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THE SITE OF EXCITATION IN THE NERVE TRUNK OF THE FROG

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When the sciatic nerve of a frog is stimulated through one pair of electrodes and the action potential is recorded at another pair, then upon reversing the direction of the stimulating current, the arrival time of the recorded impulse will be changed. This obviously is related to the fact that excitation occurs at the cathode, so when the stimulus is reversed and the cathode is shifted from one electrode site to the other, the conduction distance to the recording leads is changed. If this explanation is complete, when the electrodes are extremely close together, the latency change should correspond to the shift in excitation site only from one Ranvier node to the next, say 1 mm.

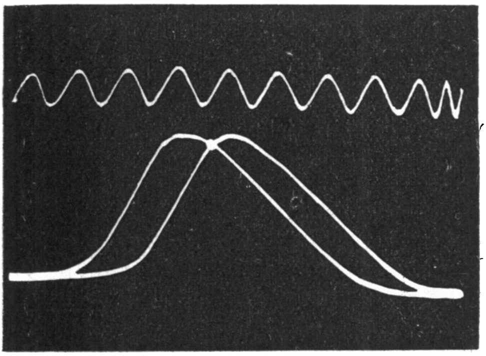

Fig. 1. Action potentials obtained by reversing the stimulating current through electrodes 1 mm. apart. Time scale: 5 kcyc. The latency change corresponds to 6 mm. difference in conduction distance.

Fig. 1 shows the result of reversing the stimulus applied through electrodes about 1 mm. apart. The two action potentials are seen to differ by about 0·2 msec. in their time of arrival, which corresponds (at the conduction velocity obtaining) not to 1 mm. but to 6 mm. shift in the site of excitation. Obviously there are two main explanations possible. (*a*) Despite the proximity of the electrodes, the sites of origin of the impulse are in fact separated by 6 mm. (*b*) The impulse starts from the manifest cathode in each case, but does not travel at the same speed. It will be observed that when the cathode is nearer the recording leads, the impulses must traverse nerve which has just been catelectrotonic. The recorded latency difference, therefore, might be the result of faster conduction through (recently) catelectrotonic nerve than through nerve (recently) anelectrotonic.

The present paper attempts to decide between these two possible explanations, in Part I by locating the site of the stimulus, in Part II by locating the site of origin of the action potential.

PART I. THE SITE OF THE STIMULUS

The possible explanations (*a*) and (*b*) above may be distinguished by an experiment which in principle is as follows.

The nerve is stimulated through a close pair of electrodes *X*, Fig. 2, with the anode towards the recording site. As seen in Fig. 1, if the current direction is reversed, the shock-spike interval will be reduced, so the electrodes may now be shifted to the left and a new position *Y* found where the increased conduction time exactly restores the shock-spike interval to its original value. Hence it will be possible to arrange two pairs of electrodes *X* and *Y* as shown such that each has the same shock-spike interval. So far this is a mere restatement of the prime observation, and does not discriminate between explanations (*a*) and (*b*). The distinguishing feature lies in the excitability of the nerve at various points.

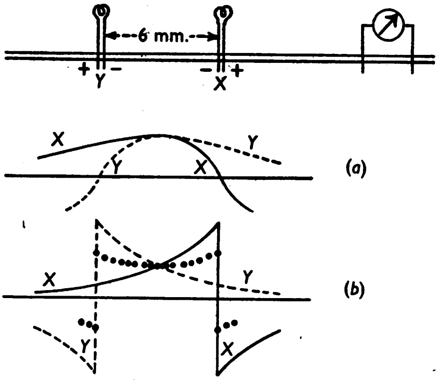

Fig. 2. Nerve excited by two electrode pairs adjusted to give the same shock-spike interval. Curves (*a*) and (*b*) indicate the excitability distribution to be expected if the excitation site is (*a*) 3 mm. extra polar, (*b*) exactly at the cathode.

According to explanation (*a*) the excitability is greatest not at the cathode, but at a point some 3 mm. away. So, if directly below the nerve in Fig. 2, a curve is represented showing the excitability of each point due to the stimulus

X alone, a relation such as the continuous curve XX, Fig. 2 (*a*), must be expected with a maximum 3 mm. to the left of the electrodes X. Similarly, the excitability due to Y alone will be given by the broken curve YY in the same figure. According to this explanation the maxima of the two curves have been adjusted to coincide, and that is why the shock-spike intervals are found equal. The critical consideration is as follows: Since X and Y each excite the same point on the nerve, their effects might be expected to be additive. In particular, if X alone is made threshold and then Y alone is made threshold, it should follow that $\frac{1}{2}(X+Y)$ should also be just threshold. For each stimulus would simultaneously contribute to the same optimum region of membrane half the current necessary just to excite it.

It would be quite otherwise with explanation (*b*). Here the excitability is maximal at the cathode and falls away with distance as shown by the curves XX and YY, Fig. 2 (*b*). But since in this case X and Y act at different and rather distant points, their simultaneous application will not be additive. For, if X and Y are each separately adjusted to threshold, then their average $\frac{1}{2}(X+Y)$, which is shown by the dotted curve of Fig. 2 (*b*), is seen hardly to exceed half-threshold at any point.

The question whether the locus of excitation is at the cathode or some 3 mm. away may be settled, therefore, simply by observing whether X and Y are additive in their effects as deduced from explanation (*a*), or nearly independent as deduced from (*b*).

METHOD

Trough. A modification of Lucas's fluid electrode was used to obtain small electrode separation with good symmetrical contact. The arrangement is shown in Fig. 3. The nerve was immersed in Ringer's fluid except for the peripheral end whence the action potential was recorded. AB and BC are silver boxes. The adjacent silver walls at B are cemented together separated by a thin layer of insulator, and a small hole is drilled through the middle, sufficient just to admit easily a frog's sciatic nerve. If the box AB is at one potential and BC at another, the change in potential of the nerve surface will be sharply localized in the region of B. So the electrodes X, Fig. 2, are realized by the hole in B, and the equivalent separation turns out to be about 1 mm. (see later Fig. 5).

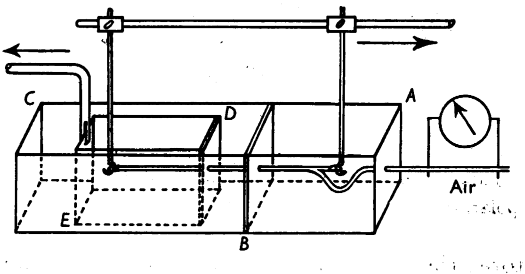

Fig. 3. Stimulating trough.

The electrodes Y, Fig. 2, result from a similar hole in the wall D of a third silver box DE. This is well insulated on the outside by paraffin wax, and the partition D is constructed, like B, of two separated silver plates, the outer of which is electrically connected to the box *BC*. A horizontal rack and pinion motion shifts the box *DE* so that the partition *D* slides over the threaded nerve. In this way the two partitions *B* and *D* may approach until their centres are 1 mm. apart. The setting is easily made correct to 0·1 mm. by means of a graduated circle on the pinion.

Preparation. The sciatic-peroneal preparation of the frog was used, dissected out in the usual way, monophasic records being led from the peroneal end, set up in moist air. The nerve was held by hooks applied to threads round the cut tibial branch and the largest of the cords of the sciatic plexus, the other cords being cut away to facilitate threading through the trough. These cuts were always far from the region stimulated. The hooks were mounted upon a second horizontal rack and pinion so that the nerve could be moved through the trough while leaving slack between the tibial hook and the recording leads. The locality chosen for excitation was the mid-thigh region which is fairly uniform.

Circuit. The box *AB* was kept at earth potential to reduce the stimulus artefact in the electric records. But it will be easier to appreciate the circuit if potentials are considered relative, not to earth, but to the potential of *BD*, since in this case the potentials of *AB* and *DE* will be proportional respectively to the stimulating currents *X* and *Y*, Fig. 2. The electrical circuit is seen in Fig. 4. A constant current pulse of a few tenths of a millisecond was passed through the primary of a step-down transformer, capacity-shunted so that the wave in the secondary was more or less square. The stimulus at *B* and *D* was adjusted by the potentiometers *X* and *Y*. The object of this experiment is to apply stimuli *X* and *Y* in varying proportions and to plot one against the other for a constant physiological result. This plotting was conveniently performed by a simple device. The plotting paper was wound upon a cylinder whose axle was fixed by a bush to the knob of a radio-type potentiometer *Y*. The setting of *Y* is thus performed by turning the cylinder. The potentiometer *X* was of the straight tubular type, fixed parallel to the cylinder axis in such a way that a pen, spring-hinged to the slider, could be pressed on to the paper. Since the lateral displacement of the pen represents *X* and the vertical (i.e. rotational) displacement of the paper represents *Y*, it is clear that the pen point plots *X* against *Y* in the conventional manner. One is saved, therefore, the labour, time and error involved in reading scales, and transferring figures to graph paper.

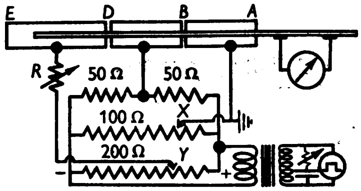

Fig. 4. Stimulating circuit.

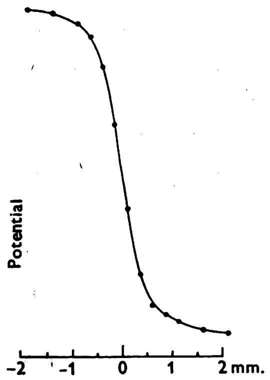

Fig. 5. Potential drop across slot *B*.

Calibration. The linearity of the potentiometer readings as plotted was compared with the readings of a precision potentiometer. The divergence was always less than 0·5 mm. in the figures of this paper.

The independence of the two settings was not quite perfect, for the potential difference across slot *D* depended slightly upon the setting of *X*, and similarly across *B* it depended slightly upon *Y*. This was measured directly by two electric probes in the appropriate fluid regions connected by cathode followers to a differential amplifier (responding only $\frac{1}{1000}$ to symmetrical inputs). The interaction between X and Y is exactly expressed by drawing the axes of the graph slightly oblique as in Figs. 6 and 8. Thus a nearly vertical line, exactly parallel to the Y axis, represents various values of the potential across D with a fixed potential drop across B. In order that an equal drop across B and D should be represented by an equal distance along the two axes, a suitable adjustment was made to the resistance R.

Potential of the fluid. In order to find how the potential varied in the fluid along the nerve in the neighbourhood of slot B, Fig. 3, an enamelled wire bare only at the tip was threaded through in place of the nerve, the motion being controlled by rack and pinion. In this way the potential of the tip could be found as it passed through the hole in B, using a null-point method of potential measurement. The electric probe was connected to the grid of a cathode follower, and the output was neutralized by an accurately measured fraction of the 'stimulus'. Fig. 5 shows the potential plotted as a function of the distance along the 'nerve'. As the recording tip entered slot B, the potential changed rapidly, and the greater part of the change is completed within the space of 1 mm. The mid-point of the slot was taken to be the place where the potential change was exactly half completed, and the distance between the slots was taken as the distance between their mid-points.

RESULTS

It will be remembered, from the discussion on Fig. 2, that the experiment consists in two determinations:

(1) The electrodes Y (i.e. slot D) must be moved to such a distance from X that the shock-spike interval is the same for both.

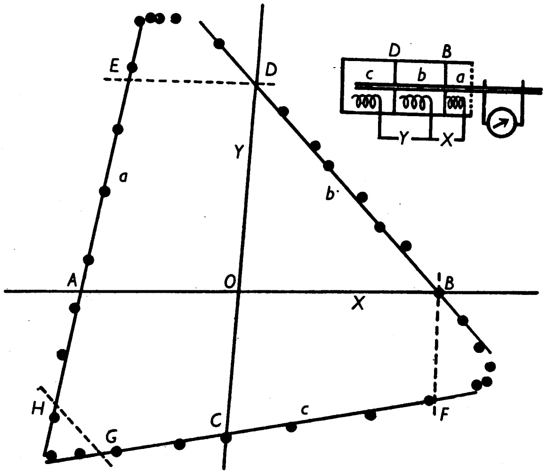

Fig. 6. Nerve excited by two slots B, D (inset) separated by 5·5 mm. At this separation the shock-spike interval was the same for excitation by X or by Y with cathode towards b (which is reckoned as the positive sign). The strength of X is plotted against Y for threshold excitation.

(2) The threshold for X alone and for Y alone is compared with the threshold for stimuli applied to X and Y simultaneously and in various proportions.

Fig. 7, frame (i) shows that condition (1) is approximately fulfilled when slot D is 5·5 mm. from B. The stimulus is a square pulse of 0·5 msec. duration starting at the downward 'escape' deflexion to the left of the record, and the action potentials are half maximal. The two superposed records are taken one from stimulus *X* only and the other from stimulus *Y* only and the two shock-spike intervals do very nearly coincide, as required by condition (1). If both *X* and *Y* are applied together in various proportions, the relation between them necessary for a half-maximal response is shown in Fig. 6, where *Y* is plotted against *X* in the usual way. The conditions of excitation are precisely as in Fig. 7, and indeed the two records of Fig. 7 are simply those from the points *B* and *D* on the curve of Fig. 6. For condition (2) of our experiment we are concerned only with the portion of Fig. 6 which lies in the first quadrant, i.e. the part between *B* and *D*. The expectations resulting from the alternative hypotheses (*a*) and (*b*) are as follows:

According to (*a*) the two stimuli *X* and *Y* both excite the same point of the nerve and hence they are simply additive. If, for example, *X* is 30% below threshold, then *Y* must be just 30% of threshold if the two combined are just to excite. Or, put graphically, if *X* is plotted against *Y*, the resulting threshold curve must be a straight line.

According to (*b*), on the other hand, the stimuli *X* and *Y* act at different and rather remote points (5·5 mm. apart in the present case) so that each stimulus would be fairly independent of the other. If they were quite independent, the curve between *B* and *D* would consist of two straight lines (the continuation of *ED* and *FB*) forming with the axes a 'square'. According to hypothesis (*b*), then, the experimental curve should approximate to this square.

It is clear, however, that the actual results of Fig. 6 fit the line *BD* rather exactly, and the 'square' not at all. The point *B* on the curve is the value when *Y* is zero and thus gives the *X* threshold for excitation at slot *B* (inset). Similarly, *D* gives the *Y* threshold for excitation at slot *D*. Intermediate points fall very close to the straight line *BD*. It may therefore be concluded that in the conditions of this experiment the site of excitation is about 3 mm. away from the manifest cathode, in the cathodal extrapolar region. Moreover, since Fig. 6 shows that the site of excitation is the same for points *B* and *D* on the curve, and Fig. 7, frame (i) shows that the shock-spike interval is the same for these two conditions of excitation, it follows that the conduction time is not appreciably influenced by passage through (recently) anelectrotonic or catelectrotonic regions of nerve.

The excitability curve

Before concluding Part I of this paper there are two further questions worth discussing. The first is this. In Fig. 6 only the portion of the curve between *B* and *D* has been considered. How is the rest of the curve to be explained? If it is assumed that the whole curve is a triangle, the interpretation is simple. The point *B* on the curve corresponds to stimulation by the slot *B* alone, which excites at the point *b* (Fig. 6, inset). Obviously the point *A* on the curve is just stimulation with this current reversed, which must excite at the point *a*. Thus the side *b* of the triangle represents stimulation at the nerve point *b*, and the side *a* presumably represents stimulation at the nerve point *a*. If point *a* were so far to the right that the stimulus *Y* could not directly affect it, then the side *a* of the triangle would be parallel to the *Y* axis. Actually it is somewhat inclined towards it, indicating that *Y* contributes about 10 % of its value to the point *a*. Analogous considerations indicate that the third side of the triangle stimulates at *c* on the nerve.

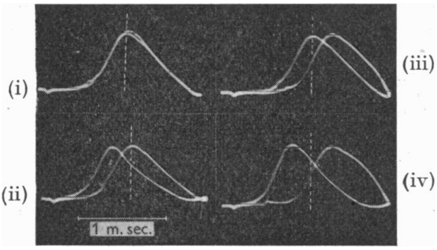

Fig. 7.

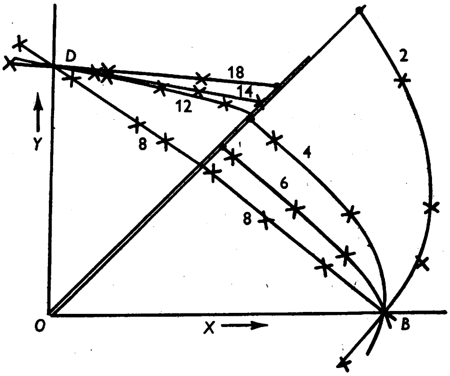

Fig. 8.

Fig. 7. Half maximal action potential records from the experiment of Fig. 6. Dotted reference line drawn perpendicular to base-line at 0·9 msec. after start of stimulus. Each frame gives superposition of two records from stimuli corresponding with the following points on the excitability triangle, Fig. 6: frame (i) *B* and *D*, frame (ii) *D* and *E*, frame (iii) *B* and *F*, frame (iv) *G* and *H*.

Fig. 8. Nerve excited by two slots as in Fig. 6. The curve relating *X* and *Y* at threshold is plotted for various slot separations indicated by the numbers (mm.). Only half the first quadrant of each curve is shown. Curve 8 in this nerve has the constant shock-spike interval.

This interpretation of the excitability triangle can be tested by measuring the shock-spike interval. For, since it has been seen that the velocity of conduction is not affected by the electrotonic changes involved, the latency of the response is simply due to conduction distance. It should therefore be expected that all stimuli lying upon one side of the excitability triangle should have the same shock-spike interval, but that this would jump suddenly to a new value on a different side of the triangle. This has been done in Fig. 7, where the vertical reference line has been drawn always 0·9 msec. after the beginning of the stimulus. Fig. 7, frame (i) shows that the latency is the same for *B* and *D*, and the impulse starts from the point *b*. Frame (ii) shows, superposed, the results of stimulating first at *D* (Fig. 6) and then at *E*. The record shows that *D* has again the latency of frame (i), and *E* has a latency about 0·2 msec. shorter, corresponding to a jump in the excitation point from *b* to *a*. Frame (iii) shows likewise an almost equal change of latency but in the reverse direction when excitation moves from *B* to *F* (Fig. 6), which corresponds to the increased conduction distance b to c. Stimuli at G and H should excite at c and a respectively, and hence the latency change in frame (iv) should be 0·4 msec. It is seen that this is approximately the case, and also that each curve in frame (iv) has about the same latency as the corresponding curve in (ii) and (iii). Thus frame (i) shows that there is no latency change along the side BD, and this last comparison of the remaining frames shows that there is no latency change along the other two sides. To a first approximation, therefore, the curve of Fig. 6 corresponds to excitation at these three different loci on the nerve.

Partition separation

The final question to be raised concerns a separation of the partitions B and D different from the critical value 5·5 mm. so far exclusively considered. According to the argument of this paper, the straight line BD, Fig. 6, is proof that X and Y at threshold each excite at the same locus b. If the slot D is shifted a little in either direction, this coincidence of loci will no longer occur. It is important therefore to be assured that the straight-line relationship obtains only for one critical value of electrode separation (namely where the two latencies are equal), otherwise the whole argument breaks down. This general assurance is given by Fig. 8, which is constructed from a series of complete excitability curves like Fig. 6, but each obtained with a different separation of slots B and D, as indicated (in mm.) by the number labelling each curve. If the nerve were uniform in every way, each curve would be symmetrical about the '45°' line through O. Also every curve would pass through the points B and D, because at these points one stimulus is zero, and hence it cannot matter how far or near it is. There is considerable variation from nerve to nerve in the value of the critical partition separation. In Fig. 6 it was 5·5 mm., the average value is perhaps 7 mm.; in Fig. 8 it was 8 mm., and the curve BD (8) is seen to be nearly straight. For compactness all the other curves have only one half plotted, since the omitted half was in fact more or less the reflexion in the '45°' line. Spontaneous changes in threshold during the determination of the whole set is compensated by scaling each curve to pass through B or D.

Turning to the results of Fig. 8, it is seen at once that the more the separation differs from the critical value 8 mm. either greater or less, the more the curves diverge from the straight line BD, and this always in the sense that threshold (=distance from O) becomes greater. This is precisely in accord with expectations, for since at 8 mm. separation the two excitation sites coincide, a movement of partition D in either direction will cause a separation of the sites and hence a less effective summation of the two stimuli. A detailed consideration of the shapes of the curve will not be undertaken here, but it will be noticed that it makes a good deal of difference whether the separation of loci results from the partition D being too close or too far. In the latter case (the upper set) each curve consists of a pair of nearly straight lines meeting (ideally) on the

'45°' line at an angle which approaches a right angle as the separation is increased. This is exactly to be expected, for as the electrodes become more separated, so Y receives a smaller and smaller fraction of X until at last it becomes quite independent, and the curve runs parallel to the X axis. Since in curve 8 the excitation sites coincide, in curve 14 they are situated 6 mm. apart, hence curve 14 shows the inclination to the X axis for a site separation of 6 mm. The side c of the triangle in Fig. 6 was interpreted as showing the same thing, since the distance between b and c was about 6 mm. The inclinations in the two figures are seen to be almost identical.

The curves for small-slot separation are much more complicated to interpret, and it will suffice here to make one observation concerning curve 2. Referring the description to the inset of Fig. 6, the site b will be 4 mm. from B (for the nerve of Fig. 8) and the distance BD 2 mm. for curve 2. Hence b lies on the anodal side of D and the effect of stimulus Y upon b is to *inhibit* excitation. That is why at the point B (Fig. 8), curve 2 is seen to run up to the right instead of up to the left. In curve 8, increase of Y involves a decrease in X, but in 2 increase of Y requires that X also be increased, i.e. Y inhibits.

The slope of each curve at B may thus be used to give information about the spread of excitability from slot D to the excitation site b. The method is developed in Part I of the next paper.

Enough has been said to indicate that the results of varying the position of the partition D are consistent with what went before.

PART II. LOCALIZATION BY DIRECT RECORDING

In Part I it was concluded that when the stimulating cathode lies very close to the anode, the impulse arises not at the cathode itself, but some 3 mm. distant on the cathodal side. This result was obtained by excitability measurements. It would be strengthened if the same could be found by recording the action potential directly at the origin of the impulse. It might perhaps be thought that it was impracticable to obtain any record from a nerve immersed in a large volume of saline, especially at the site of stimulation, but, by introducing electrodes within the connective tissue sheath (epineurium), quite large action potentials can be obtained. Their interpretation proved more difficult.

There has always been difficulty and sometimes confusion in the interpretation of experiments where one electrode was in contact with a nerve or muscle more or less immersed in fluid, and the other electrode in the fluid. This arrangement therefore was not attempted.

In the first experiments two fine insulated electrodes were inserted with their bare tips separated by 1 mm. along the axis of the nerve. The idea was to distinguish an impulse starting at the right of the tips from one to the left by noting the inversion of the recorded wave. This distinction is valid enough when the impulse runs uniformly past the electrodes in one direction or the other.

But when the impulse starts at the recording leads, both the electrodes become negative together (relative to a distant point), and that which is observed, namely the potential difference between them, is negligible. What appears in the record, therefore, is simply a long 'latency' (which in fact is simultaneous activity under both electrodes) followed by a wave of one sign or the other depending upon the direction in which the wave spreads faster. This obviously is a most misleading type of record for it strongly suggests that the nerve is quiet when in fact it is active, and that the impulse has arisen from a rather distant point when actually it starts at the recording leads. Something less paradoxical is needed.

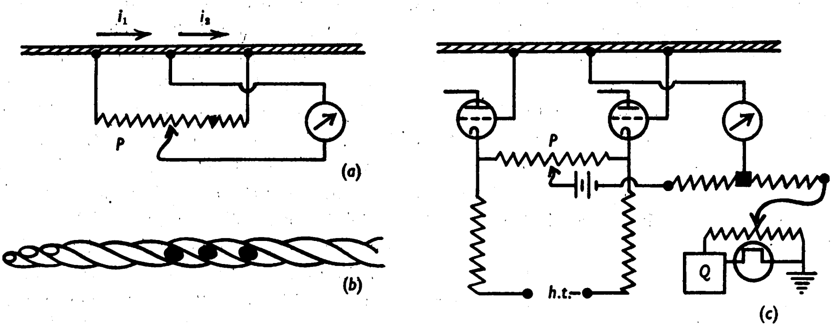

Fig. 9. (*a*) Diagrammatic circuit to measure (i_1–i_2). (*b*) Three fine insulated wires twisted together. The insulation is scraped bare on each wire at the black patch. Not at all drawn to scale. (*c*) Actual recording circuit to measure nerve membrane current. To avoid polarizing the bare areas (*b*), each lead goes to a valve grid and draws no current. Q is a suitable capacity network to balance out stimulus 'escape'.

Now the plainest property of a nerve element which indicates whether the nerve is passive, active in the element, or active in a neighbouring element, is the membrane current. This is the current which leaves or enters the nerve through its membrane (chiefly at the nodes of Ranvier). If the nerve is passive, this current is proportional to the stimulating current, and may be balanced out by a suitable capacitative network. In activity there is a local circuit where the current is inwards in the active elements and outwards in the neighbouring elements. The appearance, therefore, of an inward or an outward membrane current in records from a balanced network, signifies activity either in the recorded element or in the neighbouring region, respectively.

The membrane current may be measured by the tripolar recording arrangement shown schematically in Fig. 9(*a*). If current is passed down the nerve from an electrode far to the left of the figure to one far to the right, there will be no membrane current in the middle region where the recording electrodes lie, and (i_1–i_2) is zero. The potentiometer P is adjusted to give null deflexion in this case, and with this setting it is clear that, no matter what be the conditions of stimulation, the deflexion will always be proportional to (i_1–i_2).

Now this quantity is made up of two parts, the contribution from the stimulus, and the contribution from the action potential. The former may be eliminated from the records by using a subthreshold stimulus and feeding some fraction into a network so constructed that in the record (i_1-i_2) is exactly balanced out. The output is found to remain zero for all stimulus strengths below threshold. Above threshold the records show simply the membrane action current, and the critical question whether this is inwards or outwards is answered by noting whether the initial deflexion is up or down.

METHOD

The stimulating trough was the same as in Fig. 3, but as only one stimulation site was needed, the trough *DE* was removed. The recording leads were three fine enamelled silver wires No. 46 s.w.g. They were twisted together to form a 3-strand wire thread, and cut off obliquely to form a pointed tip (Fig. 9*b*). The whole was painted with shellac varnish, and the tip in particular was well insulated. The insulation was found to be satisfactory by testing between one strand and the next, and between the three strands together and the saline in which they were immersed. Now a small bright area was exposed by scraping away the insulation for about 0·2 mm. on the side of each wire. The three exposed areas were at intervals of 1 mm. along the axis of the wire thread and about 5 mm. from the insulated tip (see Fig. 9(*b*), which is not at all drawn to scale).

The frog's sciatic nerve was dissected out and set up upon a plane mirror so that, viewed through a binocular microscope (× 7), by reflected light, both the upper and the lower (reflected) surface could be simultaneously observed. The epineurium was pierced by a needle near the nerve junction at the knee, and the electrode inserted. The wire thread was always held in forceps lined with soft material to avoid damage to the insulation. The wires were carefully passed up the nerve within the sheath until the tip reached the cut branch to the hamstring muscles at the top of the thigh. It was fixed in this position by a ligature round the nerve with the contained wires near their point of entry. The nerve was then threaded into the stimulating trough with care to avoid much bending or relative movements between the nerve and the wires. Nevertheless, it is certain that considerable damage to the nerve fibres must have been done by this crude electrode insertion, and the action potential from the whole nerve distally was in fact rather small. The results to be described, however, were consistent and could be repeated for many hours. It was on account of the deformation of some fibres by the electrodes that the bright recording areas were placed some 5 mm. from the tip. For otherwise the region measured, instead of being uniform, would have been the junction between crushed and normal nerve, which might complicate the interpretation of the records.

Recording circuit. It is essential that no current should be drawn from the small bare areas of the recording electrodes, or they would become strongly polarized. They may not, therefore, be connected directly through the potentiometer as shown in Fig. 9*a*. The actual arrangement is shown in Fig. 9*c*. The middle electrode goes direct to the grid of the first amplifier valve (without grid leak) and the other two electrodes to cathode followers so that the potentiometer *P* may still act as in Fig. 9*a*, but without drawing electrode current. The output from *P*, suitably biased, is fed to the input of the amplifier, which may also receive a small fraction of the stimulating current either as a direct square wave, or as modified by the condenser circuit *Q*. The object was to balance out as perfectly as possible the 'stimulus artefact' and in the experiment of Fig. 10 all subthreshold records were nearly horizontal base-lines, except for the brief capacity spikes occurring at make and break of the square wave stimulus.

Procedure. (*a*) The amplifier was made truly differential by connecting all three electrodes together, varying their common potential relative to earth, and adjusting to zero any resulting output. (*b*) By a slight modification of the stimulating conditions a current pulse was passed uniformly in the fluid down the trough. The recording site was in the middle of this region, and the potentiometer P was adjusted to give no output. (c) The record now registers (i_1-i_2), and it is only necessary to ascertain the sign of the deflexion. This is found by leading a negative pulse into the fluid from a wire inserted near the middle recording lead. The downward deflexion which resulted provides the key for interpreting Fig. 10, for downward deflexion there must signify inward flowing membrane current, and hence membrane activity under the middle recording lead. (d) The stimulating trough was restored to its previous form, and action potentials recorded when the stimulating slot was in various positions relative to the nerve with its contained electrodes. Under the dissecting microscope the position of the wire tip could be directly observed through the epineurium, and the middle recording area was known to be 6·25 mm. from this. Thus the distance of the leads from the stimulating slot was exactly known. For each position the stimulating current was increased until just subthreshold. There was always some stimulus artefact, often a very large one, but this could always be exactly balanced out by suitable adjustment of the capacitative network Q (Fig. 9c).

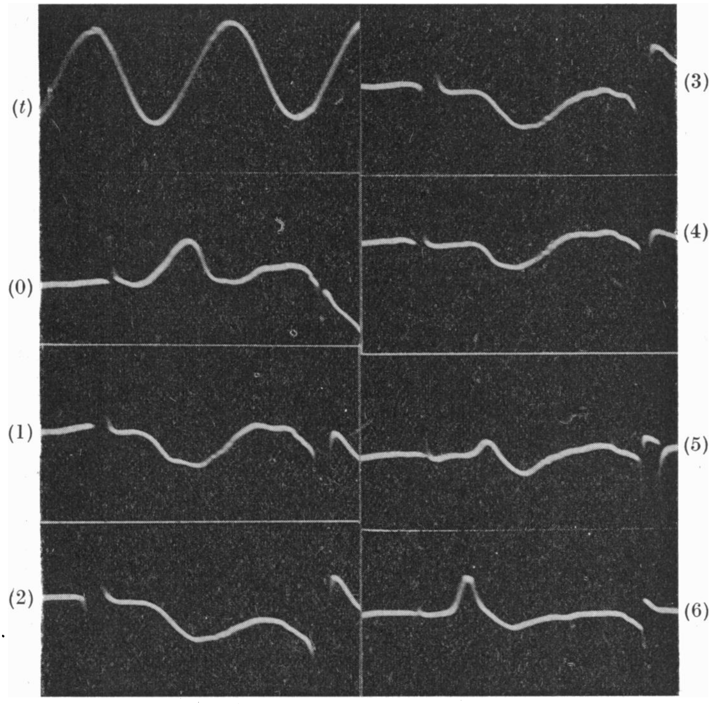

Fig. 10. Records of nerve membrane current obtained from the site of origin of the impulse when the nerve was immersed in a Ringer bath. Frame (t) 1 kcyc. Frames (0)–(6) records when the middle lead (Fig. 9c) was 0–6 mm. respectively from the centre of the stimulating slot. Downwards deflexion signifies inwards current, hence active nerve. The nerve becomes active simultaneously over the stretch 1–4 mm. (incl.) from the slot.

RESULTS

Fig. 10 shows a set of records taken at various distances from the stimulating partition. The stimulus is a square pulse of duration about 1·5 msec. The start and finish are accompanied by a capacitative spike, which does not seriously complicate the record. Apart from this all subthreshold responses were almost exactly horizontal lines at least for the first millisecond. The earliest detectable threshold responses were always in the same direction as the half-maximal responses of Fig. 10, though the latency was naturally longer because of the greater utilization time of the weaker stimulus. Strong stimuli, on the other hand, sometimes inverted a wave. This was doubtless due to the strong current spreading and exciting directly a region which formerly was only brought into activity by the propagated impulse.

Turning to the details of Fig. 10, frame (*t*) shows the time-scale of 1000 cycles. Frames (0)–(6) were obtained when the middle recording lead was 0, 1, 2, 3, 4, 5, and 6 mm. respectively from the centre of the stimulating slot. It is seen that when the distance is 1, 2, 3, 4 mm., the current crossing the nerve sheath starts downwards, i.e. in the inwards direction. So over this region the nerve becomes excitable at once, and the current flows out in the remoter regions seen at 0, 5, and 6 mm., and also beyond this, not here reproduced.

Conclusions. The results of this experiment stand in fair agreement with those of Part I. In view of the distortion due to electrode insertion some quantitative discrepancies are not surprising, and will not be discussed.

The main conclusion seems supported by both lines of investigation, for the impulse starts some 3 mm. from the manifest cathode in every case.

SUMMARY

1. It is found that when a nerve is excited through a pair of electrodes 1 mm. apart, the shock-spike interval depends upon the direction of the current. We should expect a change in conduction time due to 1 or 2 mm., but the change found corresponds to 6 or 8 mm. Is this due to the excitation site not being located at the manifest cathode, or to the conduction velocity not being the same in the two cases?

2. Two pairs of electrodes are arranged (Fig. 2) with opposite polarity to give the same shock-spike interval. If they really act both at the same place on the nerve, then the two stimuli will be additive in all proportions. If each acts at its manifest cathode the two will be more or less independent. They are found to be nearly exactly additive. Thus the locus of excitation is some 2 mm. or more from the manifest cathode, and conduction velocity is unaffected.

3. This conclusion is confirmed by recording the action potential at the excitation site. This was done in a nerve immersed in Ringer's fluid by passing the recording leads up within the epineurium. Excitation at threshold arose simultaneously over the whole stretch of nerve extending from the cathode for a distance of 3 mm.