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LOCAL RESPONSES IN SINGLE MEDULLATED NERVE FIBRES

BY J. DEL CASTILLO AND L. STARK

From the Department of Physiology, University College, London

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There is now good evidence that the membrane of medullated as well as non-medullated nerve fibres can be partially excited and give a non-propagated electric response to subthreshold stimuli. One way of showing this is to study the electric potential changes at the point of stimulation, where a small localized action potential can be demonstrated at the cathode, provided the stimulus is close to threshold strength. The phenomenon has been observed very clearly in isolated non-medullated axons (Hodgkin, 1937, 1938). In the case of medullated nerve, the evidence was for a long time of an indirect kind (Katz, 1937, 1939), and only in recent years has it been possible to overcome certain technical difficulties and establish the existence of local excitation in medullated nerve beyond dispute (Katz, 1947; Rosenblueth & Luco, 1950; Schoepfle & Erlanger, 1950, 1951*a, b*; Huxley & Stämpfli, 1951; del Castillo & Stark, 1951). It is important that the presence of a local action potential could be demonstrated by some of these investigators in the intact nerve trunk, so that we need not be seriously concerned with the argument that the phenomenon might be 'abnormal' and due to damage by microdissection or other special manipulation. It is still necessary, however, for a more accurate study of local action potentials to employ isolated nerve axons; this has been the purpose of the present work.

METHODS

Single myelinated fibres, isolated from the sciatic nerve of the frog *Rana temporaria*, were employed throughout this investigation. The fibres were dissected from a sciatic-gastrocnemius preparation in the manner described by Tasaki (1939) and modified by Stämpfli (1946*a*). A distal portion of the nerve trunk is reduced to a single motor fibre still attached to the muscle, which continues to twitch on stimulating the proximal end of the nerve. The single fibre is then placed across three pools of Ringer's solution separated by vaseline ridges similar to the 'ridge-insulator' of Tasaki (1939). Two vaseline threads (about 0.7 mm in diameter), obtained by squeezing vaseline from a syringe, are laid on a glass slide with their centres about 1 mm apart. By gently warming the vaseline they can be made to adhere to the glass surface. The vaseline ridges built in

this way provide an adequate electrical insulation between the pools of Ringer, the resistance between them being of the order of 10–15 M Ω . The isolated fibre is placed so that the central pool contains a single node of Ranvier (see Fig. 1). The internodes resting on the ridges are protected by small droplets of paraffin oil, no part of the fibre being exposed to the air. When setting up the preparation, the Ringer's solution surrounding the nerve fibre was frequently renewed in order to avoid tonicity changes due to evaporation. Later, the whole preparation was enclosed in a moist chamber provided with adjustable electrodes. The experiments were performed on winter and spring frogs, at a room temperature of 18–21° C.

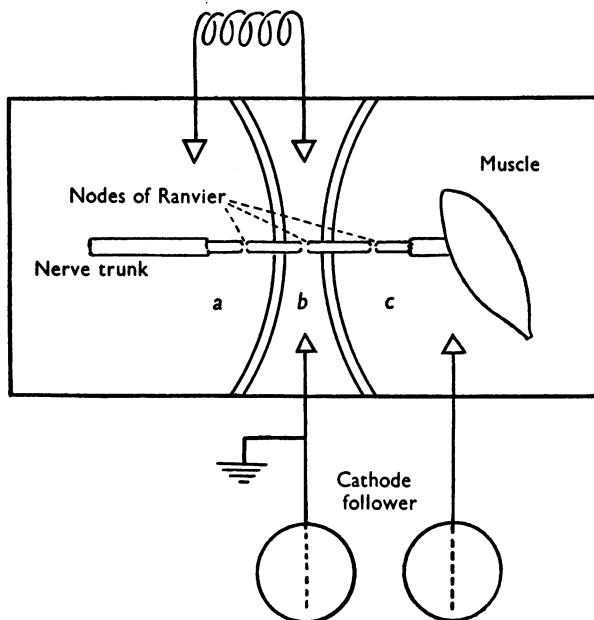


Fig. 1. Arrangement for recording potential changes at a single node of Ranvier of an isolated myelinated axon.

The part of the axon in the central pool (*b* in Fig. 1) was stimulated by means of platinum electrodes dipped into the pools *a* and *b*. Brief electric pulses, about 65 μ sec in duration at a frequency of 3–5 per sec, were delivered to the preparation via a shielded air-cored transformer and a reversing switch. The potential changes developed by the longitudinal action currents across the high resistance ridge separating pools *b* and *c* were amplified and recorded.

The recording apparatus included platinum electrodes, input cathode followers of low grid current and reduced grid-to-earth capacitance, similar to those described by Huxley & Stämpfli (1949), a d.c. amplifier, and a cathode-ray oscilloscope with attached camera. It has been unnecessary to use the recording system as a balanced instrument and one of the inputs was earthed. The other grid input of the cathode follower was connected with the electrode dipped into pool *c*.

Square pulse calibrations indicated that the time constant of the delay of the recording system varied in the different experiments and was rather long, due mainly to the high resistance of the preparation. The deflexion due to a square pulse delivered through the preparation and the electrodes reached 63.2% of its final value in about 0.12 msec. A further decrease of this time constant, down to 0.08 msec, could be obtained by a reduction of the stray capacitances of the electrode at pool *c* and by shunting the preparation by a 10 M Ω resistance.

The Ringer's fluid used in all the experiments was made up with the following ionic composition expressed in m.mole/l.: Na⁺, 115; K⁺, 2.1; Ca⁺⁺, 1.8; Cl⁻, 120.7.

RESULTS

If a brief cathodic shock of sufficient strength is delivered to the part of the axon occupying the central pool, a nerve impulse is elicited which causes the muscle to twitch. Its site of origin is the node of Ranvier, as Lillie (1925) suggested and Huxley & Stämpfli (1949) have demonstrated. At this point the active processes take place which give rise to propagated spikes in a medullated nerve fibre.

The action potential developed at this node rises almost instantly after the initial shock artifact, and is shown in Fig. 2A. Its duration (3–5 msec) is longer than that previously found by other authors in similar preparations (Huxley & Stämpfli, 1949; Frankenhaeuser & Schneider, 1951) and exhibits

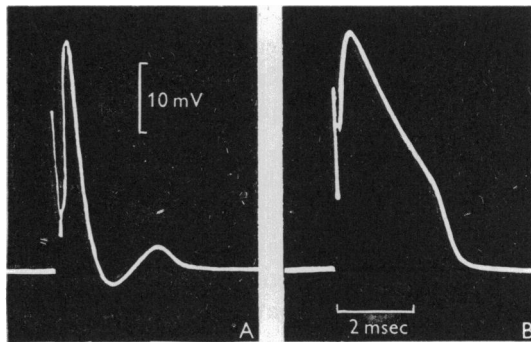


Fig. 2. Action potential at a single node of Ranvier as recorded with Fig. 1 arrangement. In B the nodes of Ranvier at pool *c* were blocked by procaine. (See text.)

a positive deflexion following the initial negative rise. In some cases, after this positive deflexion the potential may swing until reaching a negative value only slightly lower than the initial one, although usually, as in Fig. 2A, only a small negative wave of variable size marks the end of the action potential. It can be demonstrated that this positive deflexion is due to the activity of the adjacent node of Ranvier in the pool *c*. If this pool is filled with a 0.2% solution of procaine in Ringer's fluid, the positive deflexion disappears and the action potential becomes that represented in Fig. 2B. A comparison of both records shows that, under the present experimental conditions, the response given by the node in pool *b* is different both in shape and duration from the action potential recorded from the node in pool *c*. A satisfactory explanation of these results cannot yet be given. The form of the action potential originated at node *b* and the difference existing between this potential and that arising at the adjacent node at pool *c* does not seem to be due either to abnormal changes in the tonicity of the Ringer's solution in contact with the fibre or to the high

value of the external resistance between the adjacent nodes. Further investigations are, therefore, required. It is, however, important to note that the local response, as recorded from this preparation, did not significantly differ either from those recorded from non-medullated axons or medullated nerve trunks. All the experiments have been performed in preparations in which the nodes in pool *c* have been blocked by procaine; in this way, the positive deflexion, which occurs very soon after the initiation of the action potential (about 0.5 msec), is excluded from the records. Furthermore, control experiments have shown that application of procaine to these nodes has no appreciable influence on the action and subthreshold potentials recorded from the central pool, at least during the first few hours.

If the strength of the stimuli is decreased, the action potentials begin to rise with an increasing delay and eventually they disappear, being replaced by small subthreshold non-propagated potentials.

As has been previously demonstrated in non-medullated axons (Hodgkin, 1938) and myelinated nerve trunks (Katz, 1947), these subliminal potentials can be regarded as a combination of passive depolarization potentials and a local response of the nerve membrane. Its dual nature becomes apparent by varying the strength and polarity of the applied subthreshold shocks.

In Fig. 3 are represented the potentials which follow shocks of various strengths and opposite polarity. Cathodic shocks of weak intensity and anodic shocks of any subthreshold strength give rise to potentials of similar shape, which decay in a simple, approximately exponential, fashion. When the stimuli approach threshold, the cathodic potentials change in a characteristic way: their duration increases and a hump appears, the curve becoming rounded as seen in Fig. 3. The anodic shock of the same strength still produces a potential of simple exponential form, similar to that elicited at the cathode by weak shocks.

If the subthreshold potentials are measured at a given time after the beginning of the stimulus and plotted against stimulus strength, a curve is obtained which is approximately linear over a range comprising anodic and weak cathodic shocks, and bends up from about 0.5 threshold cathodic strength (Fig. 4). This curve gives an indication of the size of the local response at different stimulus strength. It is difficult to determine exactly where the subliminal response begins to appear over and above the polarization potential, since the departure from linearity is extremely gradual. Above 0.6 threshold, however, the response is quite clear, increasing at accelerating rate until it reaches propagating size.

When the strength of the cathodic shock approaches threshold, i.e. when the resulting potential almost reaches propagating size, the subliminal responses begin to vary in both amplitude and duration; successive stimuli of the same strength produce a random display of potential changes. This is particularly

evident in Fig. 5A. It can be seen that successive shocks of 0.87 threshold produce potentials which vary in size by as much as 20%, measured at 0.2 msec after the beginning of the shock artifact. With higher stimulus strength the fluctuation of the potentials becomes even more marked. Fig. 5B

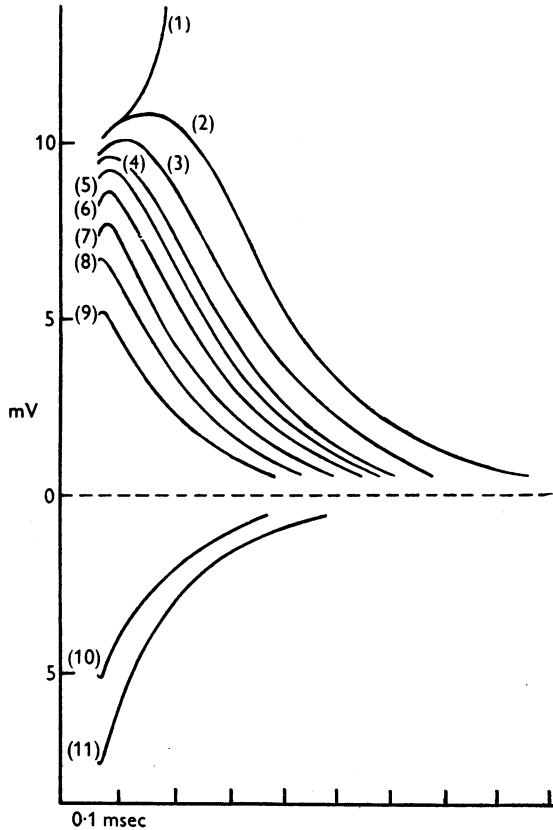


Fig. 3. Tracings of potential changes at a single node of Ranvier (at pool *b* in Fig. 1) following stimulation by brief electric shocks. Ordinates: potential in mV. Broken line: base line, zero potential. Abscissae: time in 0.1 msec. In superimposing the records the stimulus artifact was taken as the common point, starting at zero time. The records were obtained with the following stimuli strength, expressed as percentage of threshold from above: cathodic, (1) action potential and (2) 100, (3) 95, (4) 92.5, (5) 90, (6) 87.5, (7) 81, (8), 76, (9) 65; anodic, (10) 65 and (11) 100.

illustrates the effect of twenty successive shocks of just threshold strength; nearly one-half of the responses succeeded in growing up into propagated action potentials, whereas the rest died out as local non-propagated responses whose amplitude, measured at 0.35 msec after the beginning of the stimuli varied by as much as 60%. Fig. 6A shows local responses whose amplitude and duration varied very much; they were obtained in a preparation which

had been stimulated during several hours. This fluctuation of the subthreshold responses seems to be more pronounced in these medullated fibres than in the non-medullated axons studied by Hodgkin (1938) where at strength of stimuli up to 0.96 the responses varied by less than 1%.

The transition from local subthreshold responses to propagated action potentials seems to occur at a fairly constant level. This is particularly clear in Fig. 5 B. It can be seen in this record, how action potentials rise, with different

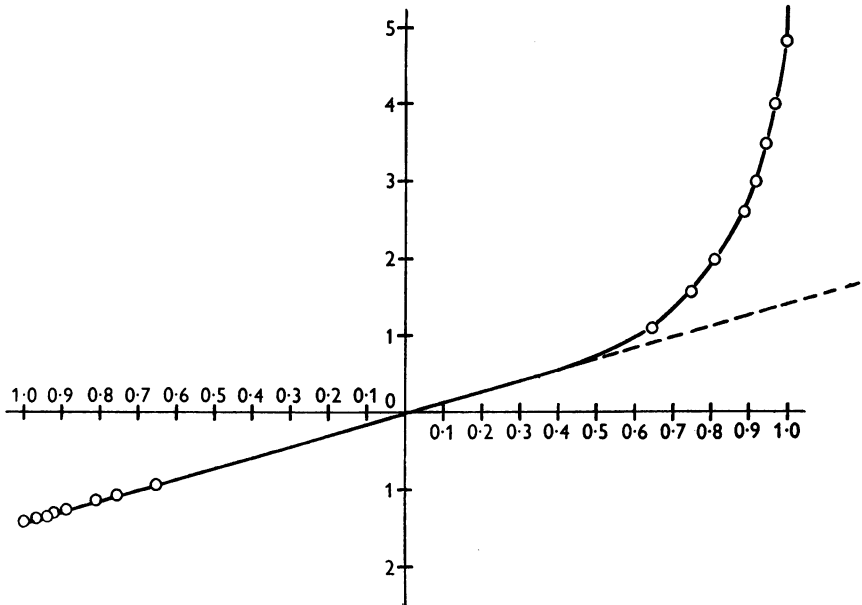


Fig. 4. Ordinates: potentials elicited by anodal and cathodal stimuli of different strengths as measured at 0.3 msec after the initiation of the shock artifact (same experiment as in Fig. 3) expressed in arbitrary units. Abscissae: strength of the applied shocks taking threshold as unity.

latencies (0.12–0.4 msec from the beginning of the stimuli), all at a constant critical level. Hodgkin (1938) has shown, in crustacean axons, that both propagated and local responses start out in the same way and may not separate until nearly 500 μ sec after the beginning of the shock. This observation has been confirmed in our preparations. It was particularly evident at the end of long experiments when the ratio between the height of propagated spikes and that of the local responses had become very small (see Fig. 6 B). All the responses which exceed the critical level turn into propagated impulses. In some cases, however, the potential has remained at the critical level for about 0.6 msec after it was attained, before growing up into a propagated spike.

It is not possible to give an exact figure for the relative amplitudes of spike and local response elicited by just threshold stimuli, i.e. for the ratio of the spike height to the critical potential level from where the propagated potentials rise. This ratio varies very much with the condition of the fibre and declines slowly during the experiments. The time course of its decay also depends on

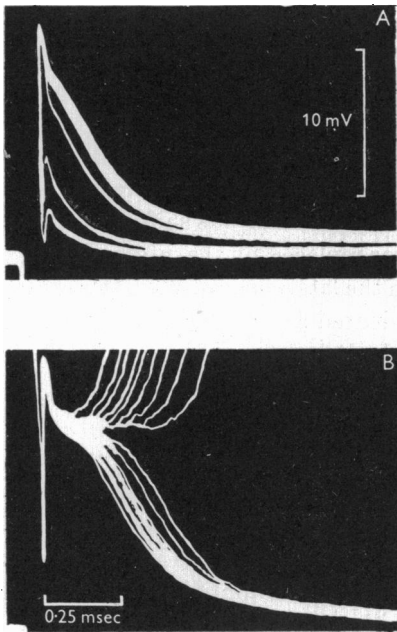


Fig. 5

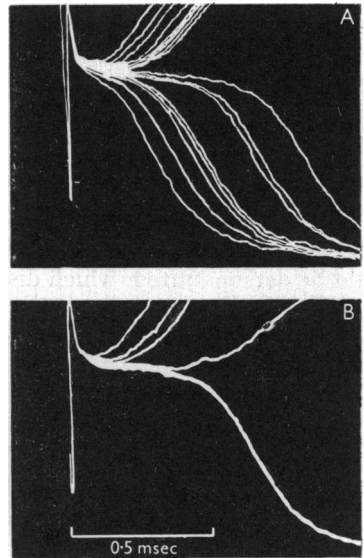


Fig. 6

Fig. 5. A: subthreshold potentials evoked at a single node of Ranvier by brief cathodic shocks of increasing strength. Each line results from the superimposition of about ten potentials. Relative strength of stimuli in order of increasing magnitude of the potentials, expressed in percentage of threshold strength: 21, 43, 76, 87. B: effect of twenty successive shocks of just threshold strength. Nearly half of them succeeded in eliciting propagated spikes. The rest produced potentials which died out as local, non-propagated, responses of fluctuating amplitude.

Fig. 6. A and B: local responses and propagated spikes (initial part) elicited at a single node of Ranvier by cathodic shocks of just threshold strength.

the condition of the fibre and the experimental treatment. This ratio has usually been of the order of 4; this figure, however, refers only to the average condition of the fibres which have been employed; the highest ratio which has been recorded was about 7. In the observations of Schoepfle & Erlanger (1951*b*) on the phalangeal nerve preparation of the frog the average ratio of the height of the humps to the height of the spikes was of 4.8, a figure comparable to that found in our experiments. They found, however, cases where the

magnitude of the spikes was about 10 times higher than that of the hump. This indicates that the condition of some fibres in their phalangeal nerve preparation was better than that of our isolated axons.

After a preparation has been kept for several hours the action potentials become so small that conduction eventually failed. The difference between action and subliminal potentials becomes gradually smaller until only local responses, i.e. 'humped potentials', can be elicited by shocks of any strength. Eventually, the 'humps' disappear and only simple exponentially decaying potentials are left. A similar state can be reached after narcotizing the node in pool *b* with procaine, as it has been done in control experiments. After blocking, shocks of any size and polarity elicited only symmetrical electrotonic potentials in which no trace of local response could be demonstrated.

Experiments were also performed in which the axon was shifted longitudinally, so that the node in the central pool was replaced by the internodal region, the two adjacent nodes of Ranvier being in the lateral pools. With this arrangement only action and local potentials originated at the nodes in *a* and *c* pools could be demonstrated, which disappeared when these nodes were blocked by procaine.

DISCUSSION

The experiments which have been described demonstrate the occurrence of local, non-propagated, responses which arise, following subthreshold stimuli, at the nodes of Ranvier of isolated motor axons of the frog. The results are in complete agreement with the findings of Katz (1937, 1947) and Hodgkin (1938), and confirm the observations of Huxley & Stämpfli (1951). The local responses recorded from isolated amphibian axons compare very well with those which take place in crustacean nerve fibres and those recorded from intact nerve trunks. They are also analogous to the 'kleiner Buckel' observed by Tasaki & Takeuchi (1942) at the cathode on similar single fibre preparations, and although these authors regarded the phenomenon as an adventitious artifact we feel that its identification as a subliminal membrane response can no longer be reasonably questioned. As Huxley & Stämpfli (1951) point out, the 'unterschwelliges Reizphänomen' described by Stämpfli (1946*b*) can be similarly identified with a local subliminal response. There is also very good agreement between our observations and the local responses described by Schoepfle & Erlanger (1950, 1951*a, b*) in single fibres of the phalangeal nerve of the frog, although the method followed by these investigators is different from that employed in the present work.

SUMMARY

1. The potential changes elicited by subthreshold stimuli (brief electric shocks) at single nodes of Ranvier of motor axons isolated from the frog sciatic nerve have been studied.

2. Local, i.e. non-propagated, responses can be demonstrated following cathodic stimuli of near threshold strength.

3. These local potentials compare well with those previously recorded from single invertebrate axons and with the local responses demonstrated in medullated nerve trunks of the frog. They are also similar to the local responses of single nerve fibres as recorded from multi-fibre preparations.

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