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THE SITE OF IMPULSE INITIATION IN A NERVE CELL OF A CRUSTACEAN STRETCH RECEPTOR

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The problem of the site of origin of conducted impulses in nerve cells has aroused considerable interest recently. Already in 1940 Gesell suggested that the post-synaptic spike potential in neurones may be initiated in the axon hillock region. Bishop (1953) advanced a similar theory after examining the potentials evoked in the lateral geniculate nucleus by electrical stimulation of the optic nerve. More recently, with the advent of intracellular recording methods, the responses of spinal motoneurones have been analysed by several investigators, and Araki & Otani (1955), Fuortes, Frank & Becker (1957) and Coombs, Curtis & Eccles (1957*a*, *b*) have concluded that in these cells conducted impulses originate in the initial axon segment. On the other hand, Fatt (1957) has suggested that the impulse is set up in the cell body.

Much of the difficulty in locating the site of origin of the nerve impulse in the motoneurone arises because the recordings have of necessity been made without visual control of the position of the electrode tip in the cell. The nerve cell of the crustacean stretch receptor (Alexandrowicz, 1951) is suitable for studies on the locus of impulse initiation, for it is readily accessible and its structural details are visible under appropriate optical conditions. Eyzaguirre & Kuffler (1955*a*) showed that deformation of the dendrites of this cell sets up a generator potential which leads to conducted impulses. They investigated the problem of the site of impulse initiation in this cell, and concluded that the conducted impulse usually arose in the dendrites or cell body soma, and that only during strong excitation did the impulse arise in the initial portion of the axon (see also Kuffler, 1958). This was based largely on the finding that the threshold for orthodromic spike initiation recorded in the cell body of the slowly adapting receptor was lower than the threshold measured by anti-

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dromic invasion. Florey (1955) suggested a similar locus of origin on the basis of findings with extracellular recordings by metal electrodes.

In the present investigation, the extracellular potentials recorded from the slowly adapting lobster stretch receptor have been analysed, and it has been found that the nerve impulse originates in the axon near to the cell body. A preliminary report of these results has already been published (Case, Edwards, Gesteland & Ottoson, 1957).

METHODS

The eighth thoracic receptor of the lobster, *Homarus americanus*, was used in all experiments. Dissection as well as recording was carried out with the preparation immersed in Cole's (1941) solution. To minimize injury the receptor was not dissected free from the surrounding tissues. The muscles covering the cell were removed and after adjacent muscles were retracted the structural details of the receptor were visible in dark-field illumination. The nerve was dissected free in its central course several millimetres from the cell body and lifted up into mineral oil above the saline on to two electrodes for antidromic stimulation. Recordings were made with conventional capillary micro-electrodes, filled with 5 M-NaCl, which were connected via a cathode follower to a condenser-coupled amplifier (Grass P5) with a frequency response of 35–10,000 c/s. An earth lead was placed on the muscle. The micro-electrodes were brought into contact with the preparation under microscopic control. When two micro-electrodes were used simultaneously, as in most experiments, one of the electrodes was ordinarily kept fixed on the cell body while the other was moved from one recording site to another. The receptor was activated either by stretch or by antidromic stimulation. The responses to both modes of activation were usually recorded at each recording site.

RESULTS

Features of potentials recorded from different parts of the stretch receptor

Response evoked by stretch. The extracellular potentials recorded from different parts of a lightly stretched slow receptor cell show significant differences (Fig. 1). Record E illustrates the general shape of the response obtained from anywhere on the cell body. The negative spike was always preceded by a positive deflexion. Potentials of essentially the same general appearance were obtained from the axon hillock region (D) and from the proximal portion of the axon (C). The initial positivity decreased in amplitude as the electrode was moved away from the cell body along the axon and was absent in the response at a distance of about 500 μ from the cell (B). The potential recorded in this latter position started with a relatively slowly rising negative potential from which the spike arose. If the electrode was moved still further along the axon to a point 1 mm (A) or more from the cell the response had the characteristic appearance of a propagating spike potential that approached and passed the recording site.

All the fourteen cells investigated in the present study gave responses of the same general characteristics as those shown in Fig. 1. There were minor differences from one cell to another, but these variations were confined more to the relative sizes of the various components of the potentials than to the features typical of the responses of different parts of the neurone.

Responses evoked by antidromic stimulation. Fig. 2 shows the antidromic responses obtained from the same cell and the same recording sites as the records shown in Fig. 1. A comparison of the orthodromic and antidromic responses shows that the potentials recorded from the axon near the cell body or from the cell body itself were essentially similar, irrespective of the mode of

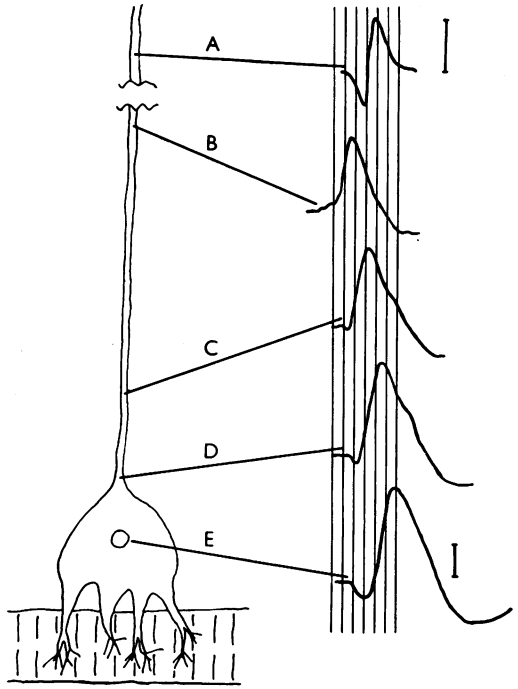

Fig. 1. Tracings of orthodromic impulses set up by stretch in the lobster stretch receptor recorded with external leads at the various indicated points on the nerve cell. One electrode was kept fixed on the cell at E, while the other was moved to the other positions. Point A was about 1·3 mm from cell body-axon boundary; B was about 500 μ distant. Time intervals are 0·1 msec. Scale (A, B, C and D recorded at the same amplification) = 0·5 mV. In this and all subsequent figures, negativity is upwards.

activation of the receptor. The point of interest in the records in Fig. 2 is that in the region of the fibre where the orthodromic potential was preceded by a negative potential (Fig. 1 B) the antidromic potential started with a positive deflexion.

The lack of initial positivity seen in the orthodromic spike at position B (Fig. 1) is regarded as evidence that the impulses originate in this region. In contrast an impulse which approaches and then conducts past an electrode sets up the following sequence of potential changes: positive, negative, positive or source, sink, source. Thus the difference between the potential con-

figuration at B (Figs. 1, 2) set up orthodromically and antidromically is adequately explained. Further, only in region B is there a significant difference between the orthodromic and the antidromic potentials.

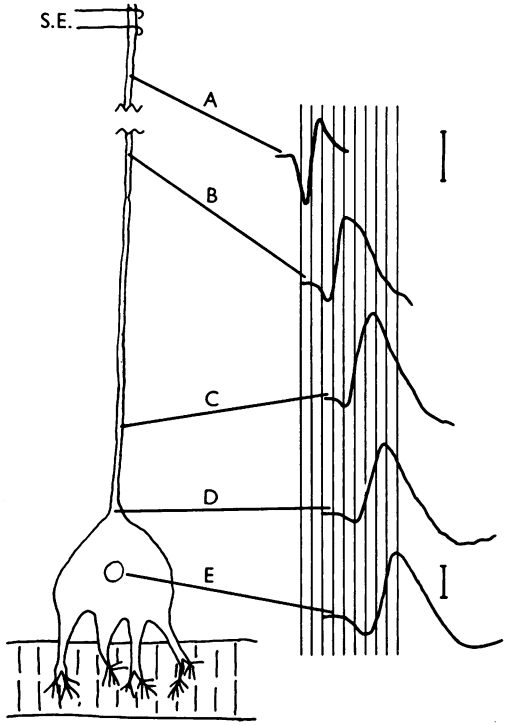

Fig. 2. Tracings of antidromic impulses in the lobster stretch receptor recorded with external leads at the various indicated points on the same nerve cell shown in Fig. 1. Time intervals, 0·1 msec. Scale (A, B, C and D recorded at the same amplification) = 0·5 mV.

Time course of potential spread

As already mentioned, the spread of the conducted impulse was studied by simultaneously recording with two micro-electrodes the activity of different parts of the stretch receptor. The records in Fig. 1 were obtained by keeping one electrode at *E* throughout the experiment and moving the other electrode stepwise from *D* to *A*. To illustrate the temporal relations of the responses of the various parts the potentials have been traced against a background of vertical lines representing the time scale. It will be observed that the earliest appearance of the impulse occurred in the axon at some distance from the cell body. This first response is the one which, as already mentioned, was characterized by the presence of an initial negative potential. In the regions of the fibre closer to the soma the negative spike potential was preceded by a positive deflexion. If the start of activity within these regions was measured from the

peak of the downward deflexion, it is apparent that the action potential spread from its site of origin centripetally back into the soma as well as centrifugally along the axon. Furthermore, the positive deflexions occurred during the time for the rise of potentials in adjacent regions of the neurone: thus the positive dip in C occurred during the rise of the spike in B, that in D during the rise of the spike in C and so on. This shows that those parts of the neurone which are at some distance from the site of initiation of the impulse acted as sources for the current flow to the sink in the active region of the fibre. Since there is also a region of sustained depolarization in the dendrites current will be drawn to two sinks from the soma and initial portion of the axon.

The finding that the impulse is seen at B earlier than elsewhere on the axon is further evidence that this is the region of the axon where the impulse originates.

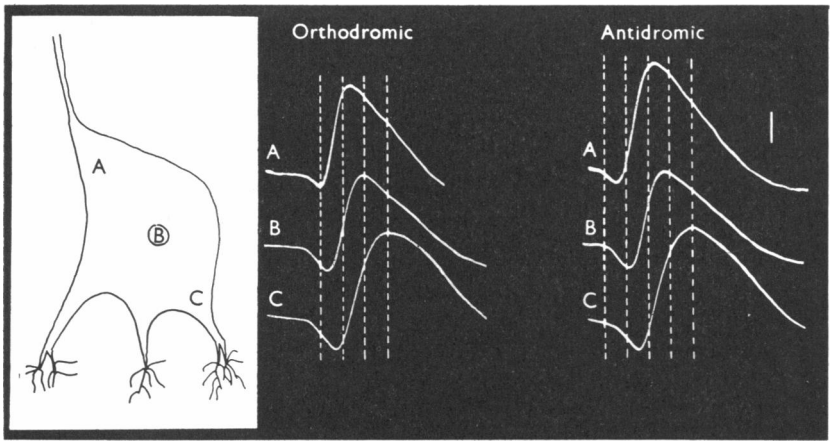

Fig. 3. Orthodromic and antidromic impulses recorded at various points on the cell body. Time intervals, 0·1 msec. Scale 0·5 mV.

In most cells the time of propagation of the orthodromic spike from the site of origin on the axon to the cell body was less than that of the antidromic impulse. This is seen in the records in Figs. 1 and 2. The time for conduction of the orthodromic spike from point B to the middle of the cell body (E) was about 0·3 msec, while it took about 0·35 msec for the antidromic spike to travel the same distance. In eight cells the antidromic latency between C and E was on the average $0{\cdot}018 \pm 0{\cdot}006$ msec (S.E.) greater than that of the orthodromic spike. The probability that this difference occurred by chance is less than 0·05.

Simultaneous recordings from different parts of the soma

The dimensions of the cell of the lobster's stretch receptor permit simultaneous recordings with two micro-electrodes from different points on the soma. The records in Fig. 3 show the orthodromic and antidromic potentials

recorded from the axonal (A) and dendritic (C) edges of the cell body and from the centre of the cell (B). It is significant that the time course and the shape of the potentials were the same whether the cell was activated by stretch or by antidromic stimulation. In both instances the axon hillock region was first activated, and from there the impulse passed over the soma toward the dendrites. The rate of propagation was, however, not the same for the two impulses. In the cell from which the records in Fig. 3 were obtained, the time for conduction, measured by the peak of the positive deflexion from the proximal to the distal end of the cell (about 150 μ) was about 0·07 msec for the orthodromic potential, whereas the antidromically evoked impulse needed about 0·1 msec to propagate the same distance. It should be noted that the initial positive deflexion increased in amplitude from the axonal to the dendritic edge. A similar observation was made in the recordings from different points on the axon, as pointed out in the discussion of the records in Fig. 1.

Double discharge

It was found by Eyzaguirre & Kuffler (1955*b*) that the slowly adapting stretch receptor cell sometimes fired in bursts instead of discharging a train of regularly spaced impulses. The grouped discharge could be initiated also by antidromic stimulation and the number of impulses in each burst was found to vary inversely with the magnitude of stretch. The multiple discharges set up by the antidromic impulse were shown to be conducted in the orthodromic direction.

In the present investigation grouped discharges were observed in many cells. When this type of discharge occurred in response to stretch it was also invariably evoked by antidromic stimulation. In some cells which gave normal responses to stretch it was found that antidromic stimulation at certain frequencies initiated the grouped discharge. In most preparations the burst consisted only of two impulses.

The records in Fig. 4 show such a grouped discharge consisting of two impulses evoked by stretch. The potentials on the upper beam were recorded from the axon while the lower record shows the responses of the cell body. The two impulses in each trace were similar if the differences in amplitude are disregarded. Thus both spikes in the axon were preceded by a slowly rising negative potential while the soma spikes had an initial positive deflexion. The similarity of the two axonal impulses strongly suggests that they were generated within the same region of the fibre.

The records in Fig. 5 show the antidromic responses obtained from the same recording sites on the axon (upper trace) and on the cell body (lower trace) as those in Fig. 4. The important point in these records was the difference in shape between the first and second axonal impulse. The first impulse in both sweeps (A and B) had the characteristic appearance of the

response evoked by antidromic stimulation, while the second impulse was in both sweeps identical with that obtained when the cell was stretched. This indicates that when a grouped discharge was elicited by antidromic stimulation the second spike was initiated near to the cell body.

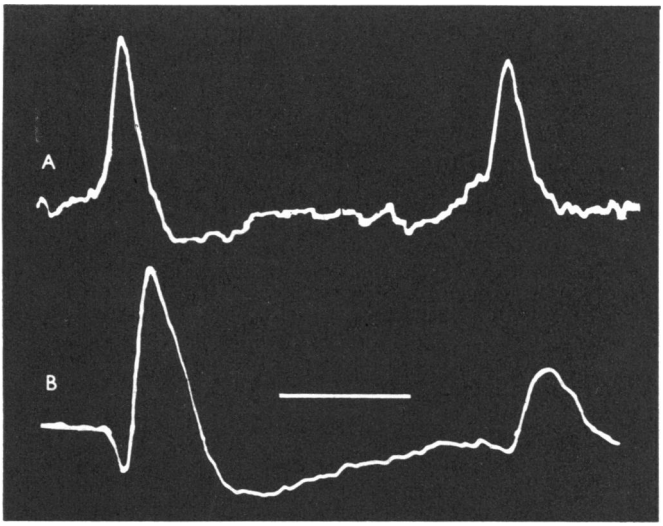

Fig. 4. Simultaneous recording of grouped orthodromic action potentials in lobster stretch receptor. A, electrode on axon; B, electrode on cell body. Time calibration, 1 msec. (Spikes retouched.)

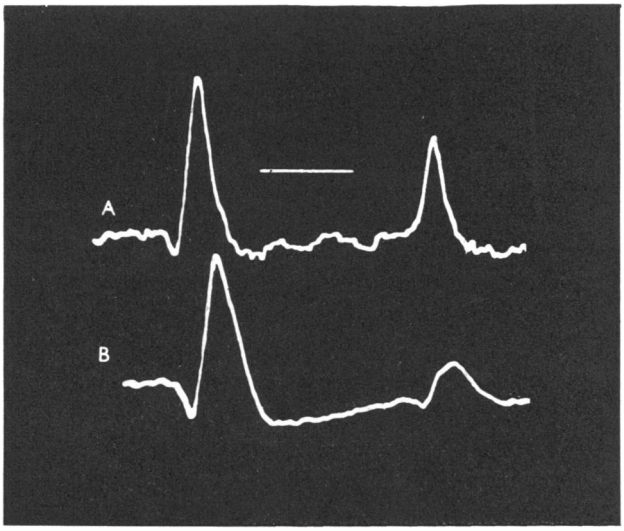

Fig. 5. Simultaneous recording of antidromic impulse and following orthodromic impulse. A, electrode on axon; B, electrode on cell body, Time calibration, 1 msec.

The second spike propagated not only in an orthodromic direction but also back into the soma. This is illustrated in Fig. 6, which shows tracings of the second impulse following an antidromic spike. These recordings were made with the same cell and in the same way as those in Figs. 1 and 2. The potentials recorded from the axon at positions B and C have no initial positivity but only the potential at B has a distinct prepotential phase. This may indicate that the site of impulse initiation of the second spike is closer to the cell body than that of the normal orthodromic impulse.

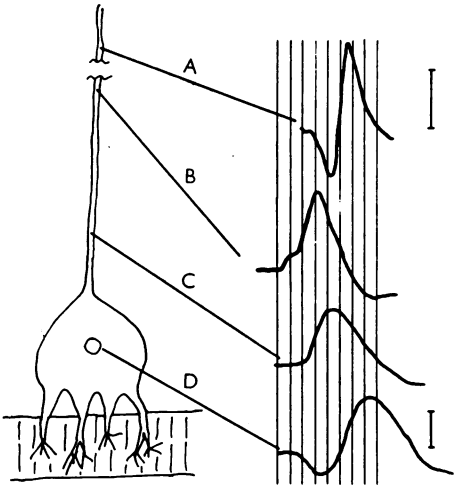

Fig. 6. Tracings of orthodromic impulse set up by antidromic impulse recorded at various indicated points on the same nerve cell shown in Fig. 1. Time intervals, 0·1 msec. Scale (A, B and C recorded at the same amplification) = 0·5 mV.

DISCUSSION

It has been shown that stretch sets up a large depolarization in the dendrites which decrements into the cell body and axon (Eyzaguirre & Kuffler, 1955*a*). Therefore the depolarization at the region where the impulse is set up is less than that in the cell body and dendrites. The present finding that the impulse originates in the axon indicates that the threshold for a conducted impulse in the axon is lower than in the cell body or dendrites. Although no direct measurements have been made, this also appears to be true of the spinal motoneurone (Araki & Otani, 1955; Coombs *et al.* 1957*a*, *b*; Fuortes *et al.* 1957).

On the basis of the present evidence and that of Eyzaguirre & Kuffler (1955*a*, *b*), the course of events leading to an impulse seems to be as follows. Stretch deformation of the dendrites produces a sustained depolarization. The current flow to this sink in the dendrites reduces the membrane potential of the soma and axon. If the threshold of the axon is lower than that of the cell

body, the depolarization would reach threshold first in the axon, where an impulse would be set up which would propagate both orthodromically along the axon and antidromically back into the soma.

In view of this picture, it would seem that the cell body and large dendrites integrate the depolarizations produced by stretch deformation of the various peripheral dendrite regions. If the total of the dendritic potential changes is sufficient to depolarize the axon to threshold, an impulse results. The magnitude of the depolarization of the axon depends primarily on the degree of stretch. In addition, the discharges are regulated by synaptic inhibitory action (Kuffler & Eyzaguirre, 1955) which apparently acts by increasing the membrane conductance in the dendrite–cell-body system. Thereby the space constant of the membrane is reduced, and the depolarization of the axon due to stretch of the dendrites is attenuated (Kuffler & Edwards, 1958).

The higher threshold of the cell body of the motoneurone has been attributed to the dense coverage of the soma membrane by synaptic knobs and glial cells (Eccles, 1957). The lobster's stretch receptor has no coverage of this kind. Therefore the threshold difference in the crustacean nerve cells must be due to some difference in the intrinsic properties of the soma membrane and of the fibre, as has also been suggested for motoneurones (Coombs *et al.* 1957*b*; Eccles, 1957). Whatever the true explanation might be it is apparent that the portion of the fibre immediately adjacent to the cell body has essentially the same excitability properties as the soma. This is evident from the observation that the impulse has not been seen to start in the very first part of the axon but always at some distance from the cell body.

There is, however, one difference between the stretch receptor neurone and the motoneurone that might be attributed to the coverage of the latter by glial cells and synaptic knobs. The records shown in Fig. 3 indicate that the cell body gives a propagated impulse. This type of response was found in all the cells examined. On the other hand, Freygang, Frank, Rall & McAlister (1958) suggest that most of the soma-dendritic membrane remains passive during the spike. The regions that remain passive could, of course, be the areas covered by glial cells and synaptic knobs.

As pointed out in Methods, the preparation was immersed in a conducting solution in our experiments. In this way spread of potential changes around the active structures was much reduced, as shown by the absence of a response when the electrode was placed in contact with the connective tissue adjacent to the cell. This method makes possible rather accurate locating of sources and sinks of current flow. In contrast, when recordings were made with the cell surrounded by paraffin oil it was possible to record potentials with the microelectrode in the connective tissue 100 μ or more from the active structure. In addition, the configuration of the response in paraffin was critically dependent on the position of the earth lead on the tissue. This appears to explain the

discrepancies between our and Florey's (1955) findings. Florey recorded from the stretch receptor in mineral oil with fine metal electrodes, and concluded that the impulse originated in the dendrites. There is one disadvantage of making the recordings with the cell fully submerged in saline. To obtain potentials of sufficient amplitude it was necessary to press the electrode against the tissue. It is difficult therefore to exclude the possibility that a pressure block was produced under the tip of the electrode. This, however, would apply equally to axon and soma and we would have to assume that the latter is more sensitive to pressure. Although it seems unlikely that pressure might have created a situation leading to impulse initiation in the axon instead of in the cell body, it is experimentally difficult to disprove or prove this assumption. It was observed, however, that variations of the electrode pressure against the soma did not change the location of the site of impulse initiation and only rarely was the discharge frequency changed.

The present finding that the orthodromic impulse propagates faster than the antidromic is consistent with observations that have been made on spinal motoneurones (Coombs *et al.* 1957*a*; Eccles, 1957; Fatt, 1957; Fuortes *et al.* 1957). It is perhaps pertinent that the cell body and dendrites are more depolarized by the generator potential during orthodromic invasion. The current flow to the dendrites during stretch has been shown to facilitate antidromic impulse invasion into the soma (Eyzaguirre & Kuffler, 1955*b*).

Eyzaguirre & Kuffler (1955*b*) suggested that the grouped discharge is produced by a delay in spread of excitation somewhere between the soma and dendrites. The present results are consistent with their findings but not with their interpretation and it appears that the grouped impulses initiated either by stretch or by antidromic excitation start in the axon. A change of the excitability properties of the cell must also be considered since the grouped discharge is not seen in normally functioning cells. The mechanisms for this are not apparent.

SUMMARY

1. The features and time course of spread of orthodromic and antidromic impulses have been studied in the slowly adapting lobster stretch receptors. Two micro-electrodes recorded simultaneously extracellular potentials from different parts of the neurone, cell body or axon.

2. When the sensory neurone is activated by stretch, the impulse recorded from the initial part of the nerve fibre starts with a negative prepotential, while in all other regions it starts with a positive deflexion. Further, the impulse appears earliest in the initial part of the fibre and from there propagates in a centrifugal direction along the axon as well as back into the soma towards the dendrites. The antidromic spike always starts with a positive deflexion, whether recorded in the axon or cell body. From these findings it is

concluded that impulses in the sensory neurone of the slowly adapting receptor are initiated in the axon near to the cell body.

3. The impulse invasion of the soma occurs more rapidly when the cell is activated by stretch than if the impulse is evoked by antidromic stimulation.

4. In a cell that gives grouped discharges the second impulse in each burst starts in the same region of the fibre where impulses are generated during stretch.

5. The present findings as to the site of origin of the impulse indicate that the threshold of the cell body is higher than that of the axon.

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