

## XIX. NEUROPHYSIOLOGY\*

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### A. MAMMALIAN NODES OF RANVIER

#### 1. RECORDING METHODS

About eight years ago, we spent a few months penetrating with microelectrodes into large myelinated fibers in normally circulated dorsal roots of the cat. At that time, the evidence suggested that the entry was at or near a node of Ranvier rather than through the myelin sheath. An electrode would stay in a single fiber very often for an hour or more with no deterioration in the electrical behavior of the fiber. Membrane potentials were usually  $\sim 70$ - $80$  mv and the height of the nerve spike  $\sim 100$ - $120$  mv, with a duration of 0.5 msec or less. It seemed that such a preparation would be useful in studying membrane properties on mammalian nerve; however, for several reasons, the work was put aside. Since that time, nobody else seems to have interested himself in this problem or to have discovered how easy the preparation is. Therefore two members of our group have revived the unreported method and W. Pickard is developing it further.

We use ordinary micropipettes with tips of 0.3 micron (I. D.), or less, and filled with 3 molar KCl in the usual fashion. These tips exhibit a resistance, in tissue fluids, 10-15 megohms, and the capacitance across the pipette wall in the fluid is  $\sim 1$  pfd/mm length of pipette independent of diameter (because in drawing the pipette the I. D./O. D. ratio tends to remain constant). Such a probe is connected to a conventional DC amplifier of high input resistance and low leakage current, which also has an adjustable "negative capacitance" compensation at the input.

A young cat's spinal cord is exposed in the usual way, grounded, and bathed in mineral oil. Over a small patch of dorsal root the arachnoid is removed between vessels. Into this patch we thrust the microelectrode and, with a little manipulation, impale a large fiber. This is seen as a sharp change of approximately  $-70$  mv with respect to ground. We stimulate the fiber through the recording electrode and cancel the stimulus artefacts in the following way: When a trapezoid of voltage is differentiated it yields two square pulses of current in opposing directions. The magnitude of the current through a fixed capacitor depends on the rate of change of the voltage, the duration depends on

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the duration of the ramp functions used to generate the trapezoid, and the interval between the two pulses is the interval between the two ramp functions. Thus we can control all of the parameters of a stimulus. We set the square pulses of current at  $\sim 10^{-9}$  amp, 0.1-msec duration, and 10-msec interval. These square current pulses yield square voltage swings whose magnitude measures the resistance through the electrode through the electrode to ground, and whose rise and fall times measure the capacitive shunt to ground across the electrode wall. The rise times can be adjusted to  $\sim 5$   $\mu$ sec by means of the negative capacitance without introducing distortion. These square voltage pulses can be balanced out in a push-pull amplifier by an analogue of the electrode impedance which is also capacitively connected to the trapezoid generator. Since, for such low currents, the KCl pipette shows no severe nonlinearities, the cancellation of the induced voltage changes across the electrode can be done extremely well in a push-pull amplifier. As one penetrates a nerve fiber the cancellation that served so well in the external fluid ceases to be effective because the resistivity to ground changes as soon as the tip of the electrode is up against a high-resistivity barrier such as membrane. When the membrane is traversed the cancellation is again effective as long as the electrode tip lies in axoplasm (which has about the same resistivity as the external fluids) and is not touching the other side of the fiber because of having been pushed too far. By fine adjustment after penetration, the tip can be positioned directly in the center of the fiber by using electrical monitoring. But the resistivity across a nodal membrane is several megohms and the capacitance is a few picofarads. Furthermore, the axoplasmic bridges to the nodes on either side are each several megohms. Thus, riding on top of the compensated signal there will appear an additional signal caused by the node membrane, and it will have a longer rise time than the compensated pulse. The net result of suitable compensation is that it is possible to measure the so-called electrotonic properties of the node directly. This current level is frequently sufficient to stimulate the node and one sees the nerve spike. The threshold for most nodes is fairly low and at no time is it necessary to use currents that take the electrode into nonlinear operation (e.g., currents greater than  $10^{-8}$  amp). It is possible, by using such an arrangement to measure the changes in impedance during the spike and many other properties of nervous membrane, and we have done so.

## 2. MATHEMATICAL AXONOLOGY

If the medullated axon is idealized as a periodic structure composed of lengths of lossy passive transmission line (internodes) coupled by electrically short lengths of active transmission line (nodes), then it is possible to derive by classical means an equation for the temporal variation of the nodal voltage. This resulting equation is

$$-\frac{2s}{r_1+r_2} \psi_0 \frac{e^{\delta a} - 1}{e^{\delta a} + 1} + \frac{1}{2\pi} \int_{-\infty}^{\infty} J_0(\omega) e^{j\omega t} d\omega =$$

$$bc \frac{dV_0}{dt} + bG_{Na}[V_0 + V_{Na}] + bG_K[V_0 - V_K] + bG_L[V_0 - V_0],$$

where  $V_0(t)$  is nodal voltage,  $\psi_0$  nodal resting potential,  $b$  nodal length, the  $V_j$  several ionic battery potentials, the  $G_j(t)$  several nodal ionic conductances per unit length,  $C$  nodal shunt capacitance per unit length,  $r_1$  and  $r_2$ , respectively, the resistance per unit length of the extracellular solution and the axoplasm,  $\delta^2 = (r_1+r_2)g$ , where  $g$  is the shunt conductance per unit length of internode,  $a$  is the internodal length, and

$$J_0(\omega) = -\frac{2}{a(r_1+r_2)} \Phi_0(\omega) \frac{\gamma_a}{\sinh \gamma a} [\cosh \gamma a - \cos \omega \tau],$$

where  $\Phi_0(\omega)$  is the Fourier transform of  $V_0(t)$ ,  $\gamma^2 = (r_1+r_2)(g+j\omega c)$ , where  $c$  is the internodal shunt capacitance per unit length, and  $\theta \tau = (a+b)$ , where  $\theta$  is the pulse conduction velocity along the axon.

It can be shown that this equation for nodal voltage does not, for many axons, approximate a linear second-order differential equation even if the several ionic conductances are explicitly known as functions of time.

By a limiting process, this equation can be simplified to yield the usual equation for propagation along an unmyelinated axon. This new equation can be manipulated to yield an approximate expression for the conduction velocity. For squid giant fibers the expression for  $\theta$  reduces to

$$\theta = \frac{4}{3} \left[ \frac{\mathcal{G}_0^1}{\tilde{C}^3 (\tilde{r}_1 + \tilde{r}_2)^2} \left( \frac{V_T}{2\psi_0 \psi V_T} \right)^3 \right]^{1/4},$$

where  $\mathcal{G}_0^1$  is the mean maximum rate of use of the axon's sodium conductance per unit length,  $\tilde{C}$  is the capacitance per unit length,  $(r_1+r_2)$  is the total resistance per unit length of axoplasm and intercellular medium, and  $V_T$  is the threshold voltage. This formula gives numerical predictions that agree well with experimental data.

A detailed account of these researches will appear in a paper that is now being prepared for submission to the Journal of Theoretical Biology.

W. F. Pickard

## B. UNMYELINATED FIBERS IN THE DORSAL ROOTS OF CATS

With Dr. R. C. Gesteland, who is leaving our laboratory and will be greatly missed, we have developed a method for recording the activity of unmyelinated fibers in the dorsal roots of mammals. He will take this technique to Northwestern University where he

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plans to do a major study on these fibers. Briefly, it is this: When a cat is prepared as for the study of nodes, and the arachnoid is cleaned off a patch of root, one uses, instead of a micropipette, a Dowben-Rose electrode consisting of a glass capillary filled with Wood's metal and plated at the tip with a cap of gold on which Pt black is deposited. When such an electrode just touches the surface of the root, not only can one record the fast transients caused by myelinated fibers, but also much slower diphasic and triphasic transients, lasting 10 msec or so. These slower transients can be increased in frequency by manipulation of the viscera or by pressing on the skin, and so on. They seem very frequently to be autonomic (or enteroceptive) but sometimes are somatic. If the electrode is pressed down so as to penetrate the root, then these transients disappear and never return when the pressure is relieved, although the fast transients recover. We have seen such sensitivity to pressure in other unmyelinated small-fiber systems such as the olfactory nerve of the frog or the optic nerve of the octopus. Presumably it is this sensitivity that has prevented the prior discovery of such transients by other workers. We had hit upon this technique three years ago and laid it aside until one of us felt he could handle it seriously. It certainly beats the more exacting and tedious method of dissecting down small bundles.

J. Y. Lettvin

### C. OLFACTION IN THE FROG

Our study of the frog's nose is to appear soon in the Journal of Physiology. With R. C. Gesteland as principal investigator, we report some new observations on first-order olfactory elements studied both individually and en masse. What we found was this:

- (i) Most of the axons of the olfactory neurons have a resting discharge; the probability of an impulse occurring at any moment is not extremely low.
- (ii) This probability of discharge can be increased, in any fiber, by some odors and decreased by others; that is to say, the response has two directions.
- (iii) For any odor that causes a response, its effect during insufflation may be either the same or opposed to that produced by stopping the odor.
- (iv) For any fiber, the effect of a variety of odors can be ordered in terms of increasing or diminishing the probability of firing during and following the presentation of the odor.
- (v) The probability that two fibers will show the same order decreases steadily with the number of odors used. That is to say, if we use two odors that produce the same response in each of two fibers, a short search reveals a fiber that responds differently to the odors.
- (vi) Analysis of the voltage transient known as the electro-osmogram, a "slow wave" very like the electroretinogram and others recorded from sensory tissue, reveals that

there are two processes governing the course of the transient. One can be regarded as a current-generating process across the receptor membrane and the other as a shunting process. The interaction of these two processes accounts for the families of transient shapes that we see. The two processes that are actually involved are unknown, but they are equivalent formally to what we have described; i. e., they are not similar processes simply of opposing sign.

(vii) That there are two processes involved in changing impedance across the olfactory mucosa during stimulation can be shown at certain frequencies of applied current that depend on electrode parameters. At these frequencies the phase angle and magnitude of resulting voltage swings can fluctuate independently, the amount depending on the stimulus.

(viii) The two directions of the response in the single fiber, the two processes involved in generating the electro-osmogram, and the two processes revealed in the impedance measurement have been taken by us to be different representations of a dual process in the receptors. The coincidence of results may be fortuitous, for we have proved no causal relations; but the results are suggestive of what we suspect.

(ix) This view leads to regarding the olfactory receptors as formally equivalent to neurons elsewhere in the nervous system, with the sensitive sites acting as excitatory and inhibitory postsynaptic membrane patches do in central neurons.

We do not regard every olfactory neuron as different in its smelling order from every other neuron (and there are several million in each nose). Rather, there must be some groupings if we extend our olfactive experience to the frog. Such groupings are, however, unlikely to be uncovered by a fiber-to-fiber search when one considers how many smells must be used per fiber to establish groups.

(x) Olfactory space ceases to be comparable to other sensory spaces if, as now seems possible, we must deal with what may at first appear as arbitrary ordering principles. Notions of distance in such a space may only be locally definable and this would be in accord with the psychological data.

(xi) Finally, as a practical matter, Gesteland has shown that, by using the electro-osmogram, and the phase angle and amplitude of the voltage fluctuations resulting from the application of an oscillating current to the mucosa, it is possible to make clean distinctions between many different odors. This trivariant signature, however useful empirically, implies nothing about the relation of its components to the olfactory process.

J. Y. Lettvin

#### D. RETINAL STUDIES IN THE FROG

The ganglion cells in frog retina were incompletely described by two of us several years ago. We were more concerned with the short-time invariants, neglecting the more secular processes and only sketching possible generative events. We put off the problem

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of characterizing these cells more completely until we would have a chance to work together again. This we have been able to do this spring. We chose the simplest element to analyze – the "off" fibers of Hartline (our "dimming" detectors). These units are characterized by a vigorous, rhythmic and prolonged response to the darkening of their receptive fields, and an interruption of that discharge, a complete impression, by rebrightening. At least this is the way Hartline, Barlow, and ourselves separately reported. Yet this description, however simple, raises some definite questions: What governs the rate and length of the off-response if it is a function of the size of the step to darkness? Is the response characterized by one time constant or by several? Are all off-fibers alike in most respects, or are there individual differences of a material sort? Can we essay some guess about the generative events from noting similarities between fibers?

Our preparation was, again, the curarized frog with optic nerve exposed intracranially through retraction rather than ablation of the brain (to preserve a strong circulation). Our electrodes were, as before, our modification of the Dowben-Rose probes. Our

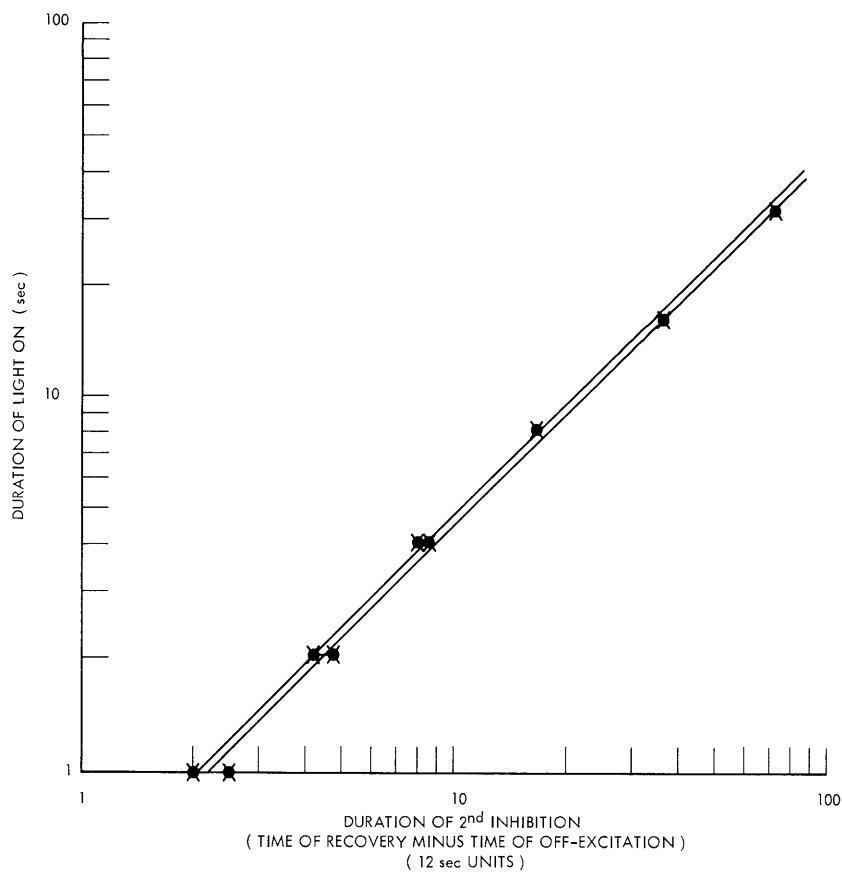


Fig. XIX-1. Time of recovery vs time of illumination.

only new apparatus was the CLOOGE (Continuous Leg Of On-Going Events). With this instrument we could record the logarithm of pulse interval over 4 decades (1 kc-0.1 cycle) and, using a sample-hold circuit and voltage comparator, generate the records shown in Fig. XIX-1. When we had isolated a single off-unit in the optic nerve by pulse-height pick-off, and were assured of its stability, we let the eye dark adapt for 35 minutes in very close to absolute darkness. At the end of this time we would administer a flash of light of 1-sec duration, wait for full recovery, then give a light of 2-sec duration, wait for recovery, and so on with 4, 8, 16, 32, and 64 sec flashes. Then we would leave the light on (it had always the same intensity in our initial experiments) until, as often happened, the fiber would begin firing again, then turn the light off to absolute darkness and watch the patterns of firing afterward. The CLOOGE yields a very easily read record of these patterns.

The most salient feature of our study is that over a wide range the time of return to resting activity in the dark is a linear function of the total amount of pigment bleached. These values were taken well below the asymptotic times of recovery for a light of this intensity left on indefinitely long and then switched off. We have not studied the asymptotes sufficiently to plot them as a function of light intensity. The data strongly suggest that the generating event is governed by a bottleneck and, whatever sign of recovery we are measuring, it changes at a fixed rate. Recently such processes have been observed in pigment changes of the frog's eye.

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