The Mechanism of Discharge Pattern Formation in Crayfish Interneurons

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ABSTRACT Excitatory and inhibitory processes which result in the generation of output impulses were analyzed in single crayfish interneurons by using intracellular recording and membrane polarizing techniques. Individual spikes which are initiated orthodromically in axon branches summate temporally and spatially to generate a main axon spike; temporally dispersed branch spikes often pace repetitive discharge of the main axon. Hyperpolarizing IPSP's sometimes suppress axonal discharge to most of these inputs, but in other cases may interact selectively with some of them. The IPSP's reverse their polarity at a hyperpolarized level of membrane potential; they sometimes exhibit two discrete time courses indicating two different input sources. Outward direct current at the main axon near branches causes repetitive discharges which may last, with optimal current intensities, for 1 to 15 seconds. The relation of discharge frequency to current intensity is linear for an early spike interval, but above 100 to 200 impulses/sec. it begins to show saturation. In one unit the current-frequency curve exhibited two linear portions, suggesting the presence of two spike-generating sites in the axon. Current threshold measurements, using test stimuli of different durations, showed that both accommodation and "early" or "residual" refractoriness contribute to the determination of discharge rate at different frequencies.

INTRODUCTION

One of the features of arthropod interneurons is the repetitive discharges which they produce in response to brief single presynaptic shocks. Such responses have been analyzed in interneurons of the crayfish (21, 22, 27); the behavior of these neurons contrasts with that of large flexor motoneurons in the same animal, which only fire singly upon orthodromic activation (28). Similar repetitive discharges are found in vertebrate interneurons (4, 5) especially in Renshaw cells, where the phenomenon is ascribed to a prolonged transmitter action (3–7).

The present experiments demonstrate that in crayfish interneurons repeti-

tive axonal spikes are generated by a different mechanism. Orthodromic all-or-none impulses in axon branches, upon which the presynaptic endings are located, summate to produce main axon discharges; branch activity often interacts with inhibitory post-synaptic potentials. The over-all frequency pattern of the axon discharge is determined by the membrane properties of the main axon to which the branch activity contributes.

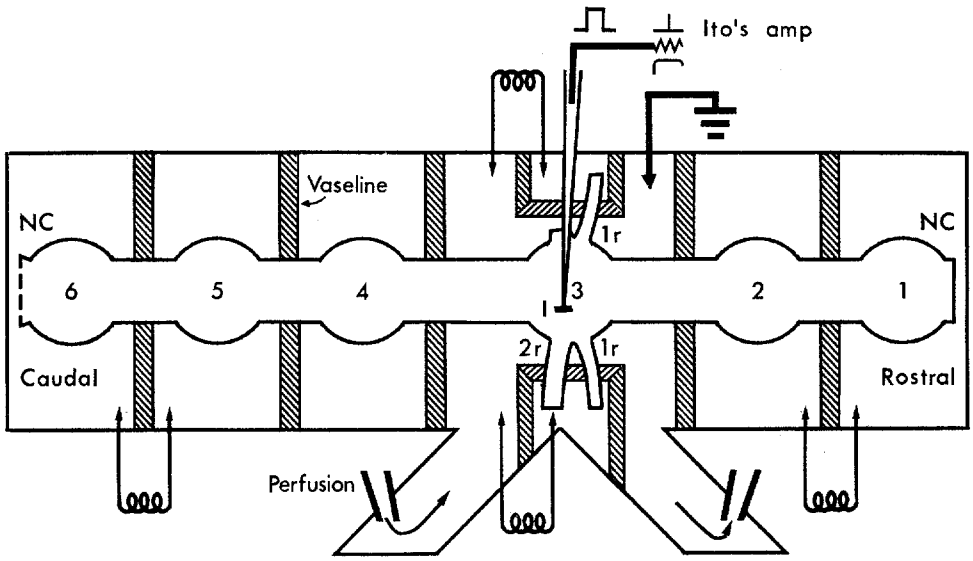

FIGURE 1. A schematic representation of the experimental arrangement. Numerals 1 to 6, abdominal ganglia; 1 *r* to 2 *r*, first and second roots; *I*, interneuron penetrated; *NC*, abdominal nerve cord. Further explanation in text.

METHODS

Interneuron fibers were penetrated by microelectrodes in the third ganglion of the isolated abdominal nerve cord of the crayfish *Procambarus clarkii* (Fig. 1). Experimental procedures and equipment were as described elsewhere (28), except for a few modifications: *i.e.*, an Ito-type preamplifier (19) was used in the present experiments to pass transmembrane current from the intracellular recording electrode, and the third abdominal ganglion was perfused with van Harreveld's solution.

To activate the penetrated units, the first and/or second root on each side of the third ganglion and the caudal or rostral ventral nerve cord were stimulated extracellularly with brief single pulses (Fig. 1). Responses were determined to be either synaptically mediated or direct (antidromic) by their ability to follow high frequency (>50/sec.) stimulation, in addition to the character of the recorded potentials with respect to latency and wave form. A unit which responded with post-synaptic discharge to first and/or second root stimulation, and with an antidromic spike to either caudal or rostral cord stimulation, was regarded as an interneuron. Many inter-

neurons showed repetitive orthodromic discharges when the microelectrode was presumed to be in the main axon (see below), and such units were the subject of the present experiments. Many of these were also activated synaptically by caudal cord stimulation. In determinations of membrane excitability, units were analyzed which showed stable responses for several hours and had resting potentials of 70 to 82 mv and action potentials of 87 to 98 mv. Some of the units used for other purposes showed lower resting and action potentials.

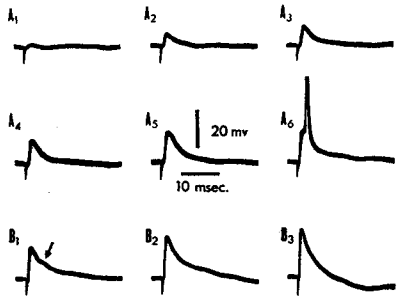

FIGURE 2. EPSP's. Ipsilateral first and second root stimulation. A_1–A_6, stimulus intensity increased. A spike is generated in A_6. B_1–B_3, hyperpolarization was applied and increased (at the stimulus intensity of A_6). Arrow in B_1 indicates the remaining regenerative component. All potential records in this and subsequent figures were obtained intracellularly.

For measurements of the relation between current and discharge frequency, the transmembrane current passed was measured directly with a differential DC amplifier of high input impedance, as the IR drop across a 500 KΩ resistor interposed between bath and ground. For threshold measurements at various intervals after a spike was generated, the current passed was measured at the output of the RF isolators. Since the current was always passed through a 100 MΩ series resistor, the effects of any possible change in electrode resistance should be very small. This was checked by demonstrating the constant appearance of the conditioning spike potential in response to a fixed, slightly suprathreshold current intensity, and by the consistent threshold current value for the test spike on repeated trials. Perfusion was stopped temporarily during current measurements to eliminate leakage of current. Experiments were done at temperatures of 17–22°C.

RESULTS

1. *Orthodromic Responses*

EPSP's The participation of slow excitatory depolarizations in the orthodromic activation of crayfish interneurons has been described in earlier papers (20, 21, 27, 31–33); but the site of origin of such subthreshold potentials was not determined. In Fig. 2, post-synaptic potentials having an excitatory function are shown. These exhibited graded increases in amplitude and maintained nearly a constant time course when the intensity of the root stimulus was increased (A_1–A_5). The potentials had a duration of approximately 10 msec., and produced a spike at a depolarization of 17 mv (A_6). The spike was blocked by hyperpolarizing the membrane (B_1), and the remaining potential was augmented in amplitude by further hyperpolarization

(B_1–B_3). Such behavior is similar to that found at other synapses (2, 8, 16), and leads to the conclusion that the subthreshold potentials represent excitatory post-synaptic potentials. Since a similar augmentation of potential by hyperpolarization could occur in the post-element of electrical junctions (30; but see reference 25) this result does not allow a conclusion about the nature of the synapses concerned. In any event, such augmentation of the depolarizing potentials by membrane hyperpolarization was not the most common finding when post-synaptic elements were penetrated in the ganglionic neuropile.

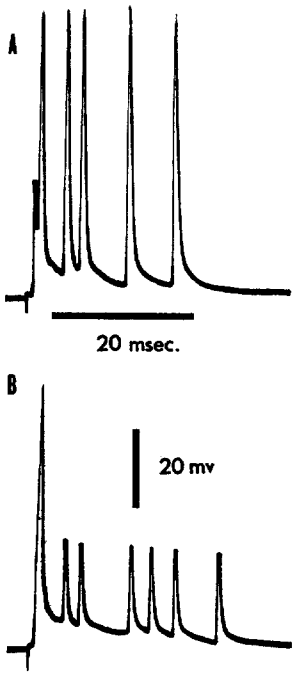

FIGURE 3. Reversal of the relative amplitudes of antidromic (first) and post-synaptic (subsequent) spikes by a mechanical shock to the preparation. Before (A) and after (B) the shock. Caudal cord stimulation.

ELECTROGENESIS IN AXON BRANCHES AND MAIN AXON Previous evidence (20, 27) suggested that the axon branches of crayfish interneurons are capable of producing independent spike potentials. The tendency of some penetrated units to produce spikes of two distinct amplitudes depending upon the route of activation, as illustrated by Fig. 3, is a manifestation of this property. A brief stimulus to the nerve cord produced first an antidromic spike in the recorded unit and then a series of orthodromic spikes (see Methods). The relative heights of the initial antidromic spike and the subsequent post-synaptic spikes were reversed after an accidental mechanical shock to the preparation (Fig. 3). The precision of this reversal suggests that the electrode tip was displaced from an axon branch to the main axon. In Fig. 3 A, the first orthodromic spike was generated on the falling phase of the electrotonic

antidromic spike. The same relation is not clearly seen in B, probably due to the short-circuiting effect of the antidromic spike or to impulse collision.

The properties of the orthodromic subthreshold potentials have been explored by analyzing the effect of hyperpolarization. This technique revealed that a large percentage of graded "slow" subthreshold potentials which would ordinarily be interpreted as EPSP's actually consisted of a number of independent all-or-none potentials which are best interpreted as spikes from branches (see Discussion). Location of the microelectrode in the main axon (or possibly in a major branch subject to antidromic invasion) was

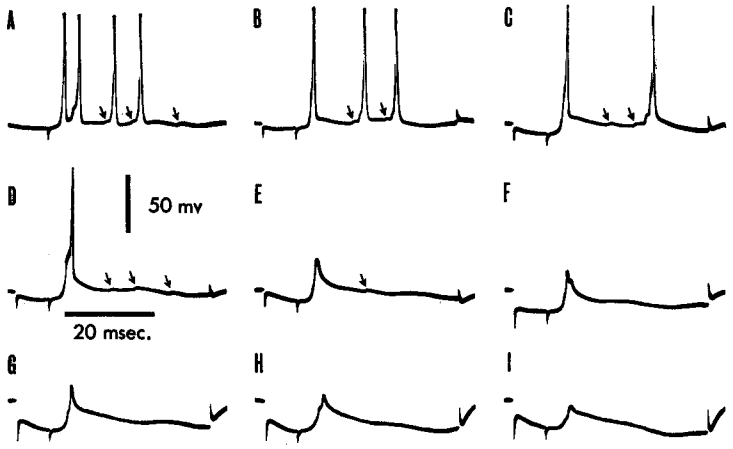

FIGURE 4. Repetitive orthodromic discharges of an axon generated by subthreshold, all-or-none potentials (A). Ipsilateral first and second root stimulation. B–I, upon increasing hyperpolarization, early and later (arrows) potentials were blocked in all-or-none fashion. The passive potential change due to transmembrane currents in this and other figures does not indicate real level of membrane potential, because of inadequacy of the compensating potential.

presumed when the amplitudes of anti- and orthodromic spikes were large and equal. When such penetrations appeared, from the response characteristics of the unit, to be near branches, patterns of activation usually resembled that shown in Fig. 4 A. In response to root stimulation, a short latency spike was generated in the main axon. Membrane hyperpolarization, which blocked the early spike, revealed the presence of an underlying summated subthreshold potential. Upon membrane hyperpolarization, the first axon spike showed an inflection on its rising phase (B–D). After the spike was blocked at the inflection point (E), further hyperpolarization caused a delay (F) and block (G) of one of the subthreshold all-or-none components, and then a delay (H) and block (I) of another. The remaining small hump in I showed similar behavior upon further hyperpolarization (not shown in the

figure). The later arriving subthreshold potentials (arrows) which caused additional discharges of the axon (A) were also blocked in all-or-none fashion by hyperpolarizations (D–F), though the time delay preceding block could not be determined because the latencies were inconsistent. The second axon spike in C shows an inflection similar to that of the first spike as well as an earlier inflection at a lower level of depolarization. The depolarization that started from the lower inflection was delayed (A – C), and was blocked in all-or-none fashion between C and D (not shown). Some units, in contrast to that shown in Fig. 4, produced large post-synaptic depolarizations underlying high frequency axon discharges. In such cases the slow potentials (as well as the axon spikes) were often augmented in amplitude at low levels of membrane hyperpolarization, as would be expected of post-synaptic potentials. However, further hyperpolarization reduced the amplitude of such slow potentials, suggesting that they too may represent summated distant spike activity, probably accompanied by local responses of the axon.

Fig. 5 provides another demonstration of the all-or-none nature of the components of the subthreshold potential. This unit responded with an all-or-none subthreshold potential to root stimulation (A_1). At a slightly higher stimulus intensity, a second such potential having a longer latency was recruited, and temporal summation of these two potentials generated an axon spike after a prolonged depolarization (up to 35 msec. from the onset of the second branch spike; A_2). That the long lasting subthreshold potential represents the local response of the nearby membrane at an unstable equilibrium condition, and is not caused by EPSP's or additional distant spikes, is shown by its absence when the axon spike was generated earlier, *i.e.*, just after the second subthreshold potential (A_3), and also by the effect of hyperpolarization (B_3, C_3). At a higher stimulus intensity, spatial summation of the early subthreshold potentials generated an axon spike (B_1). When the membrane was hyperpolarized, the subthreshold potentials remained (B_2), and their amplitudes were reduced by further hyperpolarization (B_3). At a higher stimulus intensity (C), the underlying early potential was larger (C_2) than that seen previously (B_2) when the axon spike was just blocked by hyperpolarization. This potential also consisted of distant spikes, since further hyperpolarization reduced its amplitude (C_3). In Fig. 5 D, the stimulus intensity was continuously increased from D_1 to D_5 with a fixed membrane hyperpolarization. The increments in amplitude of the potentials from D_1 to D_5 appeared in stepwise, all-or-none fashion, and their amplitude at each stimulus intensity was reduced by further hyperpolarization. Similar results were obtained consistently for every all-or-none potential at various fixed levels of hyperpolarization. Without hyperpolarization, at the stimulus intensity of D_5, a single axon spike was produced (D_6), indicating that the convergence of at least five subthreshold, all-or-none events was producing

one axon spike. A post-spike hump of depolarization (D_6) which evoked a second spike at higher stimulus intensities, is familiar in crayfish interneuron responses (*e.g.*, Fig. 4 A). It was absent in the response without hyperpolarization at the stimulus intensity of D_4, and thus must represent a strong contribution of one subthreshold component.

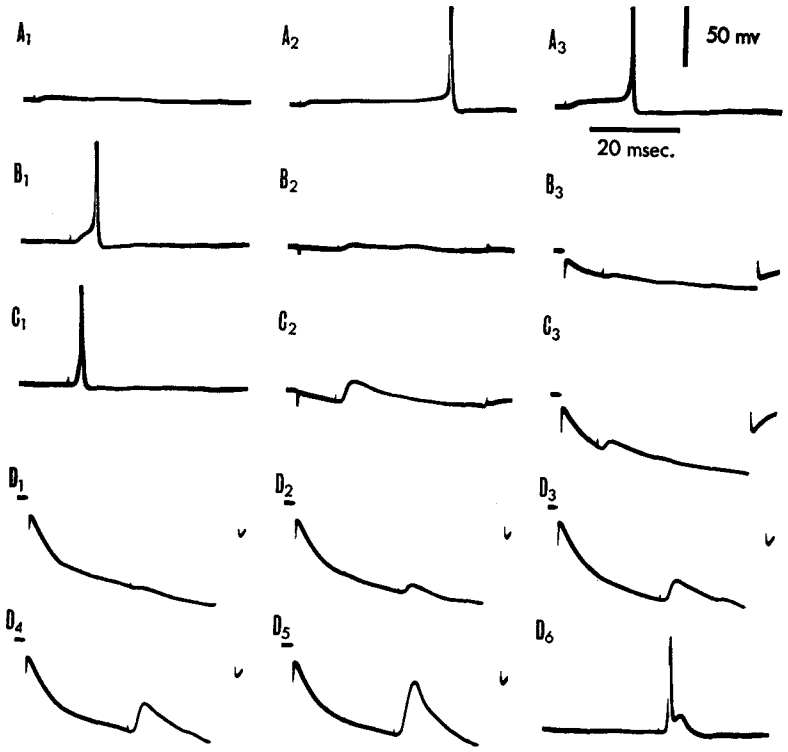

FIGURE 5. Modes of orthodromic axon spike generation by subthreshold spikes. Ipsilateral first and second root stimulation. A_1, A_2 (A_3), B_1, and C_1 form a series of increasing stimulus intensity. Hyperpolarization was added (B_2, C_2) and increased (B_3, C_3), each of two at fixed values of stimulus intensity. D_1–D_5, stimulus intensity was increased with a constant membrane hyperpolarization; D_6, response without hyperpolarization at the same stimulus intensity as D_5.

IPSP's In neurons of the lobster brain, inhibitory post-synaptic potentials which inhibited spontaneous discharges have been recorded (26). These are the only hyperpolarizing responses so far reported from arthropod neuropile, though inhibitory effects have been observed in crayfish interneurons (20, 27). In the present experiments, hyperpolarizing IPSP's were recorded in several units. The unit shown in Fig. 5 responded to contralateral first root stimulation with IPSP's (Fig. 6 A_1) which increased in amplitude at higher stimulus intensity (A_2). These IPSP's had a duration of approximately

70 msec., with a rise time of 3 to 7 msec. Their rate of rise also varied in different units, as would be expected from a recording situation in which the site of penetration might be at varying distances from the synaptic regions. Sometimes, the IPSP's showed two peaks in response to single presynaptic

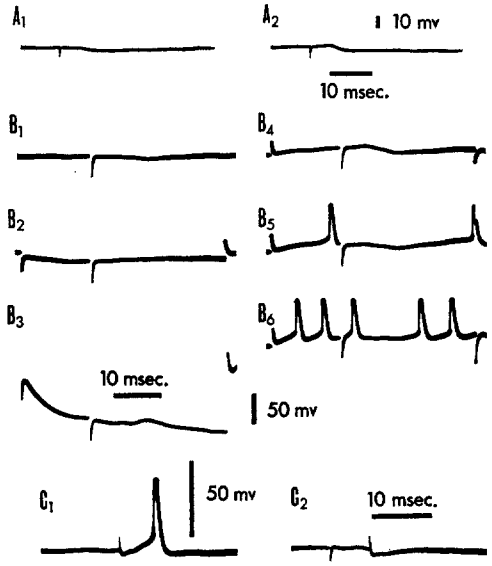

FIGURE 6. IPSP's produced by contralateral first root stimulation. A, the unit shown in Fig. 5; stimulus intensity increased in A_2. In another unit (B_1), hyperpolarization was added (B_2) and increased (B_3), and depolarization was added (B_4) and increased (B_5, B_6), all without altering stimulus intensity. C, another unit. A spike generated by ipsilateral second root stimulation (C_1) was blocked by IPSP's produced by prior stimulation of contralateral first root (C_2).

shocks (Fig. 6 B), suggesting the involvement of separate inhibitory inputs. These IPSP's generally reversed their sign under membrane hyperpolarization (B_1–B_3) and increased their amplitude upon depolarization (B_1, B_4), indicating that they drive the membrane potential toward an equilibrium level as in other preparations (1, 9, 11, 13, 23, 24). The IPSP's inhibited spike discharge, whether it was produced by transmembrane depolarization (Fig. 6 B_6) or by presynaptic activation (Fig. 6 C).

The presence of IPSP's in the axon region where many all-or-none subthreshold potentials summate to generate axon discharge (Figs. 5 and 6 A) suggests that they have a common inhibitory effect against a variety of excitatory inputs. IPSP's that exerted an action against only a specific group of inputs were also found. The unit in Fig. 7 responded with all-or-none subthreshold potentials to root stimulation, and produced one or two axon spikes at high stimulus intensities (A). Caudal cord stimulation evoked a very small (*ca.* 3 mv) depolarizing potential which did not change its sign upon imposed depolarization and enhanced the discharge produced by the depolarizing current. The amplitude of this depolarizing potential was augmented by membrane hyperpolarization. The effect of a cord stimulus, when it was combined at a fixed intensity and interval with the root stimulus, depended upon the intensity of the latter (B). The cord stimulus had no effect (B_1, B_4)

or augmented (B_2, B_3) the responses evoked by low intensity root stimuli. When high intensity root stimuli were used, on the other hand, clear inhibition was exerted by the prior cord stimulus; it reduced the first spike amplitude slightly, and blocked the later portion of the depolarization evoked

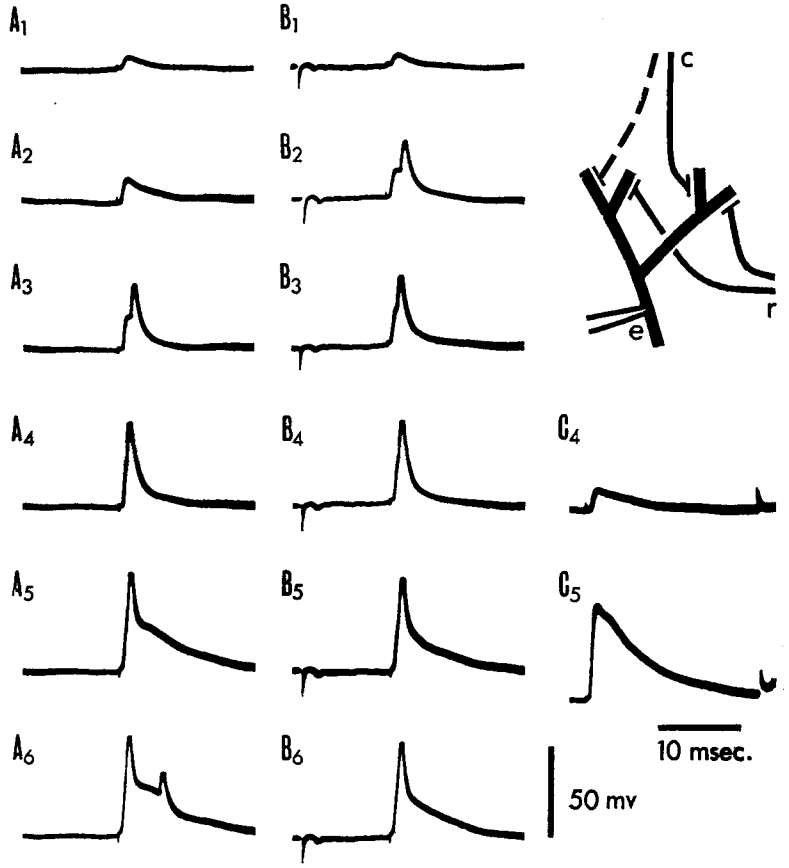

FIGURE 7. Excitatory and inhibitory inputs from caudal cord. A_1–A_6, ipsilateral second root stimulation with increasing intensity. In B_1–B_6, caudal cord stimulation at a fixed intensity was added to each root stimulus shown in the A column. In C_4 and C_5, the spike produced by root stimuli in A_4 and A_5, respectively, was just blocked by hyperpolarization. Insert, a simplified scheme to explain the results. *c*, inputs from caudal cord; *e*, penetration site; *r*, inputs from ipsilateral second root. Solid lines indicate excitatory inputs, broken line indicates inhibitory input.

by the root stimulus (B_5, B_6). In B_5 and B_6 the potentials were quite similar, in spite of the fact that the comparable responses to root stimuli alone (A_5, A_6) were very different. When the axon spike was blocked by membrane hyperpolarization, the underlying potential showed that a large subthreshold potential (see C_4, C_5) was recruited at the root stimulus intensity above which

the unit became subject to inhibition by the cord input. The illustrated records show a fixed, optimum interval between cord and root stimuli; qualitatively similar results were obtained for different stimulus intervals. The inhibitory effect of the cord stimulus thus operated selectively and effectively against one all-or-none component of the root response. This makes it unlikely that the depolarizing response to cord stimulation consists of IPSP's altered in sign by a leakage of chloride from the electrode (1, 24). Instead, the response must contain both excitatory and inhibitory components from the caudal cord (*cf.* reference 20), which synapse at different loci on the interneuron. A proposed scheme of synaptic connections which would explain these results is given in the insert of Fig. 7.

2. *Membrane Excitability*

THE RELATION BETWEEN CURRENT AND DISCHARGE FREQUENCY Constant outward transmembrane current was passed through the recording electrode which was penetrated into interneurons in the ganglion. The electrode position was again presumed to be in the main axon when the amplitudes of anti- and orthodromic spike potentials were large and equal. Such units showed underlying branch activity when activated orthodromically, and they responded to constant outward current with a train of discharges of decreasing frequency (Fig. 8), which lasted for 1 to 15 seconds in different preparations at optimal current intensities. In units with resting potentials of 70 to 82 mv, the threshold current for first spike generation ranged from 0.6 to 2.4 $\times 10^{-8}$ amp; the value for threshold current was not directly correlated with the resting potential, as would be expected from different locations of the electrode with respect to the spike-generating site (see below).

In many units the first spike, when produced by a threshold constant current, had a long latency (up to 40 msec.), arose from a maintained depolarization, and was not accompanied by a visible local response (Fig. 8 A_1). The maximum interval recorded between first and second spikes in such units was also long (375 msec.). In other cases, however, a subthreshold response of rather short duration was recorded, and the first spike was generated from it (Fig. 8 B). Repetitive slow depolarizing potentials which had a frequency of approximately 100/sec. and resembled oscillating potentials known from other excitable membranes (15, 18), were seen in this unit. The oscillations showed an increase in frequency with increased depolarizing current (B_2–B_3) before they were masked by superimposed pre-spike potentials (B_4). Their existence suggests either that the electrode position is close to the spike-generating site, making the local response visible, or—more likely—that a branch component contributes to the response.

The latency of the first spike and the subsequent spike intervals at a given current intensity were generally not directly correlated; the former value was

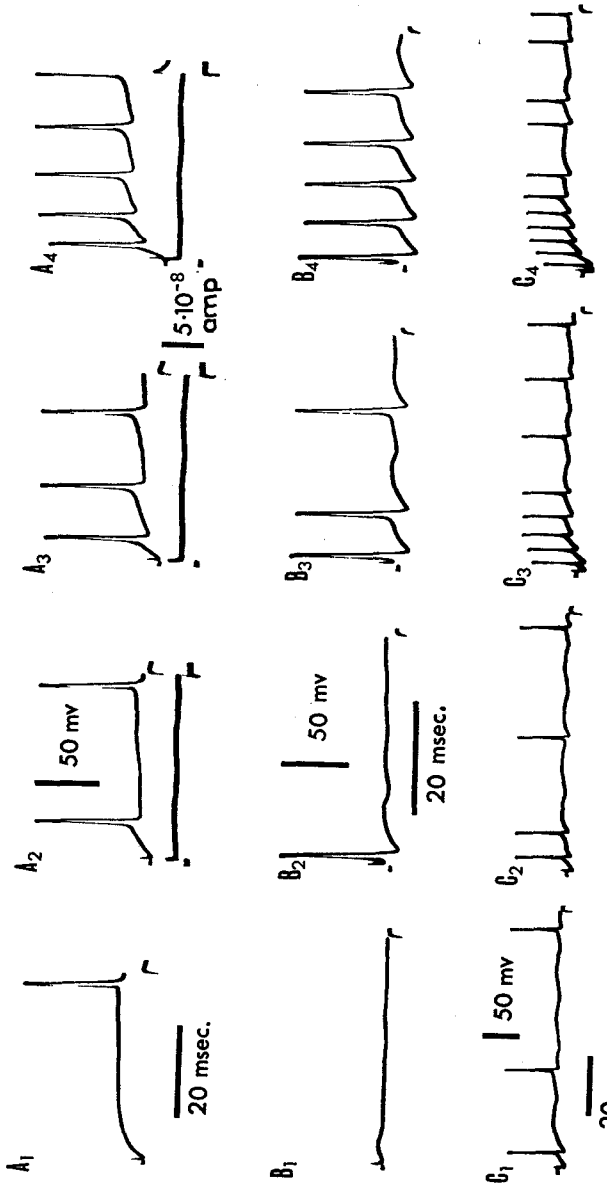

FIGURE 8. Discharges produced by outward transmembrane currents. A, B, and C, from different units. In each unit, current intensity was increased from 1 to 4. A_1, without current recording to show potential trace without 60 cycle artifacts; A_2–A_4, current records in lower traces. In C, traces include 60 cycle artifacts. Further explanation in text.

lower than the latter in many units, but higher in others. The intervals between spikes, on the other hand, were generally correlated with each other. In many units, the intervals showed a regular increase with time (Fig. 8 A), though in other cases they varied irregularly (Fig. 8 C; see below).

The current-frequency relations obtained from first and second spike intervals after the onset of current of the unit in Fig. 8 A are shown in Fig. 9 A. This and other interneurons showed a linear relation between current and frequency for an early spike interval at lower values of frequency; this is

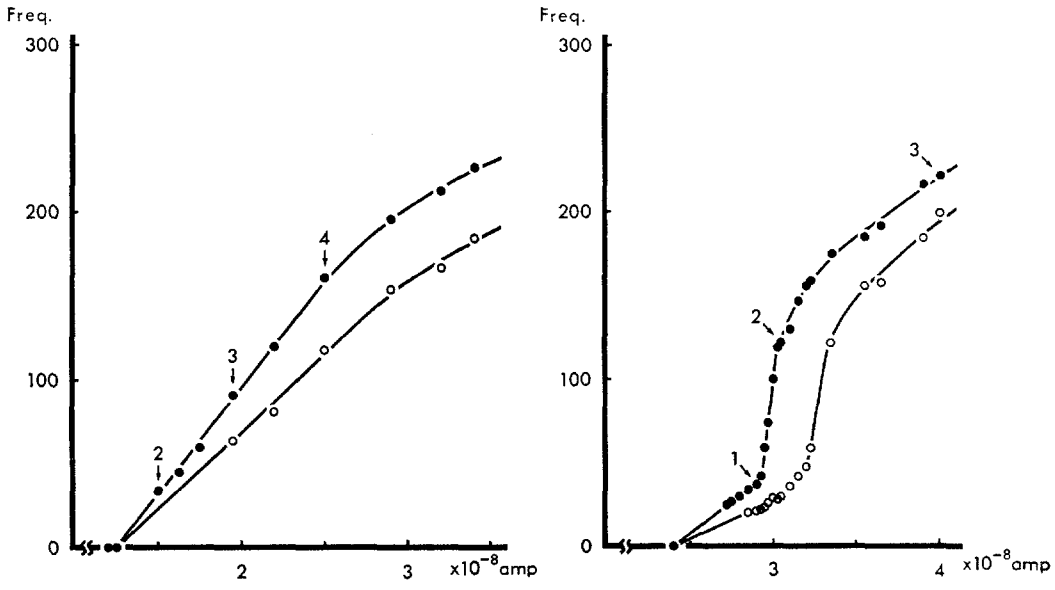

FIG. 9A FIG. 9B

FIGURE 9. Outward current (abscissa) *vs.* discharge frequency (ordinate) relations. Solid and open circles refer to the frequency values obtained from the first and second spike intervals, respectively. Fig. 9 A, the unit in Fig. 8 A. Fig. 9 B, the unit in Fig. 8 C. Values indicated by the numerals 1–4 in the figures were obtained from the records of Fig. 8 A and C with corresponding numbers.

characteristic of other nerve cells in "steady state" discharge (10, 12). The slope of the linear portion was less for the second spike interval than for the first (Fig. 9 A). Extrapolations of the linear portion of the two functions yield approximately the same intercept on the current axis; at this point the impulse frequency is zero, only one spike being generated by the current. The current required to increase the discharge frequency for the first spike interval by one impulse/sec. on the linear portion of the curve was 5 to 8 $\times$ 10^{-11} amp in the units tested. Above a frequency of 100 to 200 impulses/sec., the frequency showed a tendency to saturate. This deviation of the curves from linearity starts at approximately the same frequency for the first and

second spike intervals and at different current intensities; *i.e.*, the saturation is frequency-dependent.

Fig. 9 B shows similar current-frequency relations for the unit in Fig. 8 C; the curves exhibit two linear regions before saturation occurs. The first limb (of the curve for the first spike interval) required a current of 1.4×10^{-10} amp for a frequency increase of 1 impulse/sec.; this was followed by a second limb of augmented sensitivity (1.2×10^{-11} amp). This result may indicate the presence of two separate spike-generating sites in the axon, one of which had a higher current threshold. This interpretation is supported by the fact that occasional later impulses in the train drop out in this unit at high current intensities, a result consistent with the possibility of impulse collision (Fig. 8, C_2–C_4). Curves with a similar augmented sensitivity region were described in mammalian motoneurons by Granit *et al.* (12), who did not interpret the phenomenon. The finding of a linear current-frequency relation for *early* spike intervals, and the existence of two linear portions in one unit, suggest that these axons have fixed spike-generating sites for injected currents due to a geometrically and/or physiologically imposed distribution of excitability. These sites may not, however, necessarily be the same as those for orthodromic activation, since the current distribution would be different in the two cases. The evidence that more than one spike-generating site for synaptic activation exists along the axons of crayfish interneurons and motoneurons has been given elsewhere (20, 28).

FATIGUE As a means of examining the factors that determine spike intervals during a repetitive discharge, the firing threshold for current pulses following a conditioning impulse was measured for test stimuli of different durations. Fig. 10 shows an example, obtained from the unit shown in Fig. 8 B. Both conditioning and test stimuli were outward transmembrane currents, the former lasting 1 msec. and the latter 1, 5 or, 10 msec. With 1 msec. test stimuli, the unit had almost recovered from refractoriness 9 msec. after the conditioning spike ("early" refractoriness), but there remained a much longer period of slightly increased current threshold ("residual" refractoriness). With 5 msec. test stimuli, a somewhat longer time was required for initial recovery, and the current threshold remained at a slightly higher level (accommodation). With test stimuli of 10 msec., initial recovery had a time course similar to that seen with 5 msec. test stimuli, but the later level of threshold current was still higher. The deviation of this curve from that for 5 msec. test stimuli at shorter stimulus intervals occurred in this unit because the latency of the test spike at threshold current intensity increased at these intervals, a phenomenon probably due to a contribution from the later portion of the long test current.

In squid giant axons it is known that the local response does not contribute

significantly to spike generation at stimulus durations below 2 msec., though it does at durations of 2 to 5 msec. (14). If the results from squid axons are applicable to the present neurons, it would be reasonable to attribute the stimulus duration–dependent differences in current threshold to the presence

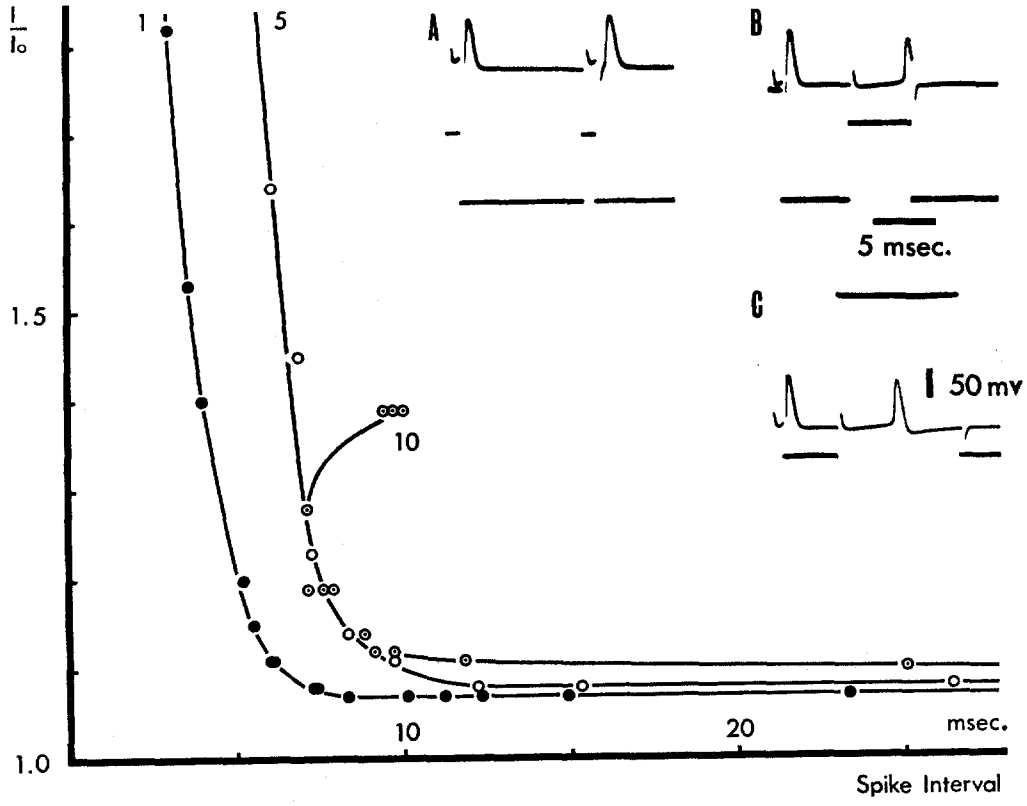

FIGURE 10. Changes of current threshold after a conditioning spike. Ordinate, $\frac{I}{I_o}$; where I is the current threshold after a conditioning spike and I_o denotes that without a previous spike. Abscissa, interval between conditioning and test spikes. Curves 1, 5, and 10 refer to the series with outward test current durations of 1, 5, and 10 msec., respectively. Insert A–C, threshold responses to test stimuli of 1, 5, and 10 msec., respectively (second spikes, upper traces), with current records at different gain for each series (lower traces). Current trace during conditioning stimulus in C was off screen.

or absence of a local response. Its effect is a dual one, involving a prolongation of the early recovery time and also a residual increase in current threshold which persists for a much longer time (compare curve 1 and curves 5, 10). Post-spike refractoriness *without* local response, however, does itself result in a prolonged elevation of the current threshold (curve 1).

The steep increase in current threshold seen with longer test pulses at inter-

vals below 10 msec. following the previous spike in Fig. 10 involves early refractoriness as well as accommodation. These phenomena would be mainly responsible for the saturation of high frequency discharge during the application of strong constant current (Fig. 9). On the other hand, the prolonged elevation of current threshold after the previous spike by longer test pulses in Fig. 10 involves residual refractoriness as well as accommodation, and both of these effects may summate after each spike discharge. These may be responsible for the frequency decrease for later spikes in the train during low frequency discharges induced by weak constant current, and for the reduction in slope of the frequency *vs.* current relation for later spike intervals (Fig. 9).

DISCUSSION

The present results demonstrate that all-or-none subthreshold potentials are a regular feature of the synaptic activation of interneurons when one records from neuropile fibers at sites determined to be along the main axon. Subdivisions of the axon spike (as in Fig. 4) indicate heterogeneity of the membrane in these neurons; morphologically, they exhibit numerous axon branches which converge upon the main axon. Since the subthreshold all-or-none potentials recorded in these experiments behaved precisely in the fashion expected of conductile membrane, it is most logical to interpret them as the spikes of axon branches, as in crayfish motoneurons (28). The alternative view that they might represent EPSP's or potentials from neighboring cells, with all-or-none block of the components being due to electrotonic effects on presynaptic terminals, seems unlikely because: (*a*) The subthreshold all-or-none potentials show entirely different behavior from that of EPSP's in the same interneurons (Fig. 2). (*b*) No *other* subthreshold depolarization is visible at many main axon recording sites. (*c*) At recording sites where fully graded potentials that increase upon hyperpolarization are seen, there are no subthreshold all-or-none components in addition (see Fig. 2). If the subthreshold all-or-none events were indeed junctional potentials, one would have to postulate that to achieve presynaptic blockade with our hyperpolarizing currents the junctions would have to be highly effective ephaptic ones; and in view of the regularity with which small all-or-none potentials are seen, they would have to be ubiquitous. This is, however, hardly consistent with our results (see *c* above).

The Integrative Significance of Axon Branches

The following conclusions may be drawn from the present results: (*a*) The graded amplitude increase of EPSP's and IPSP's achieved by increasing the intensity of presynaptic shocks confirms the integrative transfer characteristic of junctional regions in these crayfish interneurons (*cf.* references 21 and 27).

(*b*) The branches of these interneurons are capable of generating independent spikes, as are those of crayfish motoneurons (28) and of some *Aplysia* neurons (29). (*c*) In many if not all these crayfish interneurons, depolarizations produced by the branch spikes summate temporally and spatially at an impulse-generating site on the main axon which determines the output discharge of the neuron; the mode of summation is determined by the geometry of the branches involved. (*d*) Several branch spikes may converge to evoke a single axon spike, while temporally asynchronous ones may cause repetitive discharges of the main axon by bringing the level of depolarization at the spike-generating site to a critical value. (*e*) The pattern of such discharges may be rather irregular when the contribution of branch spikes is discrete, since it will depend upon the arrival time of the latter. However, firing may be regular when many branch spikes sum to cause a long lasting, smooth depolarization of the spike-generating site on the main axon. (*f*) IPSP's may inhibit a number of branch inputs together, presumably by acting upon the axonal firing site, whereas other IPSP's may act selectively against certain inputs. A similar mechanism might also operate in complex vertebrate neurons, where it has been postulated on anatomical grounds (*cf.* reference 5) that synapses of presumed inhibitory function may be distributed along dendritic branches and on the soma as well. Such a distribution would provide a morphological basis for "general" and "input-specific" inhibition. (*g*) Some units may have two separate axonal spike-generating sites in a ganglion, each presumably associated with a different set of inputs. All these interactions take place in the absence of any contribution by the soma.

Thus the mode of activation of crayfish interneurons may be more complex than that of neurons with synapses localized on or near the soma, in that excitation and inhibition can act with varying specificity upon individual input elements which in turn determine the behavior of the entire neuron. In such a situation, the axon branches take almost the integrative role of separate internuncial elements. In crayfish motoneurons a rather similar situation has been observed. These neurons also receive synaptic input on axon branches, and also give evidence of temporally asynchronous arrival of branch activity at the main axon (28). However, they only produce single efferent impulses in response to single volleys initiated in cord or root prefibers. The difference may be ascribed to the properties of spike-generating membrane of the main axon; in many cases, the "fast" flexor motoneurons of crayfish fire only once in response to maintained outward current (28).

Responses to Depolarizing Currents

In the present constant outward current experiments, the latency of the first spike was not directly correlated with the following spike intervals. This is to be expected, since an area is interposed between the site of penetration and

that of spike generation; this intervening region may be morphologically and physiologically heterogeneous. Maintained depolarization during constant current application should result in altered resistance of the interposed membrane due to local responses, and this in turn should affect the net current available at the spike generation site. After a spike is generated, it may invade this area (including any branches) to a variable extent, and its effect on the generation of the subsequent spike may thus differ from unit to unit.

It is now accepted that the spike interval in a repetitive discharge is determined primarily by the time of development of the excitatory process rather than by refractoriness due to previous spikes (17), though refractoriness and accommodation also contribute to it (10). The present results show the involvement of accommodation and of early or residual refractoriness in determining the frequency on different frequency levels. Even though we lack direct information about change in the critical depolarization level at the spike-generating site after production of an impulse, the results on current threshold change may adequately explain the observed frequency decrease during the application of constant outward currents.

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