Site of Origin and Propagation of Spike in the Giant Neuron of *Aplysia*

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ABSTRACT The form and time sequence of spikes generated by orthodromic, antidromic, and direct stimulation and during spontaneous activity have been studied with intracellular electrodes simultaneously introduced in the soma and in different parts of the axon of the giant nerve cell of *Aplysia*. Evidence was obtained that under normal conditions of excitability, the spike originates at some distance from the soma in an axonal region with a higher excitability surpassing that of the surrounding membranes. Between the trigger zone and the soma is situated a region of transitional excitability where the conduction of the spike towards the soma may be blocked at a functionally determined and variable locus. The cell body is electrically excitable, but has the highest threshold of all parts of the neuron. The inactivation or even the removal of the cell body does not suppress synaptic transmission.

The use of modern electrophysiological methods allowing intracellular and extremely localized extracellular recordings has provided recent evidence of the heterogeneity of membrane properties in different regions of a single neuron. Thus, it was shown that the site of spike initiation is not to be found in the part of the neuron in which the synaptic contacts are most dense, but in a special region in which the intrinsic membrane excitability is particularly high. Suggestions by Gesell (12) and Bishop (3) that the spike takes origin in the axon hillock region having no or few synapses, received a splendid confirmation with the work of Araki and Otani (1), Fuortes, Frank, and Becker (11), Coombs, Curtis, and Eccles (4, 5), and others on the vertebrate spinal motoneuron. In the crustacean stretch receptor Edwards and Ottoson (8) have localized the site of spike initiation in the axon relatively far from the cell body, and Diamond, Gray, and Sato (6) have concluded that the discharge of the Pacinian corpuscle originates in the first axonal node.

The *Aplysia* giant neuron (GN) with its large cell body (300 to 800 μ in

diameter) and big axonal process having no ramification in the proximity of the soma (14) offered itself as a very suitable object for the study of spike initiation. In addition the easy accessibility of this neuron permitted work under direct visual control, usually impossible in vertebrate preparations. It was therefore possible to introduce microelectrodes into the cell body and the axon and to record the electrical activity simultaneously in both structures. The results presented and analyzed in this paper show that for all modes of stimulation, the efferent spike takes its origin in the axon rather far from the cell body. Three preliminary reports on this subject have already been published (19–21).

METHODS

The abdominal ganglion of *Aplysia depilans* (Mollusca: Gastropoda) was carefully dissected from the animal, with the two connectives going to the upper ganglia being preserved, immersed into sea water, and the giant nerve cell exposed by fine dissection of the overlying conjunctive membrane. As usual in molluscs, the cell body is spheroidal with no somatic dendrites and the axonal process is devoid of myelin sheath. Intracellular potentials were recorded by capillary microelectrodes less than 1 μ filled with 2.5 M KCl; alternatively in some experiments double barreled electrodes were employed (15, 16). Applying a suitable current through one of the intracellular electrodes through a 100 MΩ series resistor permitted the membrane potential to be preset to any desired level and consequently to change the excitability of the cell. The cell body was impaled under visual control, whereas the penetration of the axon in the neuropile was verified by the presence of electrical activity synchronous with that in the cell body (for the path of the giant cell axons, see reference 14). Several experiments permitted the determination, by this method, of the position of the axon in the neuropile and these data were used to estimate the distance of the axonal electrode from the cell body. Silver wire stimulating electrodes were applied to both pleurovisceral connectives. The GN was orthodromically activated by stimulation of the left connective. With the axonal process of the GN present in the right connective as a big central nerve fiber, the stimulation of this connective was used to initiate an antidromic spike (17). The efferent response of the GN was picked up from this connective by wire electrodes at a distance 10 to 15 mm from the ganglion. Because of the diameter of the GN axon, much bigger than the other nerve fibers in this connective, the spike of the GN fiber has a relatively high amplitude, surpassing by five to ten times that of the other spikes and therefore may be recognized selectively.

RESULTS

A *Antidromic Responses*

It was shown (17) that the antidromic spike has an inflection in its rising phase when recorded in the soma (tracings in Fig. 1, *5*). When the excitabil-

ity of the cell is lowered either by applying hyperpolarizing current or by fatigue, this inflection is emphasized and two components may be clearly distinguished (s in Fig. 1, *3* and *4*): a prepotential of 20 to 35 mv followed after a variable delay by a large spike potential of 80 to 120 mv. It was proposed (17) that these two components indicate a two stage invasion of the neuron: the prepotential (or A spike) being produced, when the antidromic spike is blocked in the axon at some distance from the soma, while the large

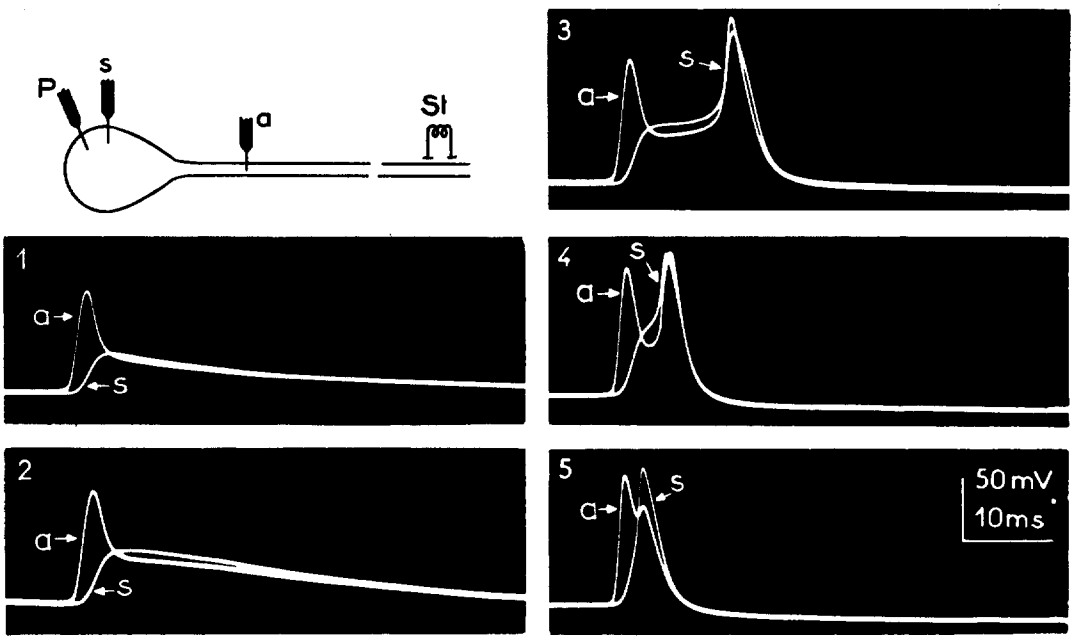

FIGURE 1. Simultaneous intracellular recordings with microelectrodes inserted in the soma (*s*) and axon (*a*) at 700 μ from the soma. The records are superimposed. The cell was slightly hyperpolarized (electrode P) in *1* and from *2* to *5* progressively brought to normal polarization. Antidromic stimulation evokes A spike alone in *1*, A and S spikes in *3* to *5* with different A–S intervals. Note in *1* the equal form of the membrane electrotonic recharge recorded in *a* and *s*; the difference of this recharge observed in *2* is due to the presence of a local somatic response.

potential (or S spike) is produced by the discharge of the soma. Thus, in spite of significant anatomical differences an analogy may be found between the GN and the vertebrate spinal motoneuron. The A spike of the GN would be equivalent to the initial segment potential (IS spike of Coombs *et al.* (4) and A spike of Fuortes *et al.* (11), and the S potential to the soma-dendrite spike (SD spike of Coombs *et al.* and B spike of Fuortes *et al.*).

It would therefore be expected that the respective sizes of A and S spikes would be reversed if the potentials are recorded not in the soma, but in the axon at some distance from the soma. Actually, the axonal records are com-

plex and do not result only from an A-S amplitude inversion. Several important observations can be made in the simultaneous intraaxonal and intrasomatic records presented in Fig. 1. There is a significant delay between A spikes recorded in the axon (*a*) at 700 μ from the cell body and in the soma (*s*). This delay suggests an active conduction of the A spike between *a* and *s*: the membrane region in *a* where the axonal electrode is inserted, would therefore be active during the A spike. However, the amplitude of the A spike recorded in the axon at *a* does not reach the expected value and is smaller than the size of the S spike in *s*. Other experiments have shown that the amplitude of A spike is still smaller when recorded closer to the soma. Since the membrane in *a* is active, the blocking of an A spike must occur between *a* and *s*. The depression of an A spike may be explained by the strong loading effect of the non-excited somatic membrane on the A spike generator. This current flow would be particularly intense in the proximity of the cell body and indeed experimental data have confirmed that the A spike is progressively more and more depressed when approaching the soma. The decrease of the A spike amplitude together with a possible change in membrane excitability results in a progressive diminution of its safety factor for continued conduction; this latter may become insufficient in the proximity of the soma in which case conduction of the A spike is blocked. It may be further shown that the region where the conduction fails may be located more or less distant from the soma, as may be concluded from the differences in amplitude of the A spike recorded in *a* (Fig. 1). The A spike becomes greater with the increase in excitability expressed by the diminution of the interval for A-S transmission; presumably the conditions of excitability are better for a further advance of the A spike towards the soma.

The presence of a non-excited somatic membrane is also responsible for the long duration of the afterdepolarization which follows the A spike recorded in the axon as well as in the soma (Fig. 1, *1*). It is clear that this represents the charging of the membrane not activated by the A spike. During this time, local responses (called basic potentials by Tauc, 18) may evolve in this latter membrane, summing with the after depolarization and increasing the amplitude of the response recorded in the soma (Fig. 1, *2*). The local responses when reaching the critical amplitude develop into the somatic spike (Fig. 1, *3–5*). The depolarizing afterpotential is abolished by the discharge, which gives good evidence that the same membrane is involved.

The equal amplitudes and similar forms of the after depolarization recorded in *a* and *s* (Fig. 1, *1*) point to a large space constant between these two regions. When a square current pulse is applied by intracellular electrode to the somatic membrane, the potential change recorded in the axon compared with that in the soma (Fig. 2) is only about 15 per cent smaller, the axonal electrode being at 700 μ from the cell. Consequently, we can assign the space constant a value of several millimeters.

Signs of somatic discharges, as seen on the axonal recordings, depend on the somatic spike latency: when the interval A–S is brief, the S component is small (Fig. 1, *5*; Fig. 3, *1*); however, the amplitude of the S component increases with a longer duration of the A-S interval (Fig. 3, *2–4*), till it reaches the amplitude of the somatic spike recorded in *s* (Fig. 1, *3–4*; Fig. 3, *5*). Fig. 3 also shows that when the S component in axonal recording reaches a definite value, an efferent response is sent down the axon (Fig. 3, *4*). It was assumed before (17) that this unexpected efferent response to antidromic stimulation could be initiated as soon as the duration of the A–S interval is longer than the absolute refractory period of the A spike membrane responsible for the A spike; so that the axonal region can be excited again by the somatic sink.

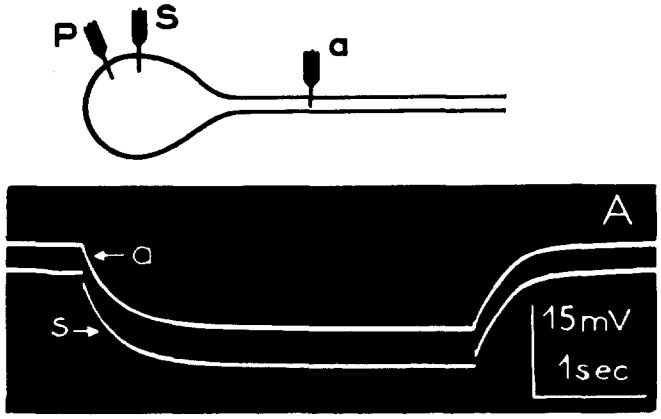

FIGURE 2. Comparison of the electrotonic charging of the somatic (recorded in *s*) and axonal (recorded in *a* at 700 μ from the soma) membranes by a square inward current pulse applied to the soma by electrode P. Simultaneous recordings.

Let us call A′ spike the S component recorded in the axon; it actually results from two factors: a passive and an active one. The passive factor is present alone during the absolute refractory period; since this period is simultaneous with the falling phase of the A spike during which great changes of conductance take place (9), the amplitude of the passive potential will be first small and will increase towards the end of the absolute refractory period. A′ represents at this moment only a passive electrotonic phenomenon, its size for the same A–S interval will decrease exponentially as the distance from the soma. In contrast, the amplitude of the active component of A′, which is present after the absolute refractory period, is less dependent on the spatial factor. When the A–S interval surpasses the relative refractory period of the A membrane, the amplitude of A′ is the same as that of the S spike recorded in the soma (Fig. 3, *5*; Fig. 4, *5*). At this moment the amplitude of A′ is even greater than the amplitude of the A spike recorded in the same place. Presumably when the soma and axon discharge nearly simultaneously there is less loading effect of the one on the other.

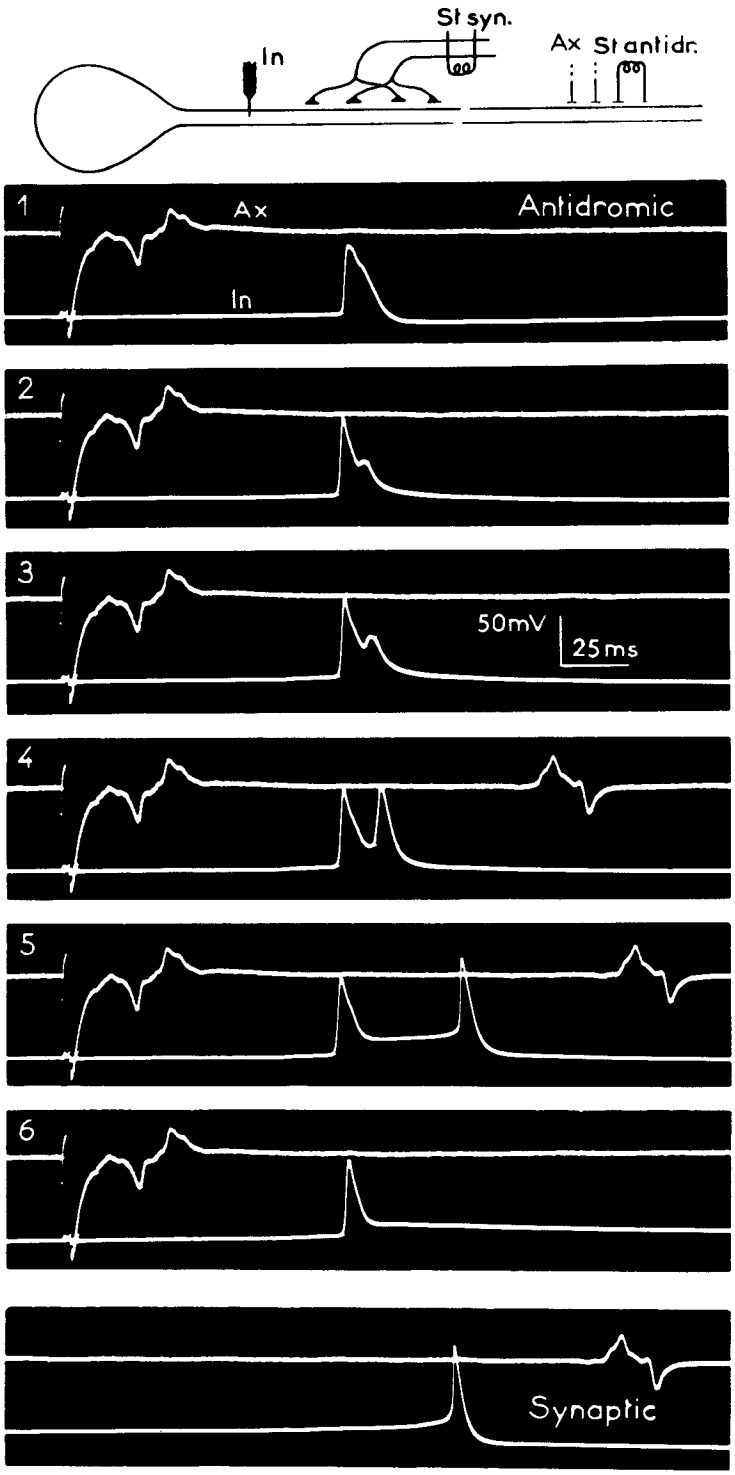
St syn.
In
Ax
St antidr.
1
Ax
Antidromic
In
2
3
50mV
25ms
4
5
6
Synaptic

FIG. 3

It would be expected that the reactivation of the axonal region can only occur after the somatic discharge has started. This is only true when the A–S interval does not go beyond the relative refractory period, as is the case in Fig. 4, *4* where S precedes A′. But when the A–S interval increases, the time by which S precedes A′ is shorter, and finally drops to zero (Fig. 4, *5*). The positions are even reversed, A′ then preceding the S spike (Fig. 4, *6*) when the A–S interval surpasses 30 to 40 ms. It seems impossible to explain this advance of the axonal discharge without assuming an intrinsic membrane excitability of the axonal region superior to the excitability of the somatic membrane. The intracellular responses in Fig. 4, *6* indicate that the depolarization of the somatic membrane by an A spike is maintained largely beyond the time of a simple electrotonic charge due to the development of a local response. Because of the large space constant, this depolarization is electrotonically communicated to the already discharged axonal membrane which, having a threshold lower than the somatic membrane, discharges again before the soma at the end of its refractory period. The soma now responds after a very brief delay, having been strongly facilitated. It may be noted that the amplitude of the second A spike in Fig. 4, *6*, is smaller than that of the S spike whereas they have the same size in Fig. 4, *5*. Obviously the smaller amplitude of the second A spike in Fig. 4, *6*, compared with that in Fig. 4, *5* results from the slight delay in the somatic discharge, leaving the A membrane for a short moment under the influence of the loading effect of the soma.

B *Orthodromic Response*

It is known (13) that in the molluscan neurons the synaptic contacts are located on the axon in the neuropile at some distance from the soma, which is supposed to be completely devoid of them. Since the presence in the axon of a membrane region of high excitability has been demonstrated, it may be anticipated that the orthodromic response will be initiated in that region. This does really happen and the records of Figs. 4 B and 5 show clearly that the axonal response precedes the somatic spike. Because of the brief of virtually non existent A–S interval due to a previous facilitation by excitatory postsynaptic potentials (EPSP's), the A′ spike is small or absent. However, with fatigue, a dissociation of A and S components is observed and the records resemble the antidromically evoked responses (Fig. 5 B, *4*). It may also be

FIGURE 3. Response of the giant cell picked up simultaneously by wire electrodes (Ax) on the axon at 12 mm from the ganglion and with intracellular electrode (In) in the axon at 800 μ from the soma. *1* to *6*, antidromic stimulation. From *1–5* the A–S interval becomes progressively longer through fatigue and the amplitude of A′ spike (see text) increases. No somatic response in *6*. Note the presence of an efferent recurrent spike in *4* and *5*. Lowest record, synaptic excitation recorded under the same experimental conditions.

seen that the EPSP's appear in the record at 2 mm from the soma (Fig. 5 B), whereas they are absent from the somatic records. This confirms the anatomical data about the axonal localization of the synaptic scale.

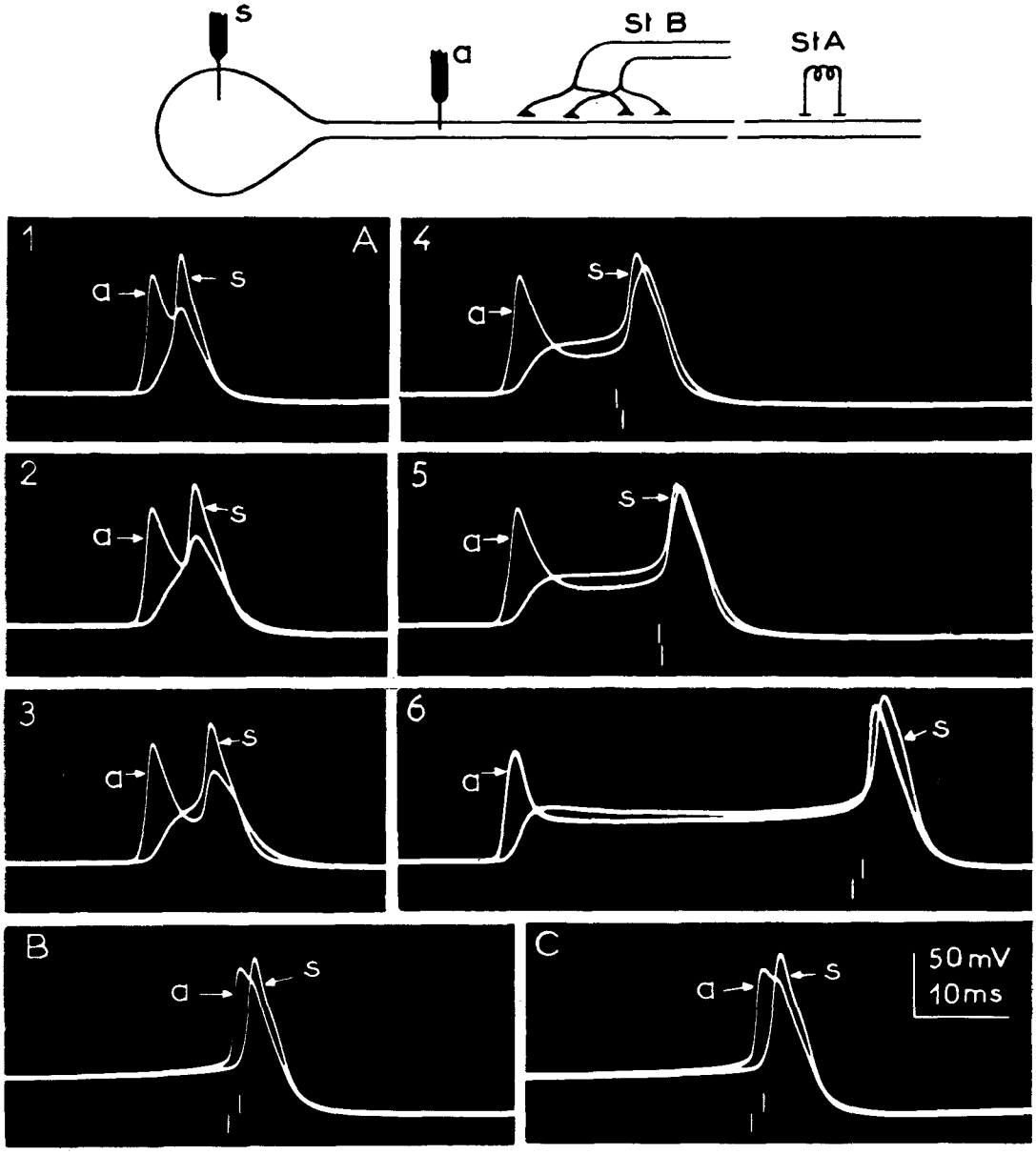

FIGURE 4. Antidromic (A), synaptic (B), and spontaneous (C) spikes simultaneously recorded in the soma (*s*) and the axon (*a*) at 800 μ from the soma. In A, from *1* to *6*, an increase in A–S interval due to fatigue is paralleled by an increase in the amplitude of the A′ spike. Note the inversion of time sequence between S and A′ spikes in *4* to *6*. In B and C the axonal spike clearly precedes the somatic response.

C *Responses to Direct Stimulation and Spontaneous Activity*

If the neuron is activated directly by a brief current pulse through intracellular electrodes inserted in the soma, the spike recorded in the axonal region precedes the somatic response, although the electrotonic gradient favors the

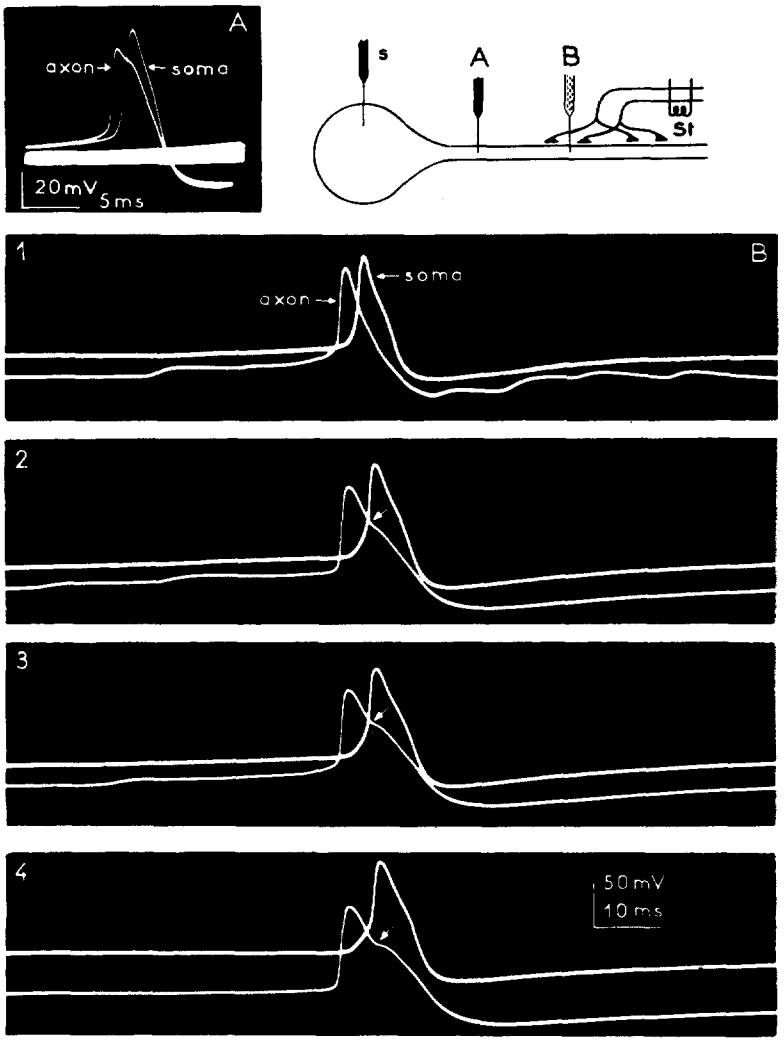

FIGURE 5. Simultaneous intracellular recordings from the soma and axon of the giant cell stimulated orthodromically (the stimulus artifact in B is outside the record). The distance of the axonal electrode is 350 μ in A, 2000 μ in B, A and B being taken from different neurons. In B, from *1* to *4*, A-S interval increasing because of fatigue. Note that the responses in *3* and *4* are similar to an antidromically evoked spike (compare to Fig. 4A, *1*). Excitatory postsynaptic potentials (decreasing with fatigue) can be seen on the record taken from the axonal region, whereas they are absent on the somatic record.

soma. If strong stimuli are applied, the interval between axonal and somatic responses is small (Fig. 6 B), but if the delay of the response increases, the interval between the two recordings also increases (Fig. 6 C). At this moment the records are similar to those obtained during rhythmic activity induced by the depolarization of the soma (Fig. 6 D). Only exceptionally strong direct

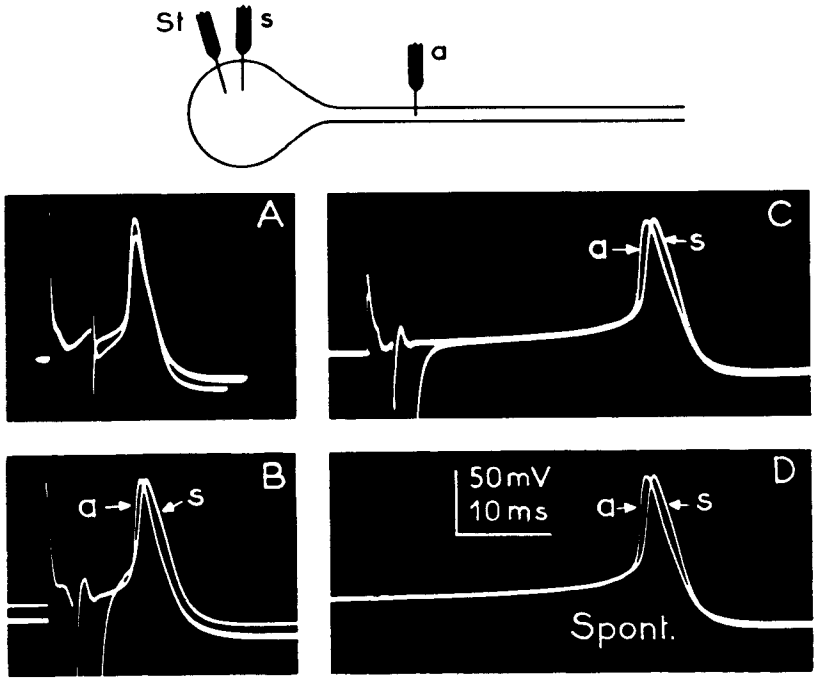

FIGURE 6. Time sequence of spikes simultaneously recorded in the soma (*s*) and in the axon (*a*) at 600 μ from the soma, evoked by direct stimulation by a transient current pulse applied by an additional electrode (*St*) inserted in the soma. In A and B, responses to relatively strong stimuli in different cells. In C the reduced intensity of the stimulus increases the latency of the spike and the delay between the spikes, if compared to B. The time sequence of spikes during spontaneous activity recorded in D is similar to C; B, C, and D being the responses of the same cell. The axonal and somatic responses are superimposed.

stimuli can produce spikes which appear simultaneously in the axon and in the soma (Fig. 6 A).

The interval between spikes recorded in *a* and *s* during spontaneous activity is particularly clear in Fig. 4 C, where the records are similar to those evoked orthodromically. Consequently the pacemaker of spontaneous activities is located in an axon region which presumably has the same localization as the site from which orthodromically evoked responses are initiated. However, the site of spike initiation during direct or spontaneous activity may spread in area or perhaps even shift towards the soma under different conditions of

excitability. When rhythmic activity is evoked by depolarizing the membrane with current applied through an intrasomatic microelectrode, the delay between axonal and somatic spikes is brief for the first response but increases progressively with time. Probably under the effect of accommodation, the level of excitability decreases progressively in the region influenced by the applied electrotonic gradient and the pacemaker is either made narrower or shifted further into the axon.

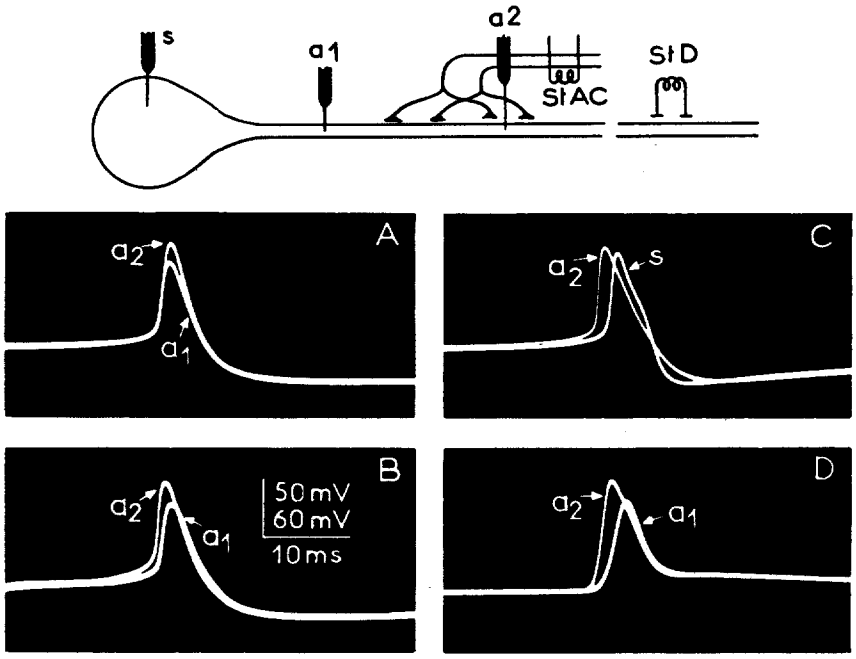

FIGURE 7. Time sequence of spikes recorded in the soma (s) and in the axon at 800 μ (a_1) and 2000 μ (a_2) from the soma. In A and B, orthodromic stimulation, electrodes in a_1 and a_2. In C orthodromic stimulation, electrodes in a_2 and s. In D antidromic stimulation, electrodes in a_1 and a_2. The records are superimposed. Further description in text.

D *Localization of the Site of Origin of the Spike*

An approximate localization of the site of origin of the spike in the axon was made possible by an experiment in which electrodes were introduced in two different places in the axon at about 800 (a_1) and 2,000 (a_2) microns from the cell body and in the soma itself. It was observed that in orthodromic activation the spike in a_2 slightly precedes that recorded in a_1 (Figs. 7 B, 8 A, 9 C); exceptionally the spikes recorded in those two regions appeared simultaneously (Fig. 7 A). The interval a_2–a_1 for orthodromic responses (Fig. 7 B) is smaller than the delay for the antidromically evoked spike (Fig. 7 D). It may therefore be concluded that the spike is not initiated further than a_2, but

between a_2 and a_1, usually closer to a_2, approximatively 1.5 mm from the soma.

It may also be seen that the EPSP's recorded at a_2 are slightly greater than those from a_1 (Fig. 8 B), with an exception marked by an arrow. The synaptic contacts are therefore to be located between a_1 and a_2 and are most dense around a_2. Consequently the spike appears to be initiated very close to or

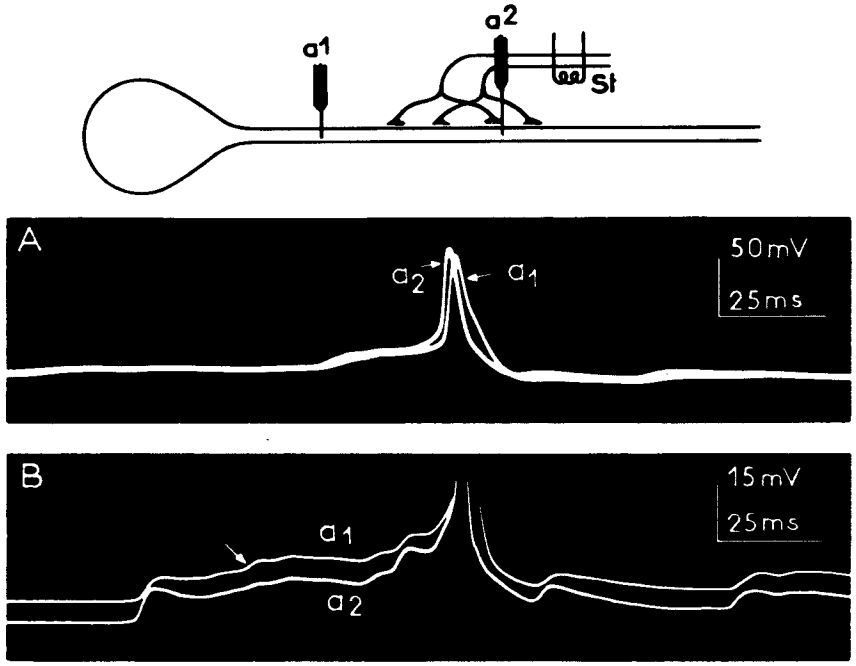

FIGURE 8. Responses to orthodromic stimulation of the giant cell (EPSP and spikes) simultaneously recorded in the axon at 800 μ (a_1) and 2000 μ (a_2) from the soma. In A the records are superimposed. The arrow in B indicates an EPSP taking origin closer to a_1 and therefore having higher amplitude in the a_1 record.

even in the region of the synaptic field. It does not seem that the excitability of the membrane is diminished by the presence of synapses; the contrary seems to be true. Further it may be shown that the interval a_2–a_1 for orthodromic responses (Fig. 9 C, *1*) and for the spontaneous spike (Fig. 9 B) is the same; the pacemaker of spontaneous activity is therefore located in the same region of the axonal synaptic field.

The diminution of the passive effect of the somatic spike with increasing distance from the soma may be demonstrated by comparing the records of Fig. 9 C. The amplitude of A′ is much smaller in a_2 than in a_1 although the spike in a_1 appears later.

E *Transmission Occurring without the Soma*

Since the spike is initiated in the axon which possesses all the synapses, it might be expected that the soma has no major functions in synaptic transmission. Indeed as is illustrated in Fig. 10, the removal of the cell body does not suppress an efferent response to orthodromic stimulation. A double control

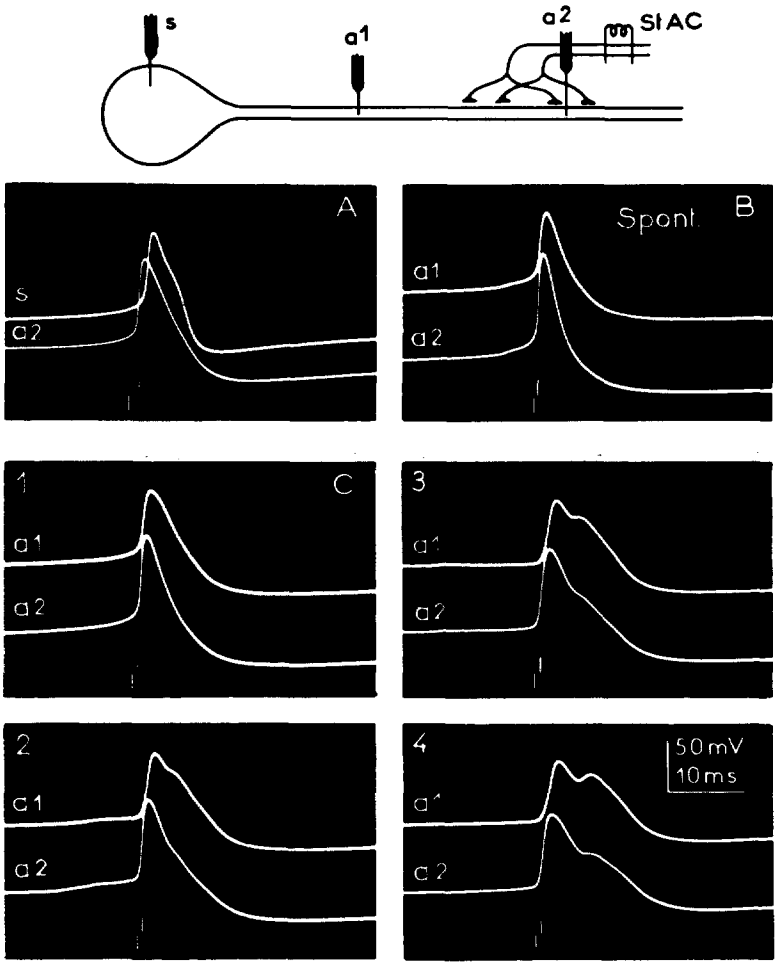

FIGURE 9. Time sequence of spikes simultaneously recorded in the soma (s) and in the axon at 800 μ (a_1) and 2000 μ (a_2) from the soma. A, orthodromic stimulation, electrodes in s and a_2. B, spontaneous activity, electrodes in a_1 and a_2. C, orthodromic stimulation, electrodes in a_1 and a_2. Note that the response in C*1* is similar to that in B. From C*1* to *4* increasing fatigue delays the somatic response; the passive A′ potential (see text) which is simultaneous with the somatic response, is smaller in a_2 than in a_1.

was made (Fig. 10) by intraaxonal recording at 600 microns from the soma (R″) and by picking up the efferent spike on the right connective at 12 mm from the ganglion (R′). After the section of the cell body, the orthodromic response remains but the A′ potential, indicator of the somatic activity, clearly visible on the axonal spike before operation (Fig. 10 A, *1*), disappears afterwards (Fig. 10 A, *2*). Furthermore, due to the injury, the resting potential becomes smaller and the conduction time of the efferent spike is increased (Fig. 10, compare A, *1* and A, *2*). Antidromically evoked spikes in Fig. 10 B are identical to the orthodromic responses except for the level of firing due to

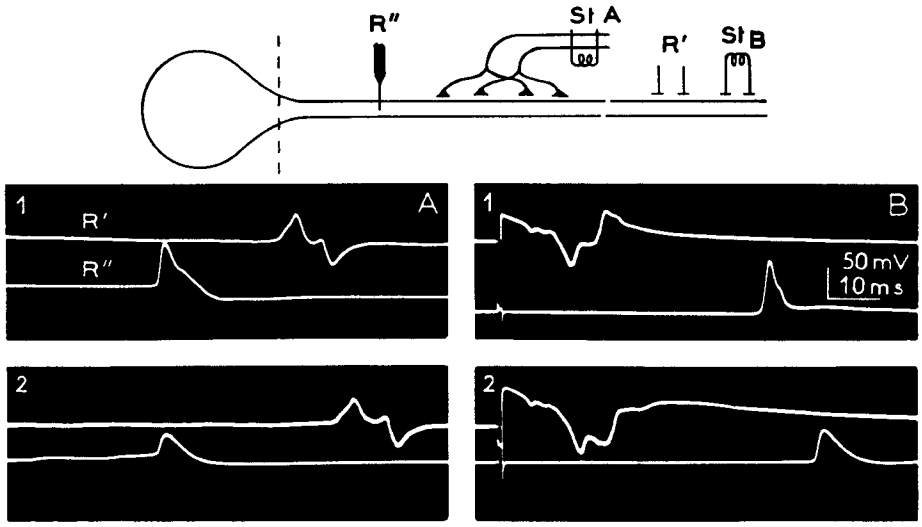

FIGURE 10. Responses to orthodromic (A) and antidromic (B) stimulations recorded intracellularly in the axon (R″) at 600 μ from the soma and picked up with wire electrodes (R′) on the axon at 12 mm from the cell body. A, *1* and B, *1* are recorded before, A, *2* and B, *2* after the removal of the cell body by section. Note in A, *2* that the absence of the soma does not suppress the synaptic transmission. The stimulus artifact in A is outside the record.

the EPSP, and prove the all or none character of the response. However, the removal of the soma causes serious injury to the neuron and the synaptic transmission will usually not be possible for more than 30 minutes.

The exclusion of the cell body from the process of synaptic transmission may be obtained by a less direct method, which avoids its destruction. It is indeed possible to inactivate selectively the soma by applying hyperpolarizing current of critical value. In this condition, it is possible to suppress the somatic response to orthodromic stimulation, yet the A spike may persist and give an afferent response (Fig. 11 D). Under less critical conditions a clear dissociation of the A and S components obtained in response to synaptic stimulation may take place (Fig. 11 B, C) so that the intrasomatic records will be similar to antidromically evoked spikes (Fig. 11 E). The efferent re-

sponse is undoubtedly engendered by the A spike, as may be seen from the delays of axonal spikes picked up far on the axon (Fig. 11, upper lines). It is remarkable to observe two afferent responses to one A–S complex if the A–S

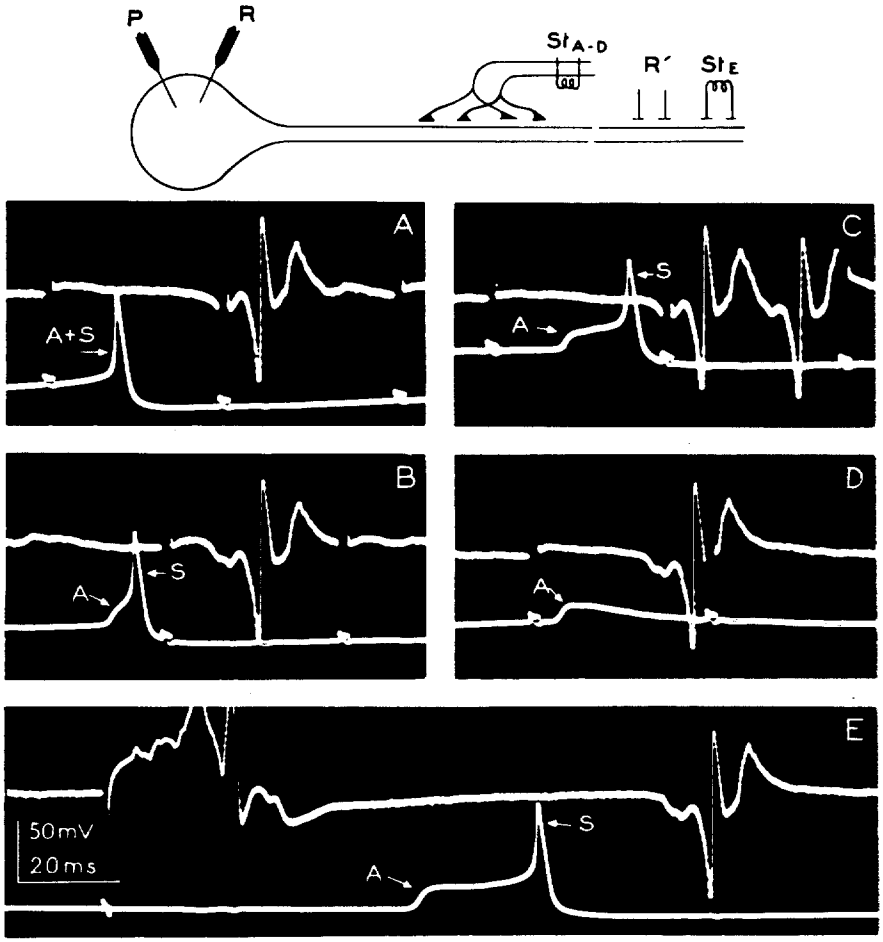

FIGURE 11. Responses of the giant cell to orthodromic (A–D) and antidromic (E) stimulations, recorded with an intrasomatic electrode (R) and picked up on the axon with wire electrodes (R′) at a distance of 12 mm from the cell body. In A to D the orthodromic response necessitates a repetitive stimulation. The intracellular electrode P is used to preset the membrane potential to a desired level. A, no polarizing current, the somatic spike presents a simple form. B, under slight hyperpolarization the orthodromic spike shows the A and S components. The axonal potential in R′ follows the A potential at the same interval (compare with record in A). C, increase of polarization increases the A–S interval of the intracellular spike. The axonal record shows two responses corresponding respectively to the A and S spikes. D, further increase of polarization inhibits the somatic discharge; however, the A potential remains and gives origin to one afferent spike. E, antidromically evoked response shows a long A–S interval similar to that recorded in C. Note the presence on the axon of a recurrent efferent response.

interval is sufficiently great, so that the S spike is able to reactivate the axonal A region.

If a microelectrode is introduced into a soma whose axon has been removed, no reaction is seen to orthodromic stimulation, as obviously no synaptic contact is present on the somatic membrane. It is, however, possible to stimulate directly an isolated soma. Because of the injury, strong depolarizing currents are necessary to evoke a response which is then of an oscillatory nature, but true spikes are produced which have the usual amplitude. From this we can

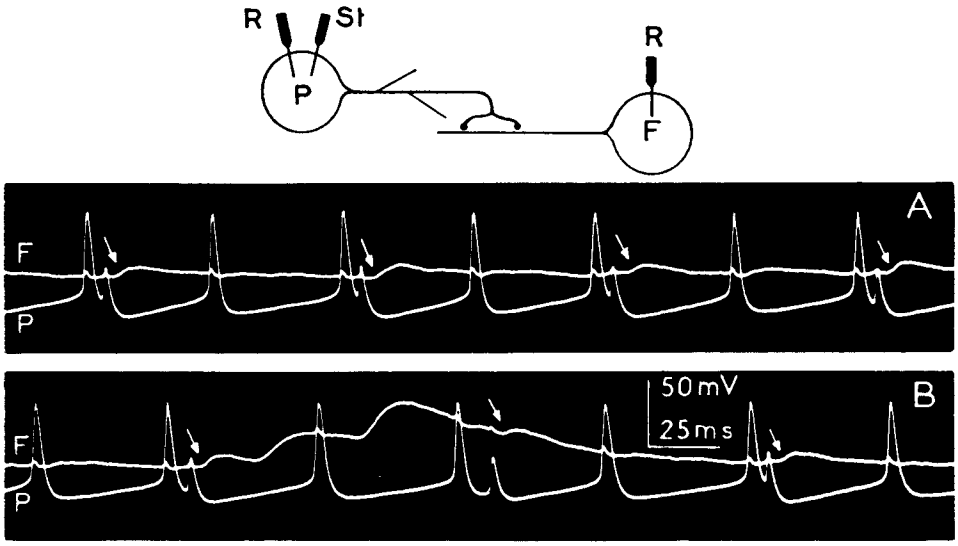

FIGURE 12. A, simultaneous intracellular recordings from two different nerve cells, one of which (F) receives the efferent fibers of the other (P). The neuron P is artificially depolarized by a current applied through electrode St and shows rhythmic activity during which appear, besides the spike, smaller separate A spikes. Note that in the cell F the excitatory postsynaptic potentials (arrows) appear only following the A spikes, indicating that the somatic discharge does not activate the axons coming to the cell F. The big EPSP's in B are due to input coming from another direction.

deduce that the somatic membrane is an electrically excitable structure similar to the axonal membrane.

It was shown (17) that during rhythmic activity evoked in the GN by a strong depolarizing current applied to the soma membrane, the somatic response did not always give an efferent spike. It may be assumed that the depolarization brought into existence a pacemaker in the cell body or in the axon hillock, which apparently was able to discharge at a higher frequency than the more distant axonal region. In this condition the normal way of AS activation is reversed and the soma discharges before the axon. The discontinuity is, however, maintained, so that the A activation may occur with some delay and sometimes completely fails. In the experiment illustrated in Fig. 12,

two different neurons were penetrated, one of which (F) receives the efferent fibers of the other (P). It can be seen in F from the alternate presence of EPSP's indicating an efferent activity of P, that in P the efferent response is not initiated by the S spike, but by an A spike which follows only every second S spike. So, paradoxically the less excitable somatic or axon hillock membrane seems to be able to maintain a higher spike frequency than the natural axonal pacemaker membrane. It may be observed, however, that in spite of the electrotonic drop, the depolarizing current more easily inactivates the axonal membrane, with its low threshold, than the less excitable somatic membrane.

DISCUSSION

In a delicate experiment, Bethe (2) succeeded in showing that the presence of the cell bodies of afferent neurons is not indispensable for the reflex movement of the crab antenna. This observation was confirmed by Young (22) for the axo-axonal transmission in the stellate ganglion of the squid. Our results obtained by electrophysiological methods agree with those of Bethe and Young, but disclose in addition that the axonal origin of the spike is not due simply to the axonal localization of the synaptic field, but to a difference in excitability of the somatic and axonal membranes. Fuortes *et al.* (11) and Coombs *et al.* (4) arrived at a similar conclusion for the vertebrate spinal motoneurons. The schema of Fig. 13, similar to a diagram of Coombs *et al.* (4), displays on an hypothetical basis the distribution of excitability levels along the structures. The membrane region with the lowest threshold, where the spike is initiated, is to be found at a distance of about 1.5 mm from the soma. The spike propagates normally away from the soma, whereas when approaching the soma its amplitude is depressed by the loading effect of the non-excited somatic membrane and, if the safety factor becomes too small, the spike is blocked in the vicinity of the soma. The upper solid line in Fig. 13 indicates the maximum amplitude of the spike recorded at different distances from the soma when the spike is blocked in the axon in the area marked by an arrow.

The A spike acts as an electrical stimulus for the undischarged axonal and somatic membrane, where local responses develop during the A-S interval. The somatic discharge occurs with a delay shorter for orthodromic than for antidromic activation because of a larger initial depolarization due to the EPSP's. The amplitude of the S spike recorded in the soma is higher than the amplitude of the A spike recorded in the axon. Presumably during the S spike, the loading effect of the axon on the S spike generator is small.

The expected similarity of electrical events during activity of the GN and spinal motoneuron is confirmed only in its main aspects. The depression of the IS spike amplitude when recorded closer to the soma, as described by

Coombs and collaborators (4), was verified in our preparations thanks to the possibility of inserting the electrodes into the axon at any desired place. However, because of the greater time constant (about 100 to 200 msec. (9) against 4 msec. in the motoneuron) the A–S interval in GN may reach considerable values even with orthodromic stimulation; thus not only passive, but also active repercussions of the somatic discharge are seen in the axon (see paragraph *a* of the Results); the axonal region may be reactivated by the S spike and an efferent impulse elicited by antidromic stimulation. But for a

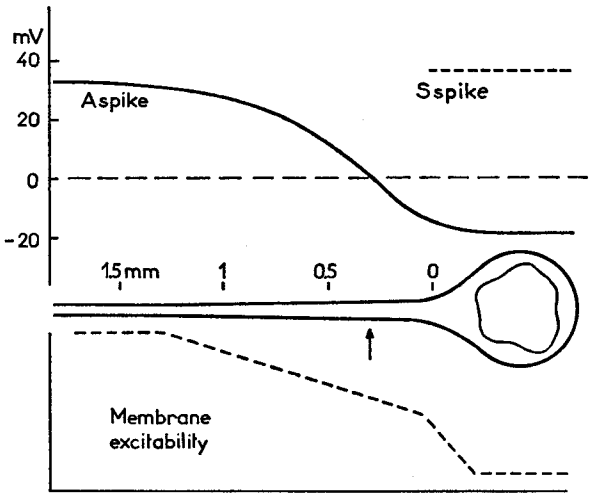

FIGURE 13. Schematic representation of the maximal height of the A spike (continuous upper line), recorded in the axon at different distances from the soma, indicated in millimeters. The upper horizontal broken line represents the maximal amplitude of the S spike recorded in the soma. The resting potential is the same all over the soma and proximal axon and has a value of about −60 mv. The lower dotted line represents an hypothetical distribution of the relative values of the membrane excitability in different regions of the neuron. The arrow indicates the probable area where the antidromically evoked spike is blocked under normal conditions of membrane polarization.

very long A–S interval, the S spike is not even necessary to reactivate the axonal region. Subliminal local responses occurring in the somatic membrane after the A spike are able to maintain a sufficient depolarization, electrotonically communicated to the axonal membrane. This membrane, because of its high excitability, can give rise to a second discharge preceding that of the cell body (Fig. 4 A, *6*). The above data and the fact that the response of the axon hillock is included in the S spike give an explanation for the similarity of critical levels for the orthodromically, directly or synaptically evoked spikes, as pointed out in a former publication (11), especially when the antidromic S spike was considered after a long A–S interval.

The site of origin of the spike in the GN seems to be located rather far from the soma, although the distance represents only three or four times the diameter of the cell body. Between that area and the soma, an intermediary region exists where the spike is conducted and may be blocked. Yet no rigid barrier for the conduction is present (paragraph *A* of the Results). The blocking may occur not only because of the decrease in the size of the spike, but also because of the drop of membrane excitability in the vicinity of the soma. It seems that this decrease is progressive, since we know that the spike may be blocked at a variable distance from the soma, and also that the "trigger zone" may become larger or shift towards the soma during direct stimulation (paragraph *C* of the Results). This finding supports the assumption of Fuortes *et al.* (11) who proposed a transitional excitability zone between the initial segment and the soma in the spinal motoneuron, whereas the model of Coombs *et al.* (4) postulates a sharp transition of thresholds. Nevertheless, a rather abrupt decrease in excitability must exist on the axo-somatic boundary, the soma having undoubtedly a very high threshold as even very strong electrical stimuli applied to the soma initiate the spike in the proximal axon.

The experiments with an isolated cell body confirm the excitability of the somatic membrane. Older experiments (16) have shown that after fatigue or during applied hyperpolarizing current, the S spike may reach different amplitudes, which, in view of the present experiments, suggest that the spike does not invade the whole somatic membrane. It cannot be decided whether during this partial response, the activated region is that contiguous with the axon hillock or corresponds to some patch or patches of different localization. The latter hypothesis is not excluded since, because of the relative values of the resistance of the soma membrane and cytoplasm, the cell body is very nearly isopotential.

In contrast to the motoneuron, the region of GN with lowest threshold is located in the area in which the synaptic contacts are most dense. It seems therefore that the presence of synapses is not a factor determining a lower excitability of the membrane as was suggested for the spinal motoneuron by Eccles (7): yet anatomical data are lacking and the presence of axonal dendrites is not excluded.

The proximity of the synaptic field to the site of initiation of the spike is not an obstacle for neuronal integration. It was shown (Fig. 2) that the space constant is very great and therefore the electrotonic effects are only slightly reduced between the pacemaker and the soma. For a synaptically evoked spike recorded in the axon and in the soma, the apparent critical potentials are practically the same and, although the individual synaptic potentials are not always visible in the soma (Fig. 5), the total depolarization which they produce is nearly identical in both regions. The presence of a somatic membrane of large surface in the giant neuron has a great influence upon the

integration of synaptic events: the currents coming from the extrasynaptic somatic and, to a lesser degree, axonal membranes depress considerably the amplitude of the EPSP's or IPSP's and minimize their local action. But on the other hand, the duration of PSP's is considerably increased beyond the time of the effective transmitter action so that their influence exceeds several hundreds of milliseconds (10, 18). In small *Aplysia* cells with a time constant of only a few milliseconds, the PSP duration is relatively brief (50 msec.) and their integrative properties are different from those of GN.

It may be concluded that in the central nervous cell of *Aplysia* and under normal conditions of excitability, the spike originates at some distance from the soma in an axonal region, because its higher excitability surpasses that of the surrounding membranes. Between the site of origin of the spike and the soma is situated a region of transitional excitability where the conduction of the spike towards the soma may be blocked at a functionally determined and variable locus. The cell body is electrically excitable but has the highest threshold of all parts of the neuron.

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