# NATURE OF THE ACTION CURRENT IN NITELLA

## II. Special Cases

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In the first paper of this series the general features of the action curve of *Nitella* were interpreted as due to the movement of potassium ions accompanied by an increase in permeability.

Can this idea explain the variations which the action curve exhibits? For this purpose we may consider the successive movements. We may begin with a fairly typical action curve[1] such as is shown in Fig. 1. This was obtained by leading off as shown in Fig. 2 (the stimulus consisting of an outgoing electrical current at *B*). In this, as in all other records shown in this paper, the common contact *F* was killed with chloroform (unless otherwise stated), making the records monophasic. Inasmuch as curves like those shown in this paper have been obtained with cells in which no spot had been killed or injured it is evident that these forms of the action curve are not influenced by the killing of *F*.

*The first movement* (*o*, Fig. 1). If this is due to the passage of $K^+$ across the inner protoplasmic surface *Y*, causing a loss of P.D., its speed and extent will depend on the rate of movement of $K^+$ and on the uniformity of behavior of the region which is being recorded.[2] If at one spot $K^+$ begins to move rapidly the start of the *o* movement will show corresponding speed but the latter part of the movement may be slow because the process will be incomplete until *Y* reaches the same state over the whole region involved. Before this happens $K^+$ may reach the outer non-aqueous layer in some part of the region

[1] We regard as fairly typical an action curve in which both the first and second peaks approach zero but there is a good deal of variation in this respect.

[2] Very frequently we lead off from an area of cell 1 cm. in length all of which is in contact with the solution.

so that a positive potential may be built up at $X$ which will prevent the $o$ movement of the curve from reaching zero (such a positive potential may exist from the start: this will be discussed in a later paper). Furthermore, if $K^+$ moves so rapidly[3] across $W$ that its concentration at the outer surface of $Y$ does not reach the same level as at the inner surface the $o$ movement may not go to zero.[4] The curve sometimes lacks a good deal of going to zero as shown in Fig. 3, but in general the approach to zero is closer than this.[5]

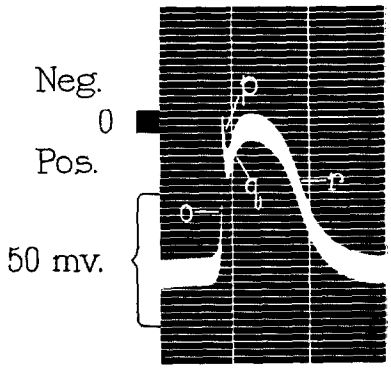


FIG. 1. Photographic record showing a fairly typical action curve in which the $o$ and $q$ movements go to zero. The experiment was arranged as in Fig. 2, employing contacts $C$ and $F$ only. $F$ was killed with $CHCl_3$ before the record started to secure monophasic recording at $C$. $C$ was in contact with 0.001 M KCl. The cell had been kept in tap water before use. The vertical marks are 5 seconds apart. Temperature, 23°C.

When the positive P.D. in the resting state depends in part on a positive P.D. across the outer protoplasmic surface, $X$, the $o$ movement will not go to zero since the outward movement of K (which

[3] The time required for the movement varies according to the thickness of the layers and the degree of stirring produced by the protoplasmic motion (which is always present in *Nitella*).

[4] When the action curve has a single peak and the $o$ movement does not go to zero the cause may be different. This will be discussed in a subsequent paper.

[5] When we record the P.D. of a spot $A$ with reference to another spot $B$ in contact with chloroform the action curve of $A$ sometimes appears to go beyond zero and become negative, but this is regarded as due not to negativity at $A$ but to positivity at $B$.

causes the loss of the P.D. across $Y$) will not cause the P.D. across $X$ to disappear at once. As $K^+$ moves on across $W$ to $X$ it will increase the positive P.D. across $X$, thus causing the $p$ movement. When it reaches the outside of $X$ it may cause the P.D. across $X$ to disappear, thus carrying the $q$ movement to zero, or nearly to zero, as in Fig. 3.

As previously explained[6] there may be a breakdown (partial or complete) in the $Y$ layer which might cause the $o$ movement quite independently of any migration of $K^+$.

*The second movement* ($p$, Fig. 1). If $K^+$ traveled outward in the form of a sharply defined moving boundary we should expect a sudden increase of positive P.D. when it reached the inner surface of $X$ and this might carry the $p$ movement back to the starting point of the

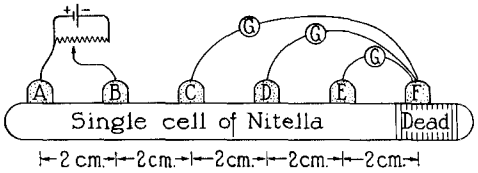


FIG. 2. Arrangement for testing *Nitella* cells. *GGG* represent string galvanometers with vacuum tube amplifiers, arranged essentially as short period electrostatic voltmeters. For details see Osterhout, W. J. V., and Hill, S. E., *J. Gen. Physiol.*, 1933–34, **17,** 87. Absorbent cotton, moistened with the contact solution, connected the cells to saturated calomel electrodes: between contacts the cell was surrounded by air.

action curve if $K^+$ produced as much effect on $X$ as on $Y$. This seldom happens but an instance in which it goes down a good way is shown[7] in Fig. 4 and one in which it goes back to the starting point is seen in Fig. 5.

The fact that the $p$ movement does not usually descend very far is not surprising as the moving boundary tends to lose its sharpness in passing across $W$ since this is a comparatively thick aqueous layer[8] which, in *Nitella*, is continually agitated by protoplasmic motion.[9]

[6] Osterhout, W. J. V., *J. Gen. Physiol.*, 1934–35, **18,** 215.

[7] In these cases the thickness of the protoplasmic layer $W$ and the protoplasmic motion in this layer were probably smaller than usual.

[8] The total thickness of the protoplasmic layer is not much more than $10\mu$: the layers $X$ and $Y$ may be very thin.

[9] This may be true in lesser degree of *Valonia*.

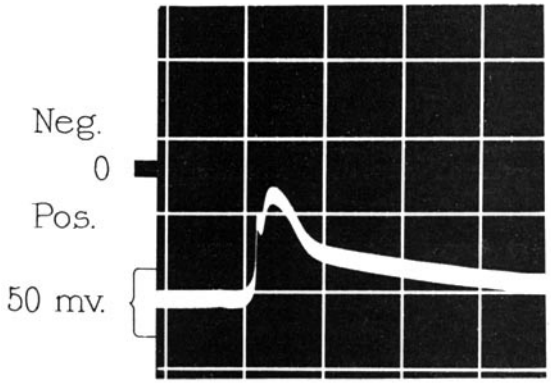


FIG. 3. Photographic record showing an *o* movement which does not go to zero. The record was arranged as in Fig. 2, employing contacts *C* and *F* only. *F* was killed with ethyl alcohol before the record started to secure monophasic recording at *C*. *C* was in contact with 0.001 M KCl. The cell had been stored in Solution A (for the composition of this see Osterhout, W. J. V., and Hill, S. E., *J. Gen. Physiol.*, 1933–34, **17,** 87) for 23 days and in 0.001 M NaOH for 1 hour before use. The vertical marks are 5 seconds apart. Temperature, 22°C.

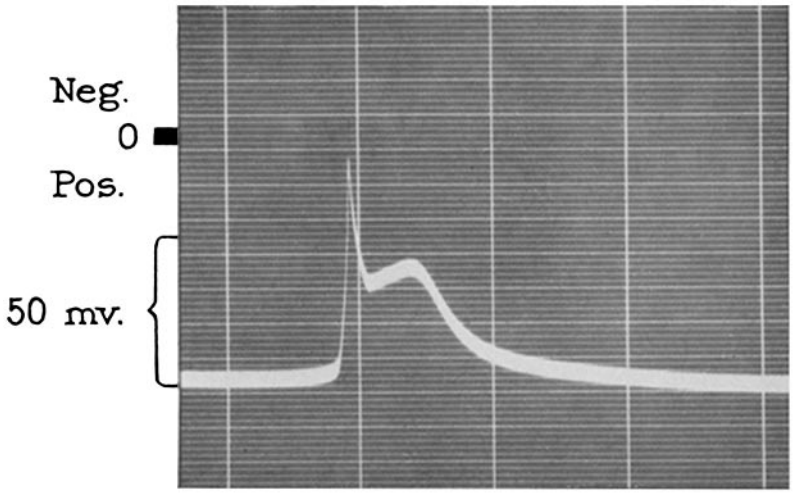


FIG. 4. Photographic record in which the *p* movement is greater than usual and the *q* movement less than usual. The experiment was arranged as in Fig. 2, employing contacts *C* and *F* only. *F* was killed with $CHCl_3$ before the record started to secure monophasic recording at *C*. *C* was in contact with Solution A, in which the cell had been kept for 10 days. The vertical marks are 5 seconds apart. Temperature, 20°C.

In consequence the concentration at the inner surface of $X$ would not as a rule reach the value found at the inner surface of $Y$ (especially as it would be lessened by outward diffusion through $X$). The same factors

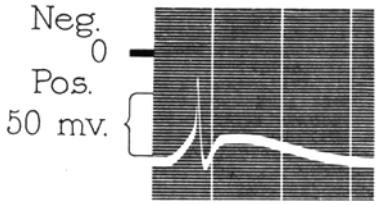


FIG. 5. Photographic record in which the $p$ movement descends to the starting point of the action curve. The experiment was arranged as shown in Fig. 2, employing contacts $C$ and $F$ only. $F$ was killed with $CHCl_3$ before the record started to secure monophasic recording at $C$. $C$ was in contact with Solution A, in which the cell had been kept for 10 days. The vertical marks are 5 seconds apart. Temperature, 20°C.

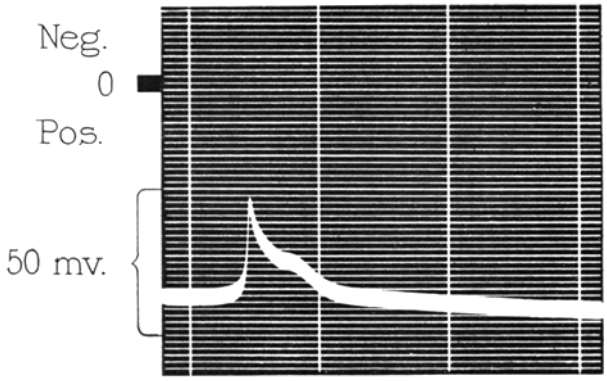


FIG. 6. Photographic record showing the $q$ movement much reduced. The experiment was arranged as shown in Fig. 2, employing contacts $C$ and $F$ only. $F$ was killed with $CHCl_3$ before the record started to secure monophasic recording at $C$. $C$ was in contact with 0.001 M KCl. The cell had been kept in Solution A for 13 days before use. The vertical marks are 5 seconds apart. Temperature, 20°C. This figure recalls some of the records obtained by Gasser with nerve.

would tend to make the slope of the curve less than in the $o$ movement.[10]

[10] Lack of uniformity in the various parts of the region recorded would also diminish the rapidity since the movement would not be finished until all of the region responded.

*The third movement* (*q*, Fig. 1). This is presumably due to the continued outward movement of K which increases the concentration of $K^+$ just outside *X*. This process would ordinarily be slow since $K^+$ would be carried away by convection currents in the film of solution between the protoplasm and the cellulose wall as well as by diffusion through the cellulose wall (which is very rapid).[11] The final level of concentration (which determines the height reached by the *q* movement) depends on how much $K^+$ reaches the outside of *X* before the process of recovery begins so that it may be expected to show considerable variation. This is actually the case (Figs. 1, 3 to 6).

*The fourth movement* (*r*, Fig. 1). This movement involves the return of the cell to the resting state in which the concentration of potassium in *W* is small. This can no doubt be brought about by those forces which in the resting state of the cell cause potassium to move from the external solution to the sap and accumulate there. Such forces could move potassium from *W* into the sap until the original resting state was achieved. This may possibly be hastened by the ingoing electrical current which, according to the local circuit theory, occurs during recovery. Recovery is, however, a relatively slow and variable process. Normally it takes about 15 seconds but it may be shorter and it is sometimes greatly prolonged.

When recovery remains permanently incomplete the protoplasm is regarded as no longer in a normal state.

Some of the curves obtained with *Nitella* resemble those gotten with nerve. For example, curves like that in Fig. 6 somewhat resemble those described by Gasser.[12] If such records were obtained with a single nerve fibre it might raise the question whether there may possibly be two surfaces, *X* and *Y*, in nerve.

If this assumption were made it might explain the form of the action curve and also the current of injury which is otherwise hard to account for. If the nerve fibre consisted wholly of protoplasm, cutting it might

[11] When 0.01 M KCl is substituted for 0.001 M the change of P.D. is nearly complete in 5 seconds, showing that diffusion through the cell wall is very rapid. *Cf.* Osterhout, W. J. V., *J. Gen. Physiol.*, 1929–30, **13,** 715.

[12] Gasser, H. S., *Am. J. Physiol.*, 1931, **97,** 254; Cold Spring Harbor symposia on quantitative biology, Cold Spring Harbor, Long Island Biological Association, 1933, **1,** 138.

automatically give rise to a fresh protoplasmic surface which would have the same electrical properties as the normal surface.[13] In that case there would be no current of injury. But if any part of the core of the nerve fibre were non-living and ran continuously lengthwise as in *Nitella* the current of injury could be explained in the same way as in *Nitella*, for in spite of the fact that the cut surfaces of the protoplasm healed at once the circuit would pass only once through living protoplasm.

The forms of the action current discussed in this paper (except Fig. 1) are relatively infrequent and constitute less than 5 per cent of the action curves observed by us.

## SUMMARY

The action curve involves four movements each of which shows considerable variation. These variations can be accounted for on the assumption that the action curve is due to the movement of potassium ions accompanied by an increase in permeability.

[13] This is found experimentally in such cells as *Ameba* and is to be expected if the ordinary surface is formed by substances which migrate into it because they are surface-active (Osterhout, W. J. V., *Ergebn. Physiol.*, 1933, **35,** 1020).