ACTION CURVES WITH SINGLE PEAKS IN NITELLA IN
RELATION TO THE MOVEMENT OF POTASSIUM

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Although closely related, Chara\(^1\) and Nitella\(^2\) show striking differences. The action curve in Nitella has two peaks but in Chara there is only one. The outer protoplasmic surface in Nitella is sensitive to K\(^+\) but this is not true of Chara.

It is of decided interest to find that these differences can be abolished by appropriate treatment. When cells of Nitella are leached in distilled water the outer surface becomes insensitive\(^3\) to K\(^+\) and at the same time the form of the action curve approaches that of Chara and shows only a single peak. The treatment with distilled water\(^3\) commonly removes the irritability\(^4\) as well as the sensitivity\(^5\) to K\(^+\) but cells are occasionally met with in which the irritability persists after the sensitivity to K\(^+\) disappears. These present some interesting features.

Under normal conditions, cells of Nitella have an outwardly directed (positive\(^6\)) p.d. of about 100 mV. due chiefly to the outwardly directed concentration gradient\(^7\) of K\(^+\) across the inner protoplasmic surface Y.

When an action current appears this p.d. disappears, partially or completely, producing the first movement of the action curve, i.e. the spike, or o movement, as seen in Fig. 1.

This is presumably due to an increase in the permeability of Y which

\(^1\) Chara coronata, Ziz. The large cells, resembling those of Nitella, are not covered with a layer of small cells as in most species of Chara.

\(^2\) Nitella flexilis, Ag.


\(^4\) By this is meant the ability to give propagated action currents on electrical stimulation.

\(^5\) By this is meant the large change in p.d. when 0.01 M KCl is replaced by 0.001 M KCl or by 0.01 M NaCl (the latter is called for convenience the potassium effect).

\(^6\) The p.d. is called positive when the positive current tends to flow from the sap across the protoplasm to the external solution.

\(^7\) Since the effect of K\(^+\) predominates the other cations are omitted from the discussion. Cf. Osterhout, W. J. V., J. Gen. Physiol., 1934–35, 18, 215.
allows K⁺ to migrate outward in the form of a moving boundary. This destroys the concentration gradient of K⁺ across Y and produces a loss of P.D.

On reaching X (when X is sensitive to K⁺) K⁺ will set up an outwardly directed (positive) potential, causing the curve to fall and producing the movement of the first peak. This movement will last until K⁺ reaches the outer surface of X and thus diminishes the concentration gradient of K⁺ across X.

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**FIG. 1.** The unbroken line shows changes in P.D. during the action current in *Nitella*, supposedly due to the outward movement of potassium. The broken line shows the P.D. in the resting state, before the outward movement of potassium begins.

In the diagrams the symbol K denotes the outwardly moving potassium (reduction in concentration is shown by reduction in the number of symbols). Each stage of its progress is marked by a change in P.D.: for example, in Diagram A the observed P.D. is due to the relatively high concentration of potassium at the inner surface of Y; in Diagram B we see that potassium has reached the outer surface of Y and in consequence the P.D. has disappeared.

The duration of the action current is usually about 15 seconds.

The duration of this downward movement will depend on the speed with which K⁺ moves across X. Its magnitude will depend on the sensitivity of X to K⁺, i.e. on the mobility ratio $\mu_K - \nu_{Cl}$, and on the partition coefficient $S_K$ ($S_K = $ concentration of K⁺ in the non-aqueous protoplasmic surface ÷ concentration of K⁺ in the adjacent aqueous solution).

It will be larger when the moving boundary is sharp for then the concen-

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tration gradient of $K^+$ across $X$ will be larger. If the front of the moving boundary becomes more diffuse the concentration of $K^+$ as it strikes $X$ will diminish and if it scarcely exceeds the concentration of $K^+$ already present in $W$ the $p$ movement will practically disappear.

This may explain such a curve as is seen in Fig. 2 which may occur even when $X$ is sensitive to $K^+$. Such curves are occasionally found, especially in cells exposed to 0.01 M NaCl which may increase the protoplasmic motion and render the moving boundary more diffuse. In such cases it is no longer possible to distinguish sharply between the fall of the curve due to the $p$ movement and the fall due to the $r$ movement (i.e. to the recovery which is presumably due to the movement of $K^+$ back into the sap). In such cases the only evidence of the $p$ movement lies in a sudden change in the course of the curve, as in Fig. 2.

If the outer protoplasmic surface $X$ loses part of its sensitivity to $K^+$ the $p$ movement will fall off in consequence.

When $X$ has lost its sensitivity to $K^+$ (as shown by the absence of the potassium effect) there is no longer any reason to expect any abrupt change in the course of the curve.

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10 The cells, after being freed from neighboring cells, stood in the laboratory at 15° ± 1°C in Solution A (cf. Osterhout, W. J. V., and Hill, S. E., J. Gen. Physiol., 1933–34, 17, 87) for several days.

The measurements were made on Nitella flexilis, Ag., using the technique described in former papers (Hill, S. E., and Osterhout, W. J. V., J. Gen. Physiol., 1937–38, 21, 541).

11 Cf. Hill, S. E., and Osterhout, W. J. V., J. Gen. Physiol., 1938–39, 22, 91. It is here suggested that increased conductivity of the protoplasm may tend to produce single peaks.

12 I.e. when there is no change in p.d. on replacing 0.01 M KCl by 0.01 M NaCl.

13 At the start of the spike there may be an abrupt rise of the curve due to electrical leakage from the stimulating electrodes.
Fig. 3. Action curve in a cell of Nitella which has lost its potassium effect (i.e., the outer protoplasmic surface has become insensitive to K⁺) as the result of leaching in distilled water.

The spot recorded, D, was in contact with 0.01 M NaCl and was connected through the recording galvanometer with another spot, F, in contact with 0.01 M NaCl (the p.d. of the latter remained constant as evidenced by the record of another spot E not shown here). In consequence the action curve is monophasic.

The cell was freed from neighboring cells and kept for 8 days in Solution A, then for 5 days in distilled water; the temperature was 15 ± 1°C. The record was made at 23°C (electrical stimulation). Vertical marks 5 seconds apart.

Fig. 4. Action curve in a normal cell of Chara which shows no potassium effect; i.e., the outer protoplasmic surface is not sensitive to K⁺.

The spot recorded, D, was in contact with 0.001 M KCl; it was connected through the recording galvanometer with a spot F killed by chloroform and having in consequence a p.d. of zero. Hence the action curve is monophasic.

The cell was freed from neighboring cells and kept for 2 days in Solution A at 15 ± 1°C. The record was made at 24°C. Vertical marks 5 seconds apart.
FIG. 5. Action curve in a cell of *Nitella* which has lost the potassium effect (i.e. the outer protoplasmic surface has become insensitive to K⁺) as the result of leaching in distilled water.

The spot recorded, D, was in contact with 0.01 M NaCl: it was connected through the recording galvanometer with another spot F in contact with 0.01 M NaCl which had a constant P.D. during the record as evidenced by the record of another spot E (not shown here). In consequence the action curve is monophasic.

The cell was freed from neighboring cells and kept for 12 days in Solution A and then placed in distilled water for 7 days: the temperature was 15 ± 1°C. The record was made at 22°C. Vertical marks 5 seconds apart.

FIG. 6. Action curve in a cell of *Nitella* which has lost the potassium effect (i.e. the outer protoplasmic surface has become insensitive to K⁺) as the result of leaching in distilled water.

The spot recorded, D, was in contact with 0.01 M NaCl: it was connected through the recording galvanometer to another spot F in contact with 0.01 M NaCl, which had a constant P.D. during the record, as evidenced by the record of another spot E (not shown here). Hence the action curve is monophasic.

The cell was freed from neighboring cells and kept in distilled water for 6 days at 15 ± 1°C. The record was made at 23°C. Heavy vertical marks 5 seconds apart.

Under these circumstances we expect only curves with rounded tops,¹⁴ ¹⁵

¹⁴ Since there is no p movement there is no q movement.

¹⁵ This applies to action curves in which the P.D. is largely lost and the spike or q movement follows the usual course and goes nearly to zero. When only a relatively small loss of P.D. occurs rounded tops may occur even when X is sensitive to K. Such action curves are regarded as abnormal and probably involve only a small outward movement of K⁺ which may not reach X at all or only to a slight extent. See Osterhout, W. J. V., *Biol. Rev.*, 1931, 6, 369 (Fig. 12 b).
such as are actually observed\textsuperscript{18} under these conditions (Fig. 3). Fig. 3 may be compared with the normal curve\textsuperscript{17} of \textit{Chara} (Fig. 4).

The course of recovery may be shorter than is usually observed in \textit{Nitella} but this is not always the case, as is evident from Figs. 5 and 6.

These facts strongly support the suggestion previously made\textsuperscript{18} that K\textsuperscript{+} plays an important rôle in the action curve.

\textbf{SUMMARY}

In \textit{Nitella} the action curve has two peaks, apparently because both protoplasmic surfaces (inner and outer) are sensitive to K\textsuperscript{+}.

Leaching in distilled water makes the outer surface insensitive to K\textsuperscript{+}. We may therefore expect the action curve to have only one peak. This expectation is realized.

The action curve thus obtained resembles that of \textit{Chara} which has an outer protoplasmic surface that is normally insensitive to K\textsuperscript{+}.

The facts indicate that the movement of K\textsuperscript{+} plays an important part in determining the shape of the action curve.

\textsuperscript{18} In some cases the potassium effect may be restored by pressure on the cell (Hill, S. E., and Osterhout, W. J. V., \textit{J. Gen. Physiol.}, 1934–35, \textbf{18}, 687) or by an action current (Osterhout, W. J. V., and Hill, S. E., \textit{J. Gen. Physiol.}, 1934–35, \textbf{18}, 681). Hence a cell showing an action curve with one peak may sometimes show a potassium effect when tested later on.

When a cell is tested for the potassium effect with negative result but when subsequently stimulated shows a tendency to form a second peak this may be due to the fact that the action current tends to restore the potassium effect and consequently the double peak.

\textsuperscript{17} After the spike the curve in Fig. 4 falls below the original level (positive after potential) and then rises. This is not a constant feature of \textit{Chara} and it may also occur in \textit{Nitella}.

In \textit{Chara} the chief seat of the p.d. appears to be (as in \textit{Nitella}) the inner protoplasmic surface \textit{Y} which is presumably sensitive to K\textsuperscript{+} although the outer surface, \textit{X}, is not. Leached \textit{Nitella} resembles \textit{Chara} in that \textit{Y} is sensitive to K\textsuperscript{+} but \textit{X} is not.