Measurement of the Action Potential of Myelinated Nerve Fiber

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THE PEAK VALUE of the action potential of the vertebrate myelinated fiber has been previously recorded by a number of investigators. (1–5 and others). Values range from about 60–155 mv depending on the method employed. These methods may be divided into three general categories.

The action potential at the node of a single fiber may be estimated from a knowledge of the action current in the axis-cylinder and the resistances in the various parts of the nerve fiber. Action currents have been determined by measuring the potential drop across a low resistance shunting the isolated section of a nerve fiber (2) and the resistances involved have been measured to a first approximation (cf. also 6, p. 32), but the uncertainty in the latter measurements lends considerable limitation to the accuracy of the action potential estimated by this method.

A second method adopted by Huxley and Stämpfli measures the peak value of the action potential by determining the amplitude of a rectangular voltage pulse just necessary to buck out the action potential of the fiber. The method suffers from errors introduced by the capacitative flow of current across the myelin sheath (see e.g. 6, fig. 33).

The third method measures the action potential of a single fiber by introducing a hyperfine glass pipette electrode into the fiber (4, 5). Errors due to capacitative losses between the fluid inside the microelectrode and the external solution are probably adequately compensated for by the use of capacitative positive feedback in the amplifier (7–9). However, after the microelectrode is introduced into the fiber there occurs an apparent prolongation of the time required for the impulse to jump across the internode impaled and a rapid deterioration of action potential suggesting damage to the fiber. Thus, the action potential recorded by this method is also subject to some doubt.

Most of these difficulties are avoided by connecting the isolated single fiber directly to the grid of a high impedance amplifier developed for use with microelectrodes. Capacitative effects can be minimized by exposing the isolated internode to dry air and by utilizing a positive feed-back in the amplifier. Injury effects are avoided by using the fiber internode itself as a micro-electrode extension connecting the amplifier grid to the inside of the active node.

METHODS

The isolated single fiber from bullfrog (*Rana catesbeiana*) sciatic nerve was used for these experiments. A large motor-nerve fiber (12–15 μ in outside diameter) was isolated from the small nerve trunk innervating either the sartorious or semitendinosus muscle for a length of 1.5–2 mm. No node of Ranvier was exposed in the isolated region of the preparation. After cleaning the fiber under a high-powered dissecting microscope, the distal unoperated region of the nerve trunk was cut across at a point approximately 10 mm from the isolated region of the preparation. The isolated fiber of the preparation was laid across the gap between the grid electrode of the amplifier and a large glass plate (fig. 1*A*). This gap of about 1 mm was first filled with Ringer's fluid and later, when the fiber had been placed in position, the fluid was completely removed from the gap, thus exposing the myelinated portion to the air. The amplifier thus recorded the potential of the distal portion of the isolated fiber (right hand end in the diagram), with respect to the proximal end. The single fiber was excited by means of the stimulating electrodes shown. A few milliseconds after each stimulus a rectangular voltage pulse was applied across the 500-ohm resistor connected between the large pool and ground. This permitted both the amplifier response and the action potential to be presented on each sweep of the oscilloscope. The major portion of the grid electrode was enclosed in a metal shield driven by the controlled positive feed-back voltage from the amplifier. The amplifier was of the negative capacity type previously reported by Sohms, Nastuk and Alexander (7), MacNichol and Wagner (8) and Woodbury (9). Provision was made for either shorting the grid to ground or shunting it to ground with a 22-megohm resistor. By adjusting the positive voltage fed back to the driven shield the effec-

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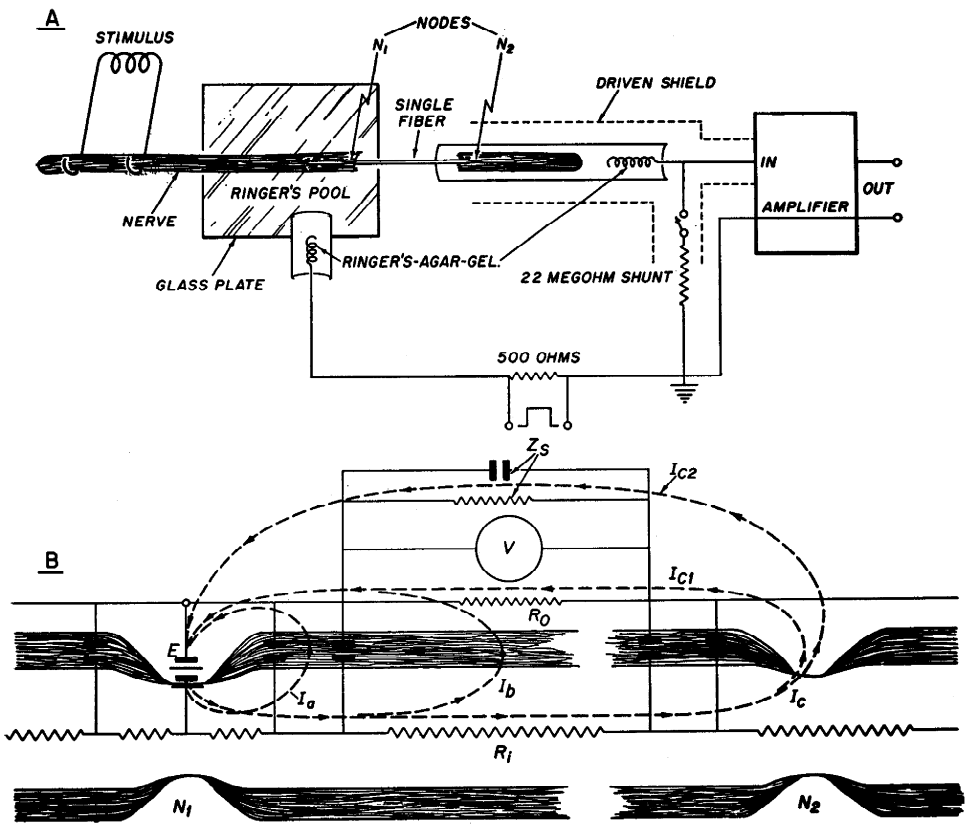

FIG. 1*A*. Experimental arrangement used for direct recording of the action potential of a single nerve fiber. *B*. Equivalent circuit used to illustrate the principle of the method. *E*, electromotive force at the node of Ranvier to be measured; R_i, resistance of the axis-cylinder between nodes N_1 and N_2; R_o, external resistance between the nodes; Z_s, impedance between the recording electrode and the ground; *I*'s, possible pathways of the current that can reduce the observed action potential. For further detail, see text.

tive input capacity could be reduced to approximately 1 $\mu\mu$f as measured by the rise-time of the response to the rectangular voltage pulse applied. Positivity of the grid electrode gave an upward deflection on the oscilloscope.

The principle of the method is illustrated in figure 1*B*. The source of the action potential, *E*, drives a current, *I*, through the longitudinal resistance of the axoplasm, R_i, in series with the longitudinal external resistance, R_o. The potential drop seen by the amplifier across R_o is,

$$V = IR_o = \frac{ER_o}{R_o + R_i}$$

As the fluid on the outside of the fiber across the air gap evaporates R_o becomes very large and in the limit $V = E$, namely,

$$\lim_{R_o \to \infty} V = \lim_{R_o \to \infty} E \frac{R_o}{R_o + R_i} = E$$

The error, $E - V$, will be the potential drop across R_i produced by any longitudinal current in R_i. Possible return paths for such currents are indicated by the arrows I_a, I_b, and I_c. As has been shown in a previous paper (10; cf. also 3, 6), the myelin sheath which separates the axis cylinder from the surrounding fluid medium has a capacity of approximately 1.6 $\mu\mu$f and a resistance of 290 megohms across a length of 1 mm. The longitudinal resistance of the axis cylinder is of the order of 15 megohms/mm. Ohmic components of the current paths I_a and I_b will be negligible due to the extremely high resistance of the myelin. Capacitative currents across the myelin are confined to the half millimeter or less of internode in the large pool of Ringer's fluid. The capacity of this part of the myelin sheath is 0.5–1 $\mu\mu$f and the resistance of the axis cylinder 5–10 megohms. This represents an RC shunt of less than 10 μsec. time constant, well below the response time of the amplifier as used. Current, I_o, leaving the axis cylinder via N_2 might return either through the fluid left on the outside of the exposed internode, I_{c1}, or through the input impedance of the amplifier, I_{c2}. If these impedances are high enough, there will be essentially no longitudinal current flowing in the axoplasm and the potential recorded will be that inside N_1.

The amplifier input impedance, Z_s, is normally greater than 10^9 ohms. When the 22-megohm shunt is

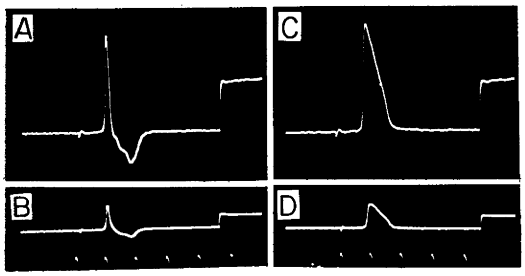

FIG. 2. Action potentials of a single nerve fiber of the bullfrog recorded by the arrangement of fig. 1, before (*A* and *B*) and after (*C* and *D*) application of an isotonic KCl solution on the distal node N_2. Lower records (*B* and *D*) were taken with the 22 megohm shunt connected between the grid and the ground of the amplifier. Rectangular pulses, 50 mv; time marks, 1 msec. apart; temperature 24°C.

applied, then V will be markedly reduced. But the rectangular calibrating voltage pulse will always be reduced in the same proportion if the resistance of the external fluid path, R_o, is large compared to R_i or Z_s. Thus if the action potential is measured by the height of the recorded calibration pulse there will be no error due to the amplifier input impedance.

RESULTS

Impulse Conduction Across the Isolated Internode. When a fresh single fiber preparation was carefully mounted across the air-gap of the apparatus illustrated in figure 1*A* and the feed-back adjusted for best response to the applied rectangular pulse, it was found that a nerve impulse gave rise to an action potential at the site of recording with a sharp rising positive phase followed by a longer negative phase, figure 2*A*. The diphasicity of the action potential observed indicates propagation of the impulse across the isolated internode. The amplitude of the action potential at this freshly prepared stage was generally between 50 and 80 mv. Both the action potential and the rectangular pulse response are reduced by connecting the 22-megohm shunt between the amplifier input and ground as seen in the lower trace *B* of figure 2. From the degree of such reduction the resistance of the preparation between electrodes was generally estimated to be between 40 and 70 megohms.

$$R = \frac{\text{reduction in pulse ht.}}{\text{reduced pulse ht.}} \times 22 \text{ (megohm)}$$

Impulse conduction was sometimes prevented by washing the distal portion of the preparation with isosmotic KCl solution, figure 2, right. The monophasic action potential obtained by this technique has a very sharp rising phase of the order of 0.1 msec. and an almost linear falling phase of approximately 1.0 msec. at 25°C. A small after-potential in the direction of continued partial depolarization of the active node is sometimes seen but was not studied carefully. The application of the KCl caused a DC potential shift of 20–50 mv.

Conduction across the internode could also be prevented by simply fanning the preparation or by sucking air past it with a small glass tube placed near the portion of the fiber across the air-gap (relative humidity of the air 35%). As the fanning proceeded the second phase of the initially diphasic response became larger and later until suddenly conduction failed and the response became monophasic as shown in figure 3*A*. If fanning was immediately discontinued conduction across the internode was sometimes re-established. When conduction had been blocked by fanning it could be restored immediately either by connecting the 22-megohm shunt across the amplifier input, figure 3*D*, or by reducing the amplitude of the feed-back voltage, thereby permitting an increase in the uncompensated input capacity, figure 3*B*. Either of these methods apparently provides a sufficiently low impedance electrical return path for excitation of the distal node by the action current of the proximal node.

Damage to the Preparation. In previous experiments by one of us, when it was discovered that the myelin sheath of the single fiber preparation is extremely resistant to desiccation, the following two phenomena were simultaneously revealed (11): *a*) when the single fiber of an isolated nerve preparation was the only electrical connection between two pools of Ringer's solution, one containing the muscle and the other the isolated or grounded stimulating electrode, there frequently occurred contractions in the muscle innervated by the fiber. If the two pools were left connected by a bridge of Ringer's soaked cotton these contractions did not occur; *b*) after the external resistance shunting the single fiber had been kept very high for a period of time, measurements of the fiber threshold and ac-

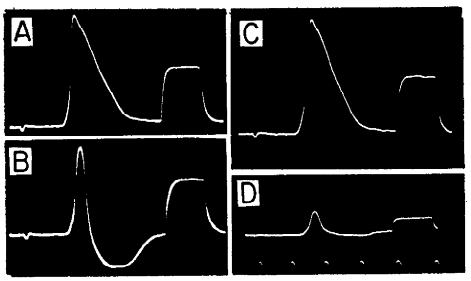

FIG. 3. Similar to fig. 2, but with the distal node kept in the normal Ringer's throughout. *Records A* and *C* were obtained when the feed-back voltage was adjusted for the best rectangular response to the applied square pulse. *Record B* was taken with the feed-back voltage reduced slightly below the optimum; note the diphasicity of the response indicating propagation of impulse across the insulated internode. *Record D* shows restoration of impulse conduction across the insulated internode by connecting a 22-megohm shunt between the grid and the ground of the amplifier. Time marks 1 msec. apart.

tion current indicated that the fiber was in abnormally poor condition. If the fiber had not been left unshunted too long the threshold and action current changes were at least partially reversible.

These phenomena are apparently due to 60 cycle or other sources of currents carried by the single fiber of the preparation due to differences in pick-up capacities of the pools at either end, particularly when one of the pools is grounded. In the present experiments this condition was avoided by keeping the isolated fiber of the preparation shunted at all times until the distal end had been placed on the shielded grid electrode of the amplifier. This shield placed around the portion of the nerve fiber on the grid-electrode apparently prevented such damaging current in the single fiber.

Damage to the preparation also results from excessive desiccation by fanning. After the action potential has become monophasic, indicating conduction-block, the amplitude rapidly reaches a maximum. Further fanning simply increases the resistance of the preparation, first without appreciable change in the spike-amplitude, later associated with a gradual loss in the amplitude. This loss in amplitude is also partially reversible if not carried too far.

Maximum Amplitude of the Action Potential. In a series of 22 experiments (performed at room temperature, 24–25°C) 10 survived the preparation stage and yielded apparently significant values of action potential. Oscillographic records were made while gently fanning the preparation and simultaneously adjusting the amplitude of the feed-back voltage to produce the optimum response to the rectangular calibrating pulse. Maximum values for the amplitude of the action potential recorded from each experiment are listed in table 1, together with an estimation of the resistance of each preparation as calculated from the reduction in amplitude with shunting, as described above. The average of the observed peak-voltages was 100.7 mv, the standard and maximum deviations being 3.4 and about 5 mv, respectively. The variation in maximum action potential between different fibers is as narrow as that found for the rheobasic voltage of different motor-nerve fibers under given experimental conditions.

The estimated resistances given indicate the practical compromise necessary with this experimental technique. Too low a fiber-resistance indicates the presence of a significant external shunt on the fiber and is associated with conduction across the isolated internode and reduced amplitude of action potential. On the other hand if the resistance of the preparation is too high the compensation for frequency loss due to the time constant of the input circuit is limited and for the observed short rise-time of approximately 0.1 msec. an amplitude loss necessarily results.

The magnitude of the error which can be introduced by failure to compensate adequately is well illustrated in figure 4. Here the

TABLE 1. PEAK ACTION POTENTIAL OF SINGLE NERVE FIBERS AT 24–25°C

Exper. No.	Max. Action Potential	Approx. Resistance
	mv	*megohms*
3	106	
8	97	100
12	99	81
15	98	78–88
16	104	72–90
17	97	64
18	97	77
19	102	77
21	105	69
22	102	57

Mean 100.7 mv; S.D. 3.4 mv.

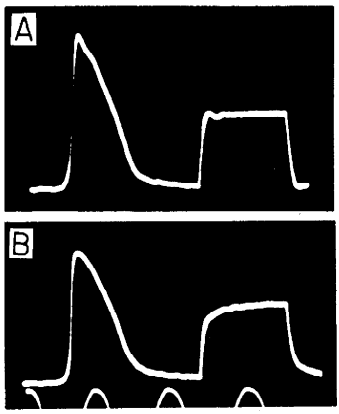

FIG. 4. Records showing the effect of changing the feed-back voltage upon the size and the shape of the observed single fiber action potential. Distal node was treated with KCl. Responses to the applied square pulses of 50 mv indicate that the feed-back was excessive in *A* and insufficient in *B*. Time marks 1 msec. apart.

difference in response illustrated in the upper and lower traces makes a difference in amplitude of recorded action potential of about 15%. We believe the variation in our results due to variation in proper compensation to be not more than 5% for these measurements.

The results of seven out of eight experiments performed at 14–17°C are shown in table 2. (Preparation 3 showed intermittent conduction block and so was excluded from the table.) The mean of the seven values is 101.6 mv with a standard deviation of 4.8 mv and a maximum deviation of 8.4 mv. The duration of these action potentials was 2–3 msec.

DISCUSSION

Problem of Reversible Conduction Block. Soon after Huxley and Stämpfli (12) published in 1949 their observation of a reversible block of nerve impulse conduction across the insulated region of a single myelinated fiber, Yamada and Maruhashi in Tokyo (personal communication) re-examined this observation. They concluded that what had been observed by Huxley and Stämpfli was the partially reversible damage caused by excessive electrical currents in the fiber picked up electrostatically when the pools of Ringer's at either end were not shorted together (see RESULTS). The Japanese physiologists measured the threshold and the action current of the fiber before and after removing the shunting resistance between the two pools and observed that the block of conduction was always associated with a deterioration of action current. Tasaki and Schoepfle (unpublished) confirmed this conclusion of Yamada and Maruhashi in 1952, showing that conduction fails only after sufficient time has elapsed for damage to occur. Wolfgram and van Harreveld (13) also reported failure to demonstrate conduction block in a nerve fiber by increasing the external resistance across the internode. (In our experience a preparation having a fiber resistance of about 50 megohms may easily pick up more than one volt; and approximately 0.2 volts is sufficient to affect the threshold and action current of the fiber if maintained for more than a few minutes.)

Reversible conduction block by insulation was also reported by Frankenhauser and Schneider (14) in 1951. These authors recorded the action potential of the fiber with an amplifier of 15 megohms input resistance. They observed that, when the impulse failed to cross the gap, lowering the amplifier input resistance restored conduction. This is in rather striking contrast to the results reported here showing conduction with an amplifier-input of 1000 megohms and compensated input capacity (fig. 2*A*). Examination of their records (their fig. 4, top), however, indicates that the time required for the impulse to jump across the internode was far longer than the normal conduction time of about 0.1 msec. and therefore that the safety factor for internodal conduction must have been low. It is probable that when the safety factor for internodal conduction is just less than unity, a slight decrease in external shunting resistance will restore conduction no matter how low the shunting resist-

TABLE 2. PEAK ACTION POTENTIAL OF SINGLE NERVE FIBERS AT 14–17°C

Exper. No.	Max. Action Potential	Approx. Resistance
	mv	*megohms*
1	110	62
2	98	72
4	101	65
5	100	70
6	97	84
7	98	75
8	107	65

Mean 101.6 mv; S.D. 4.8 mv.

ance may have been. Thus the value of the external shunting resistance (or properly impedance) at which the safety factor for internodal conduction is unity becomes a measure of the condition of the fiber.

As mentioned under RESULTS, the fresh response of figure 2*A*, indicating conduction, can be transformed into one like that of figure 3*A*, indicating block, by simply fanning the preparation and presumably thereby drying the outside of the single fiber and increasing the resistance of the external shunt. The fact that conduction can be immediately restored by connecting the 22-megohm shunt across the fiber (fig. 3*D*), is, we believe, a clearer proof of the local circuit (electrical) theory of nervous conduction in the myelinated fiber than any previously reported evidence (2, 12, 14).

Even if the shunting resistance across a single fiber is infinite, a shunting capacity is sufficient to insure conduction. Utilization time for a stimulating pulse five times rheobase is 60–70 μsec. (11), so a shunting capacity of 2 or 3 μμf should be sufficient. A capacity of this magnitude or larger does exist between two pools of Ringer's carrying a single fiber preparation unless special precautions are taken to cancel it by capacitative feed-back.

A small pool of Ringer's carrying a small semitendinosus muscle (0.4 gm) held at a distance of about 1 mm from the edge of a fairly large, grounded pool shows a capacity across the gap of 3–4 μμf. It does not seem possible, therefore, to produce conduction block in a normal nerve fiber even with perfect insulation if muscular contraction is taken as the index of conduction. *Record B* of figure 3 shows that a small reduction in degree of cancellation of capacity equivalent to an increase of a few μμf is sufficient to restore conduction. This type of restoration of conduction was consistently observed.

Action Potential. As may be seen in figure 2, right, the action potential recorded by the present method has a very simple triangular shape quite similar to that of the axial action current (6, fig. 15b). The mean amplitude of about 100 mv is probably very close to the true nodal action potential under these conditions for the local currents of fig. 1*B* are all well controlled. Due to the geometry of the preparation *Ia* is of negligibly short time constant. Components *Ib* and *Ic* are made negligible by fanning as evidenced by the fact that there is a period when further fanning fails to change the recorded action potential while continuing to increase the resistance. Ic_2 is limited by the 1000-megohm input resistance of the amplifier and 1 or 2 μμf equivalent input capacitance after compensation. As drying proceeds the 1000-megohm amplifier input resistance eventually becomes an appreciable shunt load for the increasing fiber resistance and the recorded amplitude of the action potential begins to fall. However, since the calibrating rectangular pulse is also in series with the high resistance of the fiber the measured action potential is not affected until the frequency response begins to fall.

The results of the action potential measurements at low temperatures indicate a mean value of 101.6 mv which is actually slightly higher than the mean, 100.7 mv, obtained at higher temperatures. However, the difference is not significant and the experiments simply serve to show that within the precision of these measurements the nodal action potential is not affected by a 10°C change in temperature. The absence of the temperature dependence of the action potential amplitude is well known in other excitable tissues (squid giant axon (15); heart muscle (16)). The increase in duration of the action potential at low temperatures is also well known.

D.C. Potential of the Node. Attempts to measure the d.c. potential across the resting node have been reported by Tasaki (17) using a compensation shock test method (40–70 mv); by Huxley and Stämpfli (18) using compensation with external currents (60–71 mv); and by Sato and Fukuda (19), measuring the potential directly across the air gap (15–40 mv). As reported under RESULTS, application of isosmotic KCl to the distal node in these experiments produced a d.c. potential change of only 20–50 mv. Even when the d.c. change was only 20 mv the action potential was close to 100 mv. If the source of the d.c. voltage and the action potential are located at the same nodal membrane, the presence of any shunting resistance should affect them equally. Therefore the variation in d.c. potential must be due to a potential in series with the membrane potential which does not affect the measured action potential.

SUMMARY

A method was devised for recording the membrane action potentials of an isolated myelinated nerve fiber. The peak value of the action potential developed at the node of Ranvier of the bullfrog motor nerve fiber was between 97 and 110 mv. This action potential was found to be independent of temperature between 14 and 25°C. Clearer experimental evidence was obtained in support of the electric (local circuit) theory of nervous conduction.

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