# NATURE OF THE ACTION CURRENT IN NITELLA

## V. Partial Response and the All-or-None Law

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Plate 1

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Stimulation is normally followed by a process of recovery which restores the cell to the state which existed before stimulation. If a new stimulus arrives before recovery is complete there may be no response or only a partial one.

This can be best understood if we begin by a brief discussion of the structure of the protoplasm.

Experiments indicate that the protoplasm consists of an aqueous layer $W$ bounded by an outer non-aqueous layer $X$ and an inner non-aqueous layer $Y$. Under normal conditions there is an outwardly directed concentration gradient[1] of KCl across $Y$ and a very much smaller one across $X$: hence we find a large outwardly directed potential at $Y$ and a much smaller one at $X$.[2]

Stimulation appears to be associated with an increase of the permeability[3] of $Y$ and an outward movement of $K^+$ resulting in a loss of the concentration gradient, and consequently of the potential, across $Y$. This causes the sudden rise which constitutes the spike of the action curve (*a*, Text-fig. 1).

When the outwardly moving $K^+$ reaches[4] $X$ it may set up a sufficient concentration gradient to cause an outwardly directed potential at $X$ (*b*, Text-

[1] For convenience we speak only of KCl since it is the most important substance in this connection, but other potassium salts act like KCl and to a lesser degree salts, of sodium. *Cf.* Osterhout, W. J. V., *J. Gen. Physiol.*, 1934–35, **18,** 215; 1939–40s **23,** 171.

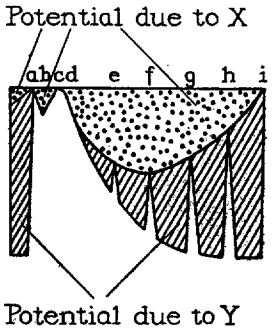
[2] This becomes evident when the potential due to $X$ is removed by leaching with distilled water (Osterhout, W. J. V., and Hill, S. E., *J. Gen. Physiol.*, 1933–34, **17,** 87) which makes $X$ insensitive to $K^+$. This does not appear to diminish the total potential.

[3] Stimulation is accompanied by a great increase in electrical conductivity. It is significant that the exit of KCl (which probably occurs) would assist this since we find that placing KCl on the outside lowers resistance. Blinks, L. R., *J. Gen. Physiol.*, 1929–30, **13,** 495; 1936–37, **20,** 229. Cole, K. S., and Curtis, H. J., *J. Gen. Physiol.*, 1938–39, **22,** 37.

[4] With $Y$ very permeable the time required for this appears to be not more than 1 second since the minimum distance between $Y$ and $X$ (*i.e.*, between the chloroplasts) is considerably less than 10 microns. When tap water saturated with chloroform is applied externally $X$ is made permeable and the chloroform may penetrate to $Y$ in less than 2 seconds.

fig. 1). This disappears as $K^+$ passes out[5] through $X$ and thus destroys the concentration gradient across $X$; in consequence we may find (as explained in previous papers[6]) a second peak in the action curve ($c$, Text-fig. 1).

The action curve now begins to descend as the process of recovery sets in. This involves the return[7] of the cell to its original state and consequently the inward movement of $K^+$ from $W$ through $Y$ into the sap. As this continues the outwardly directed concentration gradient of $K^+$ across $Y$ (and consequently the potential across $Y$) increases and the action curve descends toward the base line from which it started.




TEXT-FIG. 1. Hypothetical diagram of the distribution of potential in the protoplasm which is supposed to consist of an aqueous portion $W$ bounded by two very thin non-aqueous layers ($X$, at the outer surface, and $Y$, at the inner surface).

When stimulation occurs $Y$ loses its potential and this produces the sudden rise (spike) of the action curve at $a$. Potassium moves outward and on reaching $X$ sets up some potential ($b$) which disappears when the potassium reaches the outside of $X$ (at $c$).

The process of recovery now sets in and potassium moves back into the sap, decreasing the potassium outside of $X$ and increasing it inside of $X$ and thus increasing the potential across $X$. As potassium continues to move inward its concentration just inside $X$ decreases and the potential across $X$ decreases. Hence the potential due to $X$ first increases and then decreases.

When stimulation occurs during recovery $Y$ loses its potential but $X$ does not: hence there is no response at $d$, and the responses at $e$, $f$, $g$, and $h$ are incomplete but they increase as recovery proceeds because the potential at $Y$ increases.

[5] The rapidity of this outward movement of $K^+$ will depend on the permeability of $X$.

[6] *Cf.* Osterhout, W. J. V., *J. Gen. Physiol.*, 1934–35, **18,** 215; Osterhout, W. J. V., and Hill, S. E., *J. Gen. Physiol.*, 1939–40, **23,** 743; 1939–41, **24,** 9.

[7] This includes the return of the cell to its normal resistance and to its usual behavior in respect to apparent capacity. Blinks, L. R., *J. Gen. Physiol.*, 1936–37, **20,** 229. Cole, K. S., and Curtis, H. J., *J. Gen. Physiol.*, 1938–39, **22,** 37.

This inward movement of $K^+$ at first diminishes the concentration of $K^+$ outside of $X$ and increases its concentration just inside of $X$[8] and hence increases the outwardly directed concentration gradient of $K^+$ across $X$ and the potential across $X$. But as this process continues and $K^+$ moves inward, causing the concentration just inside $X$ to fall off, the concentration gradient—and consequently the potential—across $X$ falls off. Hence the potential across $X$ first increases and then decreases as shown in Text-fig. 1.

Let us now consider what happens if stimulation occurs during the process of recovery. We suppose that $Y$ loses its potential as usual and $K^+$ comes out of the sap into $W$. If an impulse arrives at $d$, Text-fig. 1, there will be no apparent response because $Y$ has no potential to lose. If it arrives at $e$ the action curve will be small because $Y$ has very little potential to lose. Since $W$ now has a concentration of $K^+$ much greater than usual there will be very little outward movement of $K^+$ and this can be quickly reversed in the recovery which sets in immediately afterwards. Little or no effect on the potential across $X$ can be expected because there will be little or no diffusion of $K^+$ to $X$ and any diffusion boundary will not be sharp. The net result will be a small rise in the action curve with a quick return to the base line ($e$, Text-fig. 1).

A stimulus occurring a little later finds $Y$ with more potential on hand ($f$, Text-fig. 1) and consequently the loss of this potential causes the action curve to rise more. Subsequent stimulations find $Y$ with more and more potential and therefore the action curve rises higher and higher until it reaches the magnitude it had at the start. We assume that stimulation causes no loss of potential at $X$.[9]

It is evident that an impulse arriving before recovery is complete can produce only a partial response; *i.e.*, one that is less than normal in magnitude. Even when the action curve has descended to the base line recovery may not be complete since part of the potential may be due to $X$. As stimulation does not cause a loss of this potential but only of the potential due to $Y$ the response is only a partial one[10] and does not go to zero.

Fig. 1 shows a photographic record which resembles Text-fig. 1. The

[8] This is because $K^+$ does not pass inward by simple diffusion but by a process of accumulation (requiring an expenditure of energy). This process a little later causes the concentration of $K^+$ in the sap to exceed that in $W$. It is a process which tends to go on continuously in the cell unless interrupted by stimulation which causes $K^+$ to move out through $Y$ and in some cases through $X$. *Cf.* Osterhout, W. J. V., *Bot. Rev.*, 1936, **2**, 283.

[9] Experiments have shown that the loss of potential at $Y$ does not involve loss of potential at $X$. *Cf.* Osterhout, W. J. V., *J. Gen. Physiol.*, 1938–39, **22**, 420 (Fig. 3).

[10] To what extent this explanation applies to muscle and nerve remains an open question. Regarding the possibility of a double surface in muscle see Francis, W. L., *Proc. Roy. Soc. London, Series B*, 1937, **122**, 140.

record is made from a cell in which two places, lettered from left to right *C* and *D*, were connected to a spot *F* still further to the right, as described in former papers.[11]

The cell was kept for 1 hour in 0.01 M NaCl[12] before the record was made. During this exposure the time required for full recovery was reduced from about 20 seconds to about 0.7 second and a pacemaker became established somewhere to the left of *C*. From this impulses passed to *C* and *D*, eliciting full responses at each, so that a long train of action curves appeared at the rate of 7 in each 5 seconds (these responses were practically identical with the full responses seen in the record at *C* in Figs. 1 and 2).

Then at *D* 0.01 M NaCl was replaced by 0.001 M NaCl + 0.0005 M $CaCl_2$ accompanied by some manipulation of the cell at *C*. This changed the pattern at *C* to that seen in Fig. 1. This is a variation of electrical alternans as described in a former paper[13] and consists of a full response followed as a rule by two smaller responses.

At *D* the time required for full recovery was lengthened from 0.7 to about 13 seconds thus making it possible for impulses from *C* to arrive at *D* before *D* had recovered from a previous stimulation.

In Fig. 1 we see that the third full response at *C* is not followed by a response at *D*. This may mean that the impulses coming from *C* arrived while *D* was in the absolutely refractory state and *Y* was unable to respond. But even if *Y* did respond its loss of potential might be too small to be detected (the total potential was small and it may have been all located at *X*).

The next full response at *C* is followed by a small response at *D*. As recovery at *D* progresses the successive responses become greater.

Although there is essential agreement between Text-fig. 1 and Fig. 1 there are some minor points of difference. In the first full response at *D* in Fig. 1 the curve descends sharply after the spike, presumably because $K^+$ reaches *X* and builds up a potential there. The curve then begins to rise because $K^+$ passes

[11] The cells, after being freed from neighboring cells, stood in the laboratory at 15°C. ± 1°C. in Solution A (*cf.* Osterhout, W. J. V., and Hill, S. E., *J. Gen. Physiol.*, 1933–34, **17**, 87) for several days. They belonged to Lot B (*cf.* Hill, S. E., and Osterhout, W. J. V., *Proc. Nat. Acad. Sc.*, 1938, **24**, 312).

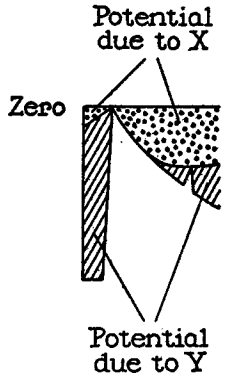
The measurements were made on *Nitella flexilis*, Ag., using the technique described in former papers (Hill, S. E., and Osterhout, W. J. V., *J. Gen. Physiol.*, 1937–38, **21**, 541). Temperature 20–26°C. Regarding the amplifier see the reference just cited. There was no indication of injury in these experiments.

[12] The effect of NaCl may be due to increased conductivity of the aqueous layer of the protoplasm *W* associated with an increased tendency of *Y* to become permeable more easily as the result of stimulation. *Cf.* Hill, S. E., and Osterhout, W. J. V., *J. Gen. Physiol.*, 1938–39, **22**, 91.

[13] Osterhout, W. J. V., *J. Gen. Physiol.*, 1942–43, **26**, 457.

out through $X$ and thus lessens the concentration gradient of $K^+$ across $X$. If this process goes far enough the curve rises to zero as in Text-fig. 1 but if recovery sets in before this happens the second peak does not reach zero: this is the state of affairs in Fig. 1.

The amount of $K^+$ passing out depends on the permeability of $X$ and on the steepness of the concentration gradient of $K^+$ as it approaches $X$, *i.e.*, on the sharpness of the outwardly moving diffusion boundary of $K^+$. If $K^+$ does not pass out through $X$ rapidly enough to lower the concentration gradient across $X$ to a sufficient extent before recovery (involving the inward movement of $K^+$) sets in[14] there will be no double peak (Text-fig. 2). It is therefore not surprising that we see single peaks in Figs. 1 and 2.




TEXT-FIG. 2. Hypothetical diagram to explain the occurrence of a single peak. We suppose that $K^+$ moving outward comes in contact with $X$ and sets up a potential but very little $K^+$ passes out through $X$ before recovery sets in, involving the inward movement of $K^+$ and the eventual loss of potential due to $X$ (this loss is not shown in the diagram).

The quick onset of potential due to $X$ produces a quick downward movement and a sharp peak as in Text-fig. 2, but it is of interest to note that we find, as expected, a rounded one in *Chara* where there is no potential due to $X$ (because $X$ is insensitive to $K^+$).[15] Here the downward movement depends solely on the

[14] In this case the potential due to $X$ increases as $K^+$ moves out of the sap into $W$, *i.e.*, at a time when the potential due to $Y$ is not increasing. But in Fig. 1 the potential due to $X$ increases as $K^+$ moves inward; *i.e.*, as the potential due to $Y$ is increasing. Hence with a double peak we may expect to see the two potentials increasing together to some extent but not so with a single peak. This corresponds to observation.

[15] Osterhout, W. J. V., *J. Gen. Physiol.*, 1934–35, **18**, 215; Osterhout, W. J. V., and Hill, S. E., *J. Gen. Physiol.*, 1940–41, **24**, 9.

inward movement of $K^+$ into the sap and the consequent increase in the potential due to *Y*. This is relatively slow and hence gives a rounded peak.

In Fig. 2 the partial responses increase in magnitude and then decrease. This is not surprising as the decrease begins at a time when the concentration of $K^+$ in *W* and the potential across *X* are falling off (*cf.* Text-fig. 1). At this time, therefore, $K^+$ coming out of the sap into *W* as the result of stimulation causes greater changes in the concentration of $K^+$ in *W* and may consequently produce greater changes in potential. Since the amount of $K^+$ coming out as well as the concentration already present in *W* would depend on a variety of conditions there is evidently considerable opportunity for variation in the action curves. This will receive attention in subsequent papers.

When there is no spontaneous activity we may employ electrical stimulation, as in Fig. 3, where the recovery time is longer and the resulting pattern is irregular.

In many cases where the cell has not been treated with NaCl and the recovery time is relatively long no partial responses are seen. The cell refuses to respond at all until recovery is complete and it then gives a full response.

Let us now consider the behavior of *C* (Figs. 1 and 2) which shows some interesting features. In the earlier part of the record (not shown here) before the cell was manipulated at *C* all the responses were full responses at the rate of 7 in 5 seconds. After manipulation the pattern at *C* changed to that seen in Fig. 1, *i.e.* a full response followed usually by 2 partial responses,[16] the latter being preceded by a positive dip in the curve. This positive dip is also seen at *D* where the 2 subsequent partial responses can be made out in some cases.

We may ask why the partial responses at *C* are so small. If the potential at *Y* is very small when they occur we may suppose that this potential is wholly lost on the all-or-none principle. Otherwise we might assume that the all-or-none law is not obeyed and that only part of the potential is lost. This might conceivably be caused by changes in ionic mobilities or in partition coefficients in *X* or *Y*. Such alterations have been observed as the result of metabolic[17] changes and of the application of reagents[18] and might conceivably be produced by stimulation. In some cases where the partial response is very small such an explanation may be indicated. Otherwise we should have to assume improbable values of the potential due to *X* especially when there are large fluctuations in this value as measured by the distance between zero and the apex of the partial response.

[16] This is a variation of electrical alternans. For somewhat similar patterns see Osterhout, W. J. V., *J. Gen. Physiol.*, 1942–43, **26,** 457, Fig. 3.

[17] Hill, S. E., and Osterhout, W. J. V., *Proc. Nat. Acad. Sc.*, 1938, **24,** 312. Osterhout, W. J. V., *J. Gen. Physiol.*, 1939–40, **23,** 429.

[18] Osterhout, W. J. V., *J. Gen. Physiol.*, 1936–37, **20,** 13, 685; 1937–38, **21,** 707; 1938–39, **22,** 417; 1939–40, **23,** 171, 569, 749; 1940–41, **24,** 699. *J. Cell. and Comp. Physiol.*, 1941, **18,** 129.

Another possibility is suggested by the work of Hodgkin,[19] who finds that an electrical disturbance in nerve may produce an electrical change beyond a block without necessarily involving any physiological response at the spot beyond the block. If the two small movements coming after each spike at *C* involve no physiological response and the similar movements at *D* are of the same sort the movements at *D* should be simultaneous with those at *C* and of smaller magnitude.[20] Whether they are simultaneous is not clear but their magnitude appears to be less and to fall off still more as the impulse travels further along the cell, as is evident in another part of the record not shown here.

## SUMMARY

When a stimulus arrives before recovery is complete there may be no response or only a partial response. A typical response appears to involve an immediate loss of potential at the inner protoplasmic surface but not at the outer surface. As long as recovery is incomplete only a part of the total potential is located at the inner protoplasmic surface and the loss of this part of the total potential can cause only a partial response; *i.e.*, one of smaller magnitude than the normal.

Even after the action curve has returned to the base line recovery may be incomplete and the response only a partial one. The return of the action curve to the base line means a recovery of total potential but if part of this is located at the outer protoplasmic surface and if this part is not lost when stimulation occurs the response can be only a partial one. During recovery there is a shift of potential from the outer to the inner protoplasmic surface. Not until this shift is completed can recovery be called complete. The response to stimulation then becomes normal because the loss of potential reaches the normal amount.

In many cases the partial responses appear to conform to the all-or-none law. In other cases this is doubtful.

[19] Hodgkin, A. L., *J. Physiol.*, 1937, **90**, 183.

[20] The distance between *C* and *D* is 1 cm.

## EXPLANATION OF PLATE 1

FIG. 1. Shows the effect of stimulation before recovery is complete.

Two places on the cell lettered from left to right *C* and *D* are connected to a spot *F* still further to the right. The cell was left in 0.01 M NaCl for 1 hour until a pacemaker was established to the left of *C* which sent impulses along the cell to *C* and to *D* (there was no electrical stimulation). *F* was in contact with 0.01 M KCl which kept the P.D. constant approximately at zero.

At *C* we find as a rule a full response going approximately to zero followed by 2 small partial responses.[16]

While *C* was left in contact with 0.01 M NaCl *D* was placed in contact with 0.005 M NaCl + 0.0025 M $CaCl_2$ which lengthened the time of recovery at *D* so that some of the impulses coming from *C* were unable to elicit a full response at *D* because they arrived before recovery was complete.

The third full response at *C* is not followed by a response at *D*: after this, responses of increasing magnitude are observed at *D* until the response becomes nearly complete and a new series begins.

It should be noted that even after the action curve has descended to the base line from which it started recovery is incomplete and the response is incomplete. For explanation see Text-fig. 1.

The cell was freed from neighboring cells and kept in Solution A at 15°C. ± 1°C. for 30 days and then placed in 0.01 M NaCl for 1 hour at about 22°C. before the record was made.

Heavy time marks 5 seconds apart.

FIG. 2. Later section of the record shown in Fig. 1. The partial responses at *D* first increase and then decrease.

FIG. 3. Shows the effect of electrical stimulation before recovery is complete: each electrical stimulus is indicated by a signal above the action curve (these signals are about 10 seconds apart). The portion of the photographic print showing the signals was cut out and pasted on: it has not shrunk evenly in drying and in consequence the lines do not all match exactly.

The stimulating electrode was 2 cm. from the recorded spot and no leakage effect (shock artifact) is evident.

The spot recorded was in contact with 0.01 M NaCl and was connected to a spot in contact with 0.01 M KCl which kept the P.D. constant approximately at zero.

The cell was freed from neighboring cells and kept in Solution A at 15°C. ± 1°C. for 9 days. It was then placed for 1 hour in 0.01 M NaCl at about 22°C. before the record was made.

Time marks 5 seconds apart.

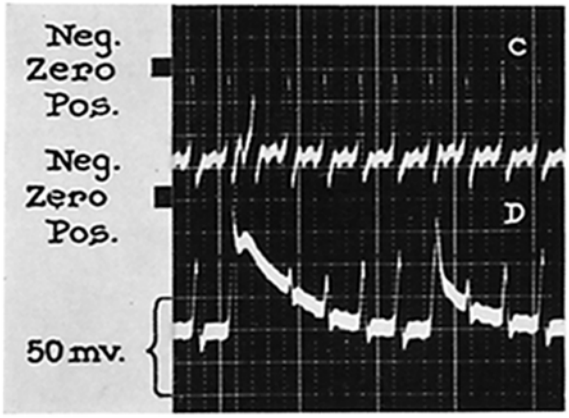



FIG. 1

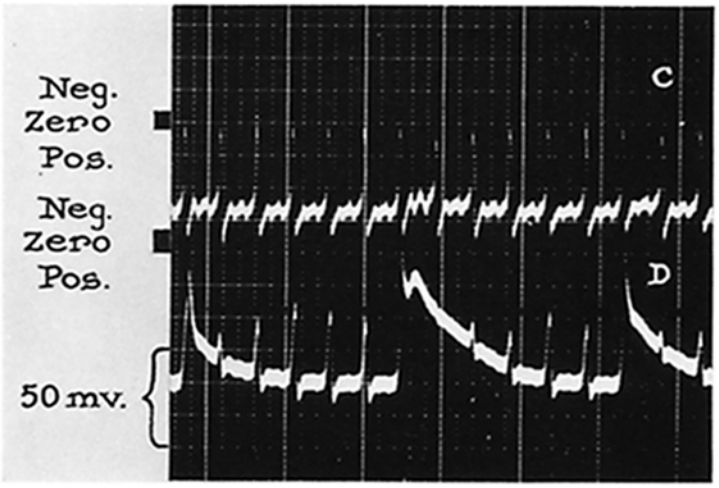



FIG. 2

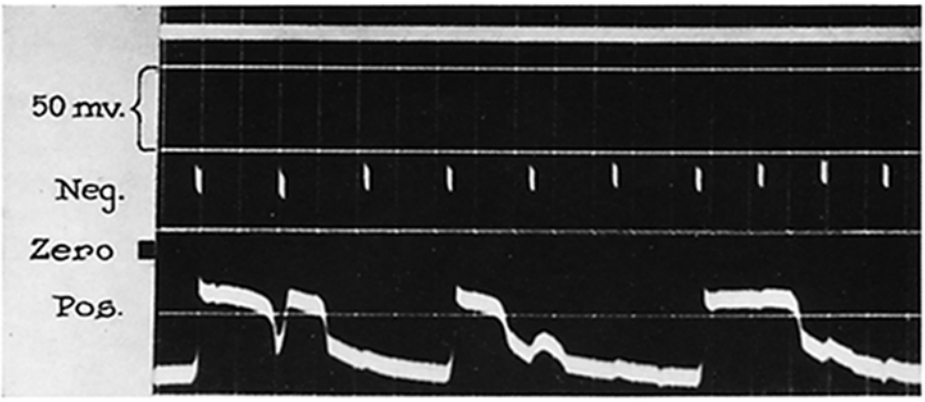



FIG. 3

(Osterhout: Nature of action current in *Nitella*. V)