmic filaments. Axoplasmic filaments are often noticeably stained at the center of an axon, several micrometers distant from the axolemma, which indicates diffusion of stain through the fixed axoplasm.

Ferric ion alone gives results similar to those obtained with the ferric ion-ferrocyanide combination. Myelin is consistently stained with ferric ion, but fewer nodes are well stained. Ferrocyanide also stains myelin, but nodal axolemmae are not stained with ferrocyanide alone.

These results are applicable to nodes of Ranvier in rat sciatic nerves and to the narrowest nodes (0.2-2 μm) in Sternarchus electric organ. Sternarchus electrocytes also have some very wide nodes (5-50 μm) that are known to be electrically inexcitable.[3,4] These nodes are not stained with ferric ion-ferrocyanide, either in blocks of tissue in which nearby narrow nodes are heavily stained or in teased fiber preparations in which adjacent narrow nodes of the same axon are well stained. Sternarchus nodes of transitional size (2-5 μm) are intermediate in their staining properties.

Our results indicate that there are distinct structural differences between nodal and internodal axolemmae. The possibility that there are differences in specific properties between the nodal and internodal axon membrane assumes special relevance in the context of the demyelinating diseases, since the conduction properties of affected axons will depend on the electrical characteristics of the demyelinated internodal axolemmae, as well as on those of the nodal membrane. It is known that the normal nodal membrane exhibits different properties than most other excitable membranes that have been studied, including those of invertebrate myelinated fibers and the unmyelinated terminals of amphibian neuromuscular junction. We emphasize that the present results do not allow us to comment on the electrical properties of the nodal and internodal regions of the axon membrane. Our results demonstrate, however, a chemical differentiation of the inner

surface of the axon membrane between nodes and internodes in normal peripheral nerve fibers, and between the inner surface of the axon membrane at active nodes, inactive nodes, and internodes in the Sternarchus electrocyte axons.

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15. ULTRASTRUCTURE AND PHYSIOLOGY OF CENTRAL AXONS

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Bell Laboratories (Grant)

Harvey A. Swadlow, Stephen G. Waxman

We have continued to examine the morphology and physiology of visual callosal axons in the adult rabbit. Axons in the posterior 3 millimeters of the splenium of the corpus callosum were examined by electron microscopy.[1] Unmyelinated fibers comprise approximately 45% of the fiber population. These axons range from 0.08 μm to 0.6 μm in diameter, and usually occur in clusters of at least 3-4 axons. Myelinated fibers comprise 55% of the axons in the splenium. The diameters of myelinated fibers range from 0.3 μm to 0.85 μm. Values of the ratio g (axon diameter/total fiber diameter) range from 0.64 to 0.87. In the majority of myelinated axons, the inner mesaxon and outer tongue of glial cytoplasm are located in the same quadrant. The unmyelinated gap at the nodes of Ranvier extends less than 2 μm, and an electron-dense undercoating is present, subjacent to the axon membrane at the node. Branching of fibers was not observed.

In our physiological studies, we examined the conduction properties of 75 visual callosal axons of the awake rabbit.[2,3] These axons were studied by measuring latency to antidromic activation of cell bodies following midline callosal and/or contralateral cortical stimulation. Seventy-three of 75 neurons (axon conduction velocities = 0.3-12.9 m/s) demonstrated decreases in antidromic latency and threshold to a test stimulus that followed an antecedent conditioning stimulus at appropriate intervals. Control experiments

indicated that the latency and threshold variations resulted from prior impulse conduction along the axon, and that the latency decrease reflected an increase in conduction velocity along the main axon trunk. On the basis of diameter spectra, we established criterion conduction velocities for the physiological identification of myelinated and unmyelinated axons. The supernormal phase is observed in both classes of fibers. The maximum magnitude of the latency decrease for different axons ranged from 3% to 22% of control values, while the duration was in the 18-169 ms range. The duration of the latency decrease was greater for slowly conducting axons than for fast conducting axons. Latency increases to an antidromic test stimulus occurred for as long as several minutes following a train of antidromic conditioning pulses. Antidromic latency shifts of lesser magnitude and duration were also observed in somatosensory callosal axons and in some cortico-tectal axons.

Our results indicate that a complex sequence of events (refractory period → supernormal phase → subnormal phase) follows the action potential in even unbranched central axons with a relatively simple morphology. Conduction properties of axonal trunks thus are not invariant but, on the contrary, reflect the history of previous impulse activity in the axon, as suggested by Chung et al.[4] Our experiments provide a body of normative data on central white matter axons. We intend now to examine the effects of epileptic spike activity and demyelination on central impulse conduction.

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16. STUDIES ON MORPHOGENESIS OF NERVE CELLS

National Institutes of Health (Grants 5 RO1 NS12307-02 and KO4 NS00010)

Stephen G. Waxman

Working with Dr. Mark A. Dichter of Harvard Medical School, we have begun to examine the development of specificity in neuro-glial interactions, using a tissue culture model. We have studied the development of dissociated cell cultures of chick dorsal root ganglia. Our studies have demonstrated that, despite initial disaggregation of

neurons and glial cells, an apparently normal neuro-glial relationship develops in the course of several weeks.[1] This includes a normal neuron-satellite cell relationship in addition to the development of compact myelin. We hope to use this system, which is highly accessible to experimental manipulation, as a model for studying the development of specificity in nerve cell development.

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