THE SITE OF IMPULSE INITIATION IN BIPOLAR RECEPTOR NEURONS OF CALLINECTES SAPIIDUS L.*

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INTRODUCTION

In the multipolar abdominal stretch receptors of the crayfish and lobster, action potentials are initiated in a segment of the axon central to the cell body (Case, Edwards, Gesteland & Ottoson, 1957; Edwards & Ottoson, 1958; Eyzaguirre & Kuffler, 1955). Since the soma lies at the periphery, close to the receptor region in the dendrites, the initial excitation set up by the mechanical stimulus must traverse the cell body to reach the spike trigger zone in the axon. A microelectrode placed in the soma of this cell detects the depolarization produced by the generator current flowing from dendrites to axon. This depolarization—the generator potential—is subsequently wiped out in the soma by the peripherally travelling action potential, only to be re-established if the excitation continues.

In an earlier paper (Mendelson, 1963) on the movement-sensitive, bipolar receptor cells of the P.D. organ of Pachygrapsus crassipes, I reported that a microelectrode in the soma does not detect a potential change that may be attributed to the flow of generator current from the mechanically excited region. Action potentials in response to adequate stimuli rise abruptly from the resting (potential) base-line without preliminary slow depolarization, nor is there a steady maintained depolarization during a prolonged stimulus that elicits a train of impulses. I therefore proposed that in the bipolar movement-receptor neuron the impulse is initiated far distal to the soma and that appreciable generator currents do not ordinarily flow through the soma. Subsequently, Mellon & Kennedy (1964) presented quite strong evidence that in the bipolar neurons of the hair-pit receptors on the crayfish branchiostegite the action potential is initiated in the distal process of the cell; the spike then traverses the soma into the central axon. The hair-pit bipolars—like the P.D. bipolars—exhibit soma action potentials, in response to mechanical stimuli, that rise sharply out of the base-line without preliminary depolarization. Mellon & Kennedy also found that when an antidromic spike left the soma refractory, an electrical stimulus to the distal process produced a small partial spike in the soma; probably the orthodromic spike was blocked in the distal process. It was not possible with the experimental arrangement they used to demonstrate that there was no action potential in the central axon at the time of the blocked spike. This leaves a slight uncertainty. This paper presents further, decisive evidence

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of spike initiation in the distal process of the bipolar P.D. receptor neuron. A preliminary report of this work has appeared (Mendelson, 1965).

MATERIALS AND METHODS

Large specimens of the blue crab, *Callinectes sapidus* L., were obtained from the Marine Biological Laboratory, Woods Hole, Mass. Single legs were removed by autotomy and prepared in the manner described for *Pachygrapsus* (Mendelson, 1963). Except for superficial differences in exoskeletal morphology, the appearance of the preparation is the same in *Callinectes* as in *Pachygrapsus*. After the P.D. organ is exposed, the P.D. bundle is separated from the main leg nerve for some distance centrally, severed at its central end, and the distal stump is pulled into a suction electrode. This electrode is connected through a relay so that it may be used for recording or switched to the output of a stimulus isolation unit to deliver antidromic
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stimuli (Fig. 1). Records from this electrode were displayed on one beam of a dual-beam oscilloscope. The other channel displayed the record of the intracellular activity of the soma picked up either by a double-barrelled electrode or a single electrode connected in a bridge circuit, so that current could be passed across the soma membrane to alter its potential. Adequate stimuli were delivered via a small glass hook contacting the elastic strand of the organ some way distal to the location of the sensory cells. The glass hook was attached to a piezoelectric crystal which, when excited with brief voltage pulses, twitched the strand. Occasionally it was found most expedient merely to tap the table lightly while the glass hook was in place. The stimulating effectiveness of this latter method was surprisingly good.

All experiments were carried out in filtered natural sea water, at room temperature (19–23° C.).

RESULTS

The first evidence for spike initiation in the distal process was the rapid initial rise of the soma impulse without visible pre-potential. Therefore the first step in this work was to analyse the requirements for initiating a soma spike. If the normal trigger zone lay in the axon central to the soma, then generator currents would depolarize the soma and the axon until the trigger zone fired. The amount of depolarization of the soma necessary to reach axon threshold would be the same whether the current came from the distal process or from an intrasomatic electrode. Thus the soma depolarization at spike threshold, as measured with current passed from the electrode, must be identical with the level of the generator potential at threshold if the spike is initiated at or central to the soma. Fig. 2 compares thresholds in an experiment where a single-barrel electrode was used in a bridge circuit to pass outward current through the cell. The first deflexion is the electrical artifact picked up from the crystal stimulator. After a delay of 7–8 msec. an action potential arose from the resting potential with no discernible inflection. (Electrical differentiation at short time-constants did not show a break on the rising phase.) Next, a long pulse of outward current was passed through the electrode. Bridge imbalance produces a large step in the potential record on which is super-imposed a slow depolarization leading to a spike. On the assumption that

Fig. 2. Determination of impulse threshold of soma using single microelectrode. In A a mechanical stimulus is followed by an intrasomatic pulse of outward current which produces a considerable bridge imbalance shift. In B the records have been realigned to eliminate most of the bridge artifact, and the level of the soma threshold is indicated by a dotted line. C, from the same cell, shows superimposed responses to three mechanical stimuli, one just subthreshold. The dotted line indicates soma threshold determined from A and B.
the equilibrium potential for the peak of the spike was not changed by the current pulse (Frank & Fuortes, 1956), the record was traced and realigned to place the peaks of the spikes at the same level (Fig. 2B) and a line was drawn in at the potential where the slow depolarization abruptly turned upward into the action potential. Finally, as shown in Fig. 2C, three mechanical stimuli of increasing strengths were applied to the cell and responses superimposed. Two of the stimuli produced spikes (at differing delays); the third stimulus was subthreshold. The dotted line indicates the spike threshold of the entire soma region of this cell as determined in Fig. 2B. A fourth sweep without stimulus shows the resting potential. This record shows that after a threshold stimulus there is no gradual fall of membrane potential toward the spike trigger threshold, and that the subthreshold stimulus did not detectably change the potential.

Fig. 3 is a record of a similar experiment. In this case a double-barrelled microelectrode was used, eliminating the problem of bridge artifact and the consequent necessity for shifting records along the potential axis. Fig. 3A is composed of two records, each of two traces. The upper member of each pair of traces is the microelectrode record; the lower is the record of the activity in the axon bundle of the whole P.D. organ. All the activity was in response to gentle tapping on the table. The upper pair of traces shows two action potentials followed by their axon response (indicated by arrows) after a slight delay due to conduction. Fig. 3B shows the same thing and is included to show a longer period of flat, silent base-line preceding the impulse. (Here the axon record coincided with the spike of another unit and is unrecognizable.) Note again the abrupt, steep rise of the potential out of the resting baseline. Figs. 3C and D, whose format is the same as Fig. 3A, show two responses of the same cell to long intrasomatic current pulses. Repetitive firing was elicited and each of the spikes recorded intracellularly in the soma was followed shortly by an axon spike in the bundle. Due to capacitive coupling between the barrels of the electrode, the potential level at which the very first spike discharged is uncertain, but all subsequent spikes were clearly preceded by a slow fall of potential to a value approximately 4-5 mV. below resting potential. The agreement in order of magnitude between the threshold of this cell and the one in Fig. 2 is quite good. The action potentials in the axon record show that the microelectrode in the soma did not degrade the performance of the cell to the point where it could not adequately propagate a signal out the axon to the C.N.S.

The next step was to show that action potentials can occur in the distal process of the P.D. bipolar cell. To this end experiments were undertaken to examine the effect of changes in the membrane potential on spike invasion into the soma. Fig. 4 shows the result of one such experiment, a result encountered much of the time. The record comprises five sweeps superimposed. One sweep was made without any stimulus to indicate the resting level; the other four first show a mechanical stimulus and the response thereto, and then an antidromic stimulus-response. A small depolarization of the soma merely shifts invasion of the mechanically produced spike to a slightly earlier time, but hyperpolarization eliminates the orthodromic response. At all levels the antidromic spike invades the soma. The mechanically evoked spike in this preparation failed in an all-or-none manner, without first becoming inflected and without evidence of a dendritic spike. This finding in isolation could fit with central spike initiation. The following experimental data cannot.
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Fig. 3. Determination of soma threshold in a different preparation using a double-barrelled microelectrode. Upper trace in each, intracellular record; lower trace, axon bundle record. A and B, mechanical stimuli; C and D, outward current. The dotted line indicates soma threshold. Arrows indicate axon spikes of the impaled cell.

Fig. 4. Effect of shifting membrane potential upon spike invasion into the soma. The orthodromic impulse, which appears first, disappears completely, although the antidromic spike still invades at all levels tested.

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Fig. 5 is a record of the results of an identical experiment on yet another cell. The responses to anti- and orthodromic stimuli have been traced and superimposed. Here the antidromic impulse shows a small inflexion even at resting potential that becomes more prominent with hyperpolarization until finally the antidromic spike cannot invade the soma and only a blocked axon spike remains. The action potential in

![Antidromic and Orthodromic responses](image)

Fig. 5. Effect of shifting membrane potential upon spike invasion into soma. In this cell both ortho- and antidromic invasion are blocked to yield similar electrotonic potentials, but at different membrane potentials.

response to mechanical stimulation is not inflected even at a hyperpolarization that blocks the antidromic spike altogether, yet it too is finally blocked by sufficient hyperpolarization to leave a potential very similar to the antidromic axon spike.

Fig. 6, from yet another cell, shows the third variety of result from such an experiment. Each trace again shows the responses to first an orthodromic and then an

![Mechanical responses](image)

Fig. 6. Effect of shifting membrane potential upon spike invasion into soma. In A, at resting potential, ortho- and antidromic impulses invade. A small hyperpolarization in B blocks invasion of the orthodromic impulse only, leaving a small electrotonic potential. Further hyperpolarization, as in C, eliminates completely the orthodromic impulse while leaving the antidromic axon spike.
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antidromic action potential. Fig. 6A is at resting potential; Fig. 6B slightly hyperpolarized and Fig. 6C strongly hyperpolarized. This cell, quite a sensitive one, often discharged two action potentials to mechanical stimulation (the third spike in Fig 6A was probably caused by unintentional vibration of the apparatus). At resting potential, spikes in response to either stimulus invade the soma, overshooting the zero level.

The first effect of hyperpolarization is to block the appearance of a full soma spike in response to a mechanical stimulus, though the antidromic potential invades the soma as well as ever. In this case the failure of a full action potential in response to mechanical stimulation results in the appearance of a small, blocked-spike potential. Note that when the soma is finally sufficiently hyperpolarized to block invasion of the antidromic action potential the small potential in response to mechanical stimulation has disappeared and, further, that in the case of this cell the blocked antidromic potential is much larger than that produced orthodromically.

In this cell it was possible to show directly the site at which the small blocked orthodromic response was generated. Fig. 7 shows the effect in this cell of passing a long-lasting current through the second barrel of the microelectrode to slightly
depolarize the soma. The upper member of each pair of traces is the microelectrode record from the soma; the lower is the record from the axon as recorded by the suction electrode. The record begins with the upper trace pair and continues with the lower pair (there is a break of several seconds between). The depolarizing current was kept on throughout the time shown. In response to this depolarization, the cell gave a series of impulses which began with all the spikes overshooting the zero level, fully invading the soma. Furthermore, the central record shows, at the arrows, that each overshooting soma spike is shortly followed by an axon spike. For some as yet unknown reason, the soma abruptly lost the capacity to generate action potentials, revealing a series of small potentials whose frequency of occurrence is approximately the same as that of the large spikes earlier. These small potentials are virtually identical with those produced by mechanical stimulation when this cell was hyperpolarized to block orthodromic invasion. They ceased to occur when the d.c. current was turned off. Since the all-or-none nature of these small potentials has already been shown, they must represent action potential activity in some part of the cell. That they are not produced in the axon is indicated not only by the fact that they differ in form from the blocked antidromic spikes, but also that when they take the place of the full-sized soma spikes, the corresponding axon spikes disappear from the central record (arrows aligned with these potentials do not show axon spikes).

Discussion

In any cell constructed like the P.D. bipolars one could reasonably expect that the action potentials are set up in the distal process close to the ultimate tip inside the scolioparium. This is the point at which the mechanical force is thought to impinge on the cell (Whitear, 1962), and any resulting generator current must spread over a great distance with little attenuation to elicit an action potential at or central to the soma. Triggering near the soma would require either a very large space constant in the distal process, or a very low potential threshold for spike initiation at the trigger zone. Since the soma action potential takes off quite abruptly from the resting level without detectable pre-potential (Mendelson, 1963; Mellon & Kennedy, 1964), only the latter possibility remained unless one accepted distal initiation. The present direct measurements of threshold in the soma region indicate the need for depolarizations of the order of 4–6 mV., and make the ultra-low threshold hypothesis untenable. Such a low threshold would also pose grave problems of stability.

Furthermore, detection of impulse activity clearly not generated in the soma or in the axon makes it clear that the distal process of the P.D. bipolar is capable of generating impulses. Production of such local impulse activity by mechanical stimuli makes it likely that the distal process is the locus at which impulses are initiated by adequate stimuli. The data at hand do not permit an assessment of where in the distal process this initiation occurs. In some cells hyperpolarization can eliminate the mechanically evoked action potential without the production of a local potential (Fig. 4). One might argue that in such a cell the trigger zone is indeed in the axon, but this requires a generator potential of 4–6 mV. in the soma. No such potential was observed. Examination of Figs. 4 and 5 reveals that mechanical stimulation of a hyperpolarized cell may
be accompanied by small phasic changes of the membrane potential (but cf. Fig. 6). It is expected that the recorded amplitude of a generator potential would be enhanced by hyperpolarization of the soma due to an increase of both the electrical gradient across the cell membrane and the gradient for current flow from the generator locus to the soma. If this is the case then it is clear that at resting potential in these cells the generator current in the soma and axon is much too small to accomplish spike triggering. Unfortunately it is equally likely that the potential changes seen in hyperpolarized cells are artifacts produced by changes in the membrane resistance caused in turn by relative motion of the electrode and the cell. These changes in resistance would interact with the hyperpolarizing current to produce potential changes. Movement of the cells due to mechanical stimulation could be observed through the microscope and no ready means was available to control this variable, so the problem of the nature of the generator currents remains unsolved.

Why can the orthodromic impulse be blocked in some cells without the production of a local potential? Quite possibly there are two populations of cells: in one group the spike trigger zone is electrically close to the soma so that hyperpolarization of the soma can spread far enough into the distal process to block the trigger zone and prevent impulse initiation by generator currents flowing from a mechanically sensitive site even farther out in the distal process; in the other group the spike trigger zone, as well as the generator region, is so far from the soma that impulses can still be initiated although they cannot enter the soma. (If the small potentials seen in hyperpolarized cells are indeed generator potentials, then one might hope that they would be more easily revealed in cells of the group that show complete orthodromic block upon hyperpolarization. Compare Figs. 4 and 6.)

Finally, one is tempted to speculate on whether a difference in location of trigger zones could have functional significance. The receptor cells of Callinectes P.D. organ appear to belong to two classes, those that are movement-sensitive and those that are position-sensitive, as has been the case in elastic joint organs of many other species (Wiersma & Boettiger, 1959; Wiersma, 1959; Bush, 1964). The property underlying this differing responsiveness is as yet unknown. If, however, one assumes that the generator currents set up in both classes of cells are like those in other sensory cells where they have been measured, then perhaps a likely explanation can be proposed. Where the spike trigger zone lies close to the generator site it will be exposed to the full dynamic phase of the generator potential as well as to a large steady depolarization during the static phase. Inactivation by the static depolarization would shut off spike firing; yet further movement, producing another dynamic peak, could elicit further spiking at a higher threshold. Such a cell would produce spikes only during the dynamic phases of generator potential, that is, during movement. Location of the spike trigger zone farther from the generator site would convey a smaller steady depolarization due to electrotonic decrement thus avoiding inactivation. Furthermore, if generator current must spread over a greater distance to depolarize the trigger zone, then the capacitance of the intervening membrane will attenuate and smooth off the dynamic phase of the generator response, reduce the response to movement and produce a response to position. During these experiments the necessary equipment was not available to distinguish movement-sensitive cells from position-sensitive cells, so a comparison of mechanical sensitivity with apparent distance to spike trigger zone cannot be made.
Although this hypothesis appears worth testing, the difficulty in impaling cells of the P.D. organ makes it unlikely that the necessary data will be available very soon.

**SUMMARY**

1. In bipolar neurons of the P.D. organ of *Callinectes sapidus* the initiation of impulses at the soma requires depolarization of at least 4–5 mV.

2. Impulses produced by mechanical stimulation of the organ arise abruptly from the resting (potential) level without prepotential, and it is impossible to demonstrate the occurrence of generator potentials of the magnitude required for spike triggering at or near the soma.

3. Anti- and orthodromic invasion of the soma may be differentially blocked by hyperpolarization; orthodromic block often reveals a small potential that appears to result from impulse activity in the distal process of the cell.

4. Blockade of the soma spike to leave only the electrotonic potential of the distal process results in interruption of axonal transmission in the impaled cell.

5. It is concluded that P.D. bipolar cells originate impulses in their distal processes with subsequent conduction through the soma into the axon.

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**REFERENCES**


