SYNAPTIC INHIBITION IN AN ISOLATED NERVE CELL*

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INTRODUCTION

The preceding two papers gave an account of some of the mechanisms which lead to the excitation of sensory neurons. These cells, described by Alexandrowicz (1, 2), are linked to stretch receptors in lobster and crayfish and consist of a nerve cell body which is situated in the periphery and sends dendritic processes into a nearby muscle strand and also gives rise to a sensory axon running centrally. Experiments have shown that stretch deformation causes a depolarization of the dendrites, the site of the generator potential, which in turn spreads to the adjoining cell portions and leads to a conducted sensory nerve impulse. It was also found that changes impressed on the dendrites can alter the resting potential of the nerve cell soma for long periods, depending on the magnitude of stretch. Thereby a sensitive mechanism is provided which controls the excitability of the sensory neuron. In contrast, but complementing this dendrite—cell body–axon excitation cycle, the present paper describes in the same cell an opposite mechanism by which the generator potential is controlled by an inhibitory axon which makes synaptic contacts with the dendrites.

While the study of inhibition in the central nervous system has been vigorously pursued by many, the mechanisms, especially in the periphery, have not aroused as much interest. At the neuromuscular junction Biedermann's classic demonstration of inhibition (3) was expanded by Hoffmann (22) and by Marmont and Wiersma (29) (*cf.* also (30)). Since then neuromuscular inhibition mechanisms were analyzed by Kuffler and Katz (26), and Fatt and Katz (15). Lately synaptic inhibition was successfully explored in spinal motoneurons of the cat by Eccles and his colleagues (4, 6). In short, at present evidence exists that inhibitory impulses may produce no change in the membrane potential of a cell, or they may produce "hyperpolarization" or even depolarization, depending on experimental conditions. It appears that synaptic inhibitory effects depend largely on the state of activation within the receptor cell. In the present preparation the "state" of the nerve cell can be adjusted

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by using its available physiological mechanisms, namely altering the dendrite depolarization by stretch. Thereby the cell membrane potential can be changed reversibly over a considerable range and variation in the synaptic inhibitory effects can be seen. Inhibitory impulses have a rather powerful action and can stop within milliseconds activity which is caused by strong excitatory stimulation. Evidence is presented that the inhibitory nerve action initiates a process which "drives" the receptor cell potential towards an equilibrium level. Thus, an inhibitory impulse may have a polarizing or depolarizing effect depending on the direction in which the postsynaptic cell is displaced from the "inhibitory equilibrium potential." These studies also help in the analysis of excitation spread between cell soma and dendrites. A preliminary report appeared a year ago (11). For an excellent review dealing with inhibitory and excitatory mechanisms Fatt's (14) survey should be consulted.

Method

The methods and preparations have been described in several preceding papers (12, 13, 25). It was assumed from the beginning that at least one of the "accessory" fibers described by Alexandrowicz (1, 2) in the lobster, would be inhibitory. This proved correct for *Homarus americanus*. However, practically all the experiments described here refer to crayfish *Procambarus alleni* (Faxon), *Orconectes virilis* (Hagen), and *Procambarus clarkii* (Girard). These species, according to Florey and Florey (16) possess only one accessory fiber which innervates the dendritic region of both sensory neurons in a paired receptor organ. The present results establish this accessory nerve in crayfish as a specific inhibitory axon. It was never possible to completely isolate inhibitory nerve fibers by dissection. In many preparations, however, the large sensory axon can be traced centrally under the microscope and at a required location it can be injured by a fine micromanipulator-operated needle, thus leaving intact the inhibitory axon. This procedure is relatively simple in the eighth thoracic receptor in which the diameter of the sensory fiber increases as it runs centrally, thus making its recognition easier. Two pairs of stimulating leads are then placed on the nerve supplying the stretch receptors, those above the injury exciting the inhibitory fiber, while nearer to the receptor cell the low threshold sensory fiber is selectively stimulated. Alternatively the common nerve was pulled apart with fine forceps centrally from the receptors and after some trials it was usually possible to obtain a strand in which the remaining functioning axon was inhibitory, tested by trains of impulses which stopped the afferent discharges (see below). Even if the initial separation was not entirely successful there could usually be found a location around the injured nerve region in which the inhibitor nerve fiber could be selectively excited. On many occasions such a procedure required many trials of placing the electrodes and choosing the correct stimulus strength and proper location of anode or cathode on the nerve. In some preparations the larger sensory nerve was blocked at the stimulating anode, a method already described in detail (27).

The simplest method of exciting an inhibitory axon is illustrated diagrammatically in Fig. 1. Since the inhibitory axon in the crayfish branches and supplies first the

"slow" and then the "fast" receptor cell (16), it can be stimulated in its distal portion before it enters the fast receptor cell region. By "axon reflex," therefore, the

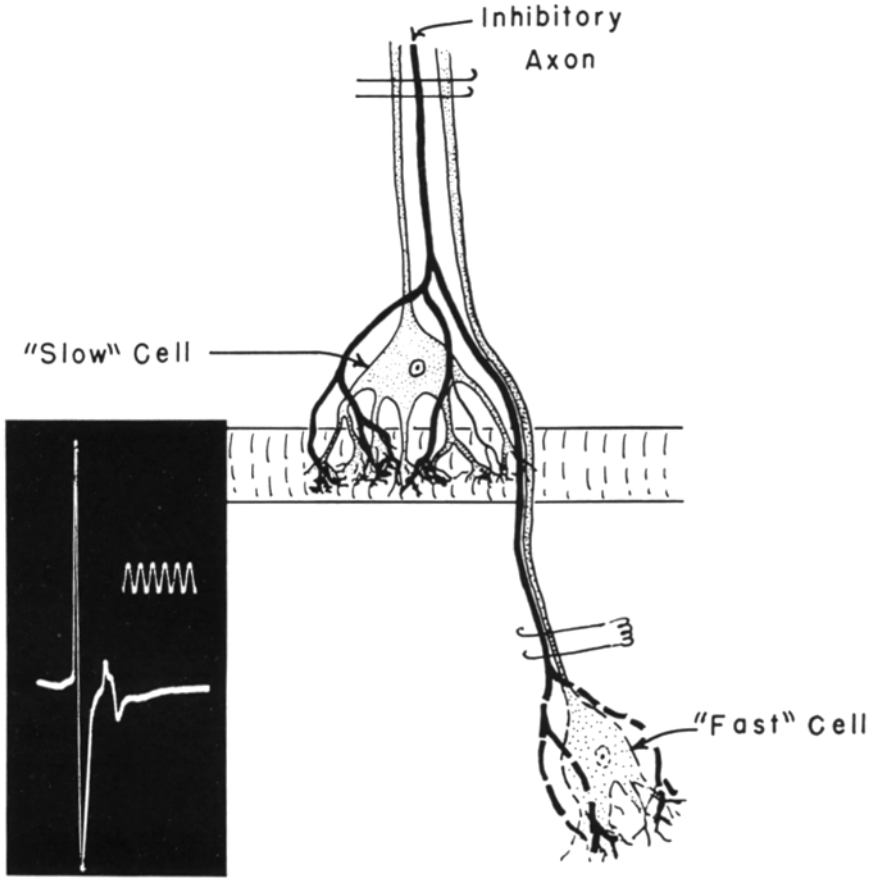

FIG. 1. Schema for stimulating the inhibitory (*I*) axon to the "slow" cell in the stretch receptor organ of the eighth thoracic segment of crayfish. The *I* fiber (black solid line) branches and innervates both receptor cells. Insert, after stimulation by the lower pair of electrodes, impulses in the fast receptor axon and in the *I* axon travel centrally and are recorded by the upper pair of electrodes. The large rapidly conducting impulse belongs to the large diameter "fast" receptor axon, the small slowly conducting impulse belongs to the small diameter *I* axon. The *I* impulse spreads by "axon reflex" into the slow cell and its appearance is correlated with inhibition of afferent discharges. Time, 1000 C.P.S.

slow nerve cell could be inhibited. The fast sensory fiber which is also excited during this procedure does not influence the slow cell discharge. This technique has been used successfully only in the eighth thoracic receptors because they are usually

sufficiently separated to provide a few millimeters of nerve for the placement of stimulating electrodes. In all experiments recording electrodes on the nerve near the receptor monitored the number of stimulated nerve fibers and it was quite simple to correlate the appearance of a particular nerve impulse with the sudden cessation of sensory discharges. The inset of Fig. 1 shows two action potentials, a rapidly conducting sensory impulse belonging to the low threshold axon of the fast receptor cell, and the smaller more slowly conducting action potential of the higher threshold inhibitory axon, excited in a manner shown in the diagram. In order to test the possible effect of chloride leaking out of the capillary leads into the cell interior (6), some of the electrodes were filled with 0.6 M K_2SO_4 instead of the usual 3 M KCl. This procedure did not change significantly the results on inhibitory activity, al-

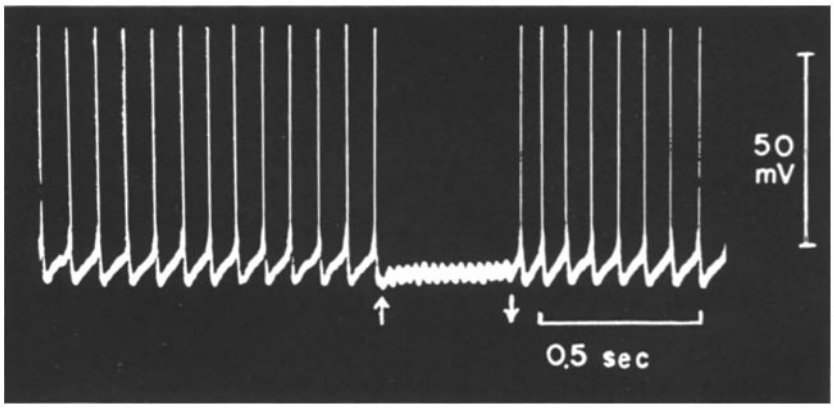

FIG. 2. Intracellular recording from a slowly adapting receptor cell at 24°C. The regular train of afferent discharges (11/sec.) set up by maintained stretch is interrupted by stimulation of the *I* axon, between arrows, at 34/sec. Small deflections are inhibitory potentials (see below).

though it seems likely that chloride diffusion did contribute to the depolarizing effects of inhibitory nerve impulses (see below).

RESULTS

1. Inhibition of a Steady Sensory Discharge

The most striking demonstration of inhibition is obtained in slowly adapting neurons while recording from the impaled cell soma. Fig. 2 illustrates such a stretched "slow" impaled receptor discharging at a regular rate of 11/sec. Between the arrows a train of inhibitory (*I*) impulses at 34/sec. abruptly stopped the sensory activity. The afferent impulses were resumed as soon as inhibitory excitation ceased. Details of such inhibition are shown in Fig. 3 under higher amplification at a faster sweep speed. The stretched slow cell was discharging at a rate of 4/sec. when eight *I* impulses at 21/sec. were set up at the beginning of the sweep of Fig. 3A. Each impulse caused a temporary 4 mv. repolarization

of the developing prepotential, back to its starting point, thus preventing it from discharging the cell. After the last *I* impulse the prepotential rose rapidly and afferent discharges were resumed. In Fig. 3 B the inhibitory train frequency was increased to 34/sec. The first *I* impulse arrived just before the cell was about to fire, at the height of the prepotential, and its repolarizing effect was greatest (5 mv.). In Fig. 3 C, 150/sec. high frequency *I* stimulation was started

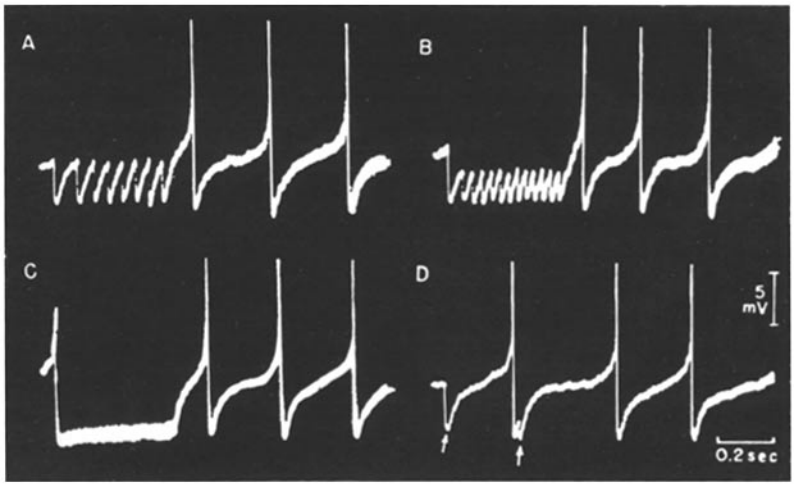

FIG. 3. Intracellular records from a slow cell in the second abdominal segment (24°C.). Recording at high amplification, only lower portions of orthodromic impulses are seen. A and B, maintained afferent impulses are inhibited by *I* stimulation at 21 and 34/sec. Each *I* impulse causes a rapid transient repolarization of the prepotential. C, *I* stimuli at 150/sec. cause and maintain repolarization. After cessation of stimulation, a prepotential redevelops at an accelerated rate and leads to prompt resumption of discharges. D, two *I* impulses (arrows). I_1 impulse arrives when the prepotential is large and it causes a large repolarization potential, while I_2, arriving as the prepotential starts to develop causes a small change. All *I* potentials reach similar levels.

during the repolarization phase of an orthodromic impulse and for the duration of inhibition the membrane potential was practically held steady, remaining repolarized, in spite of continuing stretch on the dendrites. Fig. 3 D is presented in order to show that *I* impulses produce differing amounts of repolarization, depending on their time of arrival. The first impulse (arrow 1) reached the cell just between two afferent discharges, while the second one (arrow 2) arrived before the prepotential was able to develop appreciably after a preceding orthodromic impulse. An inhibitory stimulus arriving during a conducted impulse did not affect the discharge rate significantly (see below). It appears from Fig. 3 that the effect of inhibitory impulses consists of a rapid repolariza-

tion phase after which the processes of excitation take over once more as seen from the renewed development of depolarization. At low frequencies a partial redevelopment of depolarization occurs between *I* stimuli, while at high frequencies the membrane potential is virtually clamped at a fixed level. Further, it is seen that the amount of repolarization by individual *I* impulses is greatest when the prepotential (or depolarization) is largest.

The following preliminary conclusions about the inhibitory action can be drawn: The accessory nerves of Alexandrowicz (1, 2), must be involved here since they are the only neurons which make contact with sensory nerve cells. Their synapses lie somewhere in the dendritic processes. These histological findings have been essentially confirmed and expanded by Florey and Florey (16), who, however, find only one accessory nerve in the crayfish. It has been concluded in the preceding studies (12, 13), that the sequence of events leading to excitation in the sensory neuron is as follows: Stretch deformation, acting primarily on the distal dendritic portions, sets up there the "generator potential" which leads to conducted impulses in the larger dendrite portions or in the cell soma. The finely graded generator potential, located in the dendrites and controlling the membrane potential over a wide range, is responsible for the "prepotentials" seen during the maintained steady sensory discharges of Figs. 2 and 3. It is primarily on these potentials and not on the conducted impulses that the inhibitory impulses act. *The cell discharge therefore is indirectly controlled by inhibitory impulses which act on the dendrites and reduce the generator potential.*

The effectiveness of *I* impulses in stopping sensory activity depends on the relationship between afferent discharge rate and efferent inhibitory frequency. Intermittent *I* impulses will merely prolong the gaps in a series of afferent trains, dependent on their timing. An *I* impulse which arrives at the dendrites just after the cell soma has conducted, will delay the subsequent discharge only little (Fig. 3 D, arrow 2) but if it arrives when the prepotential has already developed and the cell is about to fire, it will cause an appreciable delay because the prepotential will then have to develop anew. The effect of a certain frequency of *I* impulses on the afferent discharge rate may therefore be variable, depending on the timing of individual impulses. The essential point is that whenever the prepotential is permitted to reach the cell's firing level, afferent impulses result.

2. Inhibition at Different Levels of Membrane Potential

The above results have shown that the inhibitory repolarizing action on the receptor cell membrane increases with progressive cell depolarization. Another, perhaps simpler demonstration of the same phenomenon is made on a rapidly adapting fast receptor cell in which the membrane potential can be "set" over a wide range to different levels of depolarization, without setting up conducted

afferent discharges. Fig. 4 shows records from an impaled rapidly adapting "fast" cell from the second abdominal segment. The accurate resting potential, when these measurements were made under high amplification, is not known, but was presumably 60 to 70 mv. By appropriate stretch the resting potential of the completely relaxed cell was reduced by 16.5 mv. to a new maintained level and the inhibitory nerve fiber was excited at 20/sec. Each *I* impulse in Fig. 4A caused a peak repolarization of 9.7 mv. which was rapidly attained and then decayed almost entirely within 50 msec. If the cell was held under less stretch and therefore was initially less depolarized, the inhibitory potential became progressively smaller (4 and 1 mv., Fig. 4 B and C). When the cell was

FIG. 4. Effect of *I* impulses at different membrane potential levels. Intracellular leading, fast receptor cell of second abdominal segment. Trains of *I* impulses at 20/sec. A, cell stretched, causing initial maintained depolarization of 16.5 mv. *I* impulse repolarization peaks 9.7 mv. B and C, during progressively lowered stretch and higher membrane potential levels the same train of impulses sets up smaller *I* potentials of 4 and 1 mv. D, still further relaxation of stretch. *I* impulse sign reverses, initial depolarization follows each inhibitory stimulus. During complete relaxation of the cell *I* depolarization peaks of 7 mv. (not illustrated) were seen. For plotting of results from this cell see Fig. 5 (*cf.* also Figs. 6 and 7).

relaxed, to within about 6 mv. of its unstretched equilibrium membrane potential (see Fig. 5), each inhibitory impulse set up a small initial depolarization followed by a polarization phase. The dotted line in Fig. 4 is drawn at the membrane potential level at which the *I* potential reverses its polarity. In Fig. 5 the size of the inhibitory peak potentials is plotted as ordinate while the abscissa shows the displacement of the membrane potential. Zero stretch depolarization is indicated when the cell is completely relaxed. In that state an *I* impulse sets up a depolarization with a peak amplitude of 7 mv. (see also Figs. 6 and 7 B). As stretch increases the *I* depolarization becomes progressively smaller. Around a stretch depolarization of about 6.7 mv. the *I* potential reverses its polarity and starts to increase in amplitude in an almost linear relationship with further reduction of the resting potential. Stretch depolarization could be continued in this preparation to a level of 22.5 mv without setting up conducted impulses while the *I* polarization peaks increased to over 13 mv.

All the data for Fig. 5 were obtained from the same cell but they represent two sets of measurements taken 20 minutes apart. The nine crosses indicate the series of stretches, not starting with the receptor fully relaxed, from which the illustrations of Fig. 4 were chosen. The results in the same preparations were repeatable and remarkably similar values for *I* potentials were obtained with

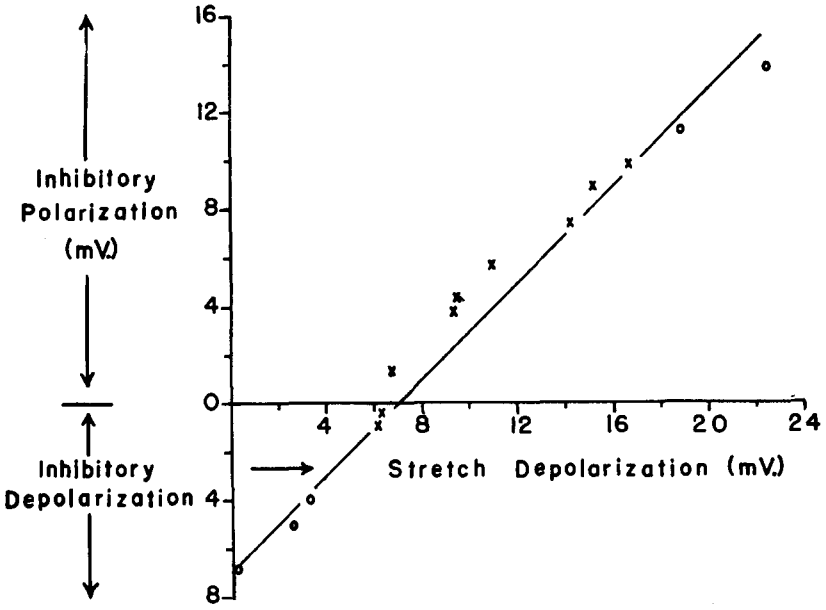

FIG. 5. Inhibitory potential amplitude at different membrane potential levels in a fast receptor cell. Abscissa, different amounts of depolarization up to 22.5 mv. were produced by stretch without discharging the cell. Zero indicates membrane potential when the cell was completely relaxed. Ordinate, inhibitory potential amplitudes. The points lie approximately along a diagonal line with a slope of 45°, crossing the abscissa at the "inhibitory equilibrium level." Note that *I* potential compensates for displacement of membrane potential in either direction from this level at about 6.4 mv. Crosses indicate set of data partly illustrated in Fig. 4. Full circles are data from same cell 20 minutes later (see text).

similar stretch depolarizations at the different times. The points of Fig. 5 lie approximately along a diagonal line which has been drawn with a slope of 1 (45°). The intersection between this line and the horizontal axis at about 6.4 mv stretch depolarization represents a constant level reached by the peaks of the *I* potential. It is at this level of membrane potential that the *I* potential reverses its polarity and it indicates an "equilibrium level" to which the inhibitory action tended to restore the membrane potential if it was displaced in *either* direction by stretch. It may perhaps express the equilibrium level of one or of several ions (see Discussion).

A further picture of inhibitory action at different levels of stretch depolarization is shown in Fig. 6. An impaled fast receptor cell was stretched while its response was tested by periodic bursts of inhibitory impulses at 30/sec. Only three segments from a moving film record, lasting altogether for 25 sec., are presented. During the relaxed state each *I* impulse caused a depolarization of 4 mv. (first 2 arrows). At arrow 3, stretch depolarized the cell to about 20 mv. and set up a short burst of three discharges. Another train of six *I* stimuli was given shortly afterwards, each impulse transiently repolarizing the membrane by 9 mv. Further stretch, immediately after the last *I* impulse, caused renewed

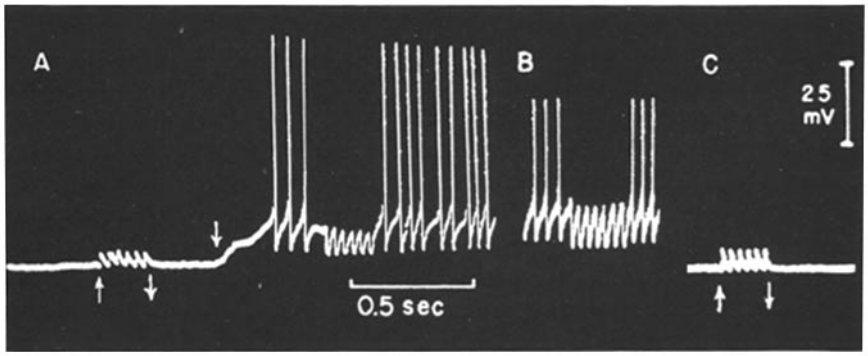

FIG. 6. *I* potentials at different levels of stretch. Fast cell from third abdominal segment, intracellular records. A, receptor almost completely relaxed, six *I* impulses at 30/sec. cause depolarization potentials (arrows). At third arrow stretch causes about 20 mv. depolarization and three discharges, followed by *I* train setting up repolarization potentials. Further continued increasing stretch results in afferent impulses which gradually decline in height. At gap 10 sec. of record cut out. In B same *I* train inhibits, the repolarization potentials reaching 12 mv. C, during complete relaxation of cell, 6.5 mv. *I* depolarization peaks are seen.

afferent discharges. During the subsequent gradually increasing stretch the impulse peaks of 73 mv. gradually declined. A section of 10 sec. was omitted during the gap following A. In B the renewed inhibitory train caused another set of polarization potentials of 12 mv. each, stopping the afferent discharge. Relaxation of the cell in C, several seconds later, increased the resting potential which, however, was higher than at the beginning of stretch in A. Accordingly each inhibitory impulse set up a reversed potential, each depolarizing the cell, this time by 6.5 mv. A gradual decline of spike potentials recorded in the cell body during repeated and long maintained stretch relaxation procedures was not uncommon and may well be attributed to a slight injury of the cell body region by the impaling electrode. In this cell the inhibitory potentials and generator potentials were only little changed and showed less sensitivity to such small but apparent damage.

These experiments demonstrate that the membrane effect produced by *I* impulses depends on the state of the sensory receptor cell. There exists a membrane potential level at which inhibition causes no electrical changes or only small ones; if the cell is displaced from this "inhibitory equilibrium level" in either direction, the *I* action tends to restore the "equilibrium" by either polarizing or depolarizing the structure. Since inhibitory potentials of both polarities were obtained with extracellular as well as with intracellular leads they are not likely to be caused by some recording artefacts, or by injury changes resulting from impalement of cells.

3. Certain Characteristics of Inhibitory Potentials

There has not been a sufficient uniformity in many of the membrane changes set up by inhibitory nerve impulses to permit a rigid description and classification of the phenomena. It appears that the processes which participate in the inhibitory events are somewhat labile and inhibitory potentials may vary appreciably in different experiments, while other characteristics of the cell, such as the conducted impulses or the slow potentials set up by the generator action show relatively small variations, as measured by the present methods. The essential feature, however, namely the ability of inhibitory impulses to stop afferent discharges or to reduce their frequency, is rather resistant to fatigue and may persist for many hours in spite of intermittent prolonged stimulation.

Time Course and Summation of I Potentials.—One type of inhibitory membrane change which is seen quite frequently is shown in Fig. 7. A single inhibitory impulse (I_1) to a fast adapting receptor cell caused a polarization peak potential of 2.5 mv. reached in less than 2.0 msec., followed by an approximately exponential decay, falling to ½ in 11 msec. A second impulse, (I_2) added its own potential which, however, never exceeded the first one. At an interval of 18.0 msec. in Fig. 7Aa, I_2 contributed a polarization potential which was similar to I_1, but at shorter intervals of 6.0 and 2.0 msec. (Fig. 7 Ab and c), I_2 contributed little and merely brought the declining potential back to the peak value reached by I_1. Fig. 7 B shows the *I* potentials in another fast cell which was completely relaxed. The *I* impulse in this cell caused a large depolarization of 10 mv., reaching its peak in 2.6 msec. and again declining to the base line in about 30 msec. with a half-time of 11.8 msec. A second impulse restored the peak depolarization, its contribution depending on the time of arrival. Fig. 7 clearly shows how a practically steady "ceiling" effect, either in the polarizing or depolarizing direction, can be attained during high frequency *I* stimulation (see Figs. 3 C, 8, and 12). Thus in Fig. 7 Ac the second *I* virtually prolonged the polarization peak and the decaying portion of the potential. In each case an *I* impulse contributes according to the momentary membrane potential level of the receptor cell.

From Fig. 7 B the equilibrium at which the *I* potential reverses its sign can be predicted. The cell was completely relaxed at a resting potential near 70 mv. giving an *I* deflection of 10 mv. At a stretch depolarization level maintained around 60 mv. the *I* depolarization potential would be quite small and beyond that value repolarization would begin. The time course of *I* potentials was not

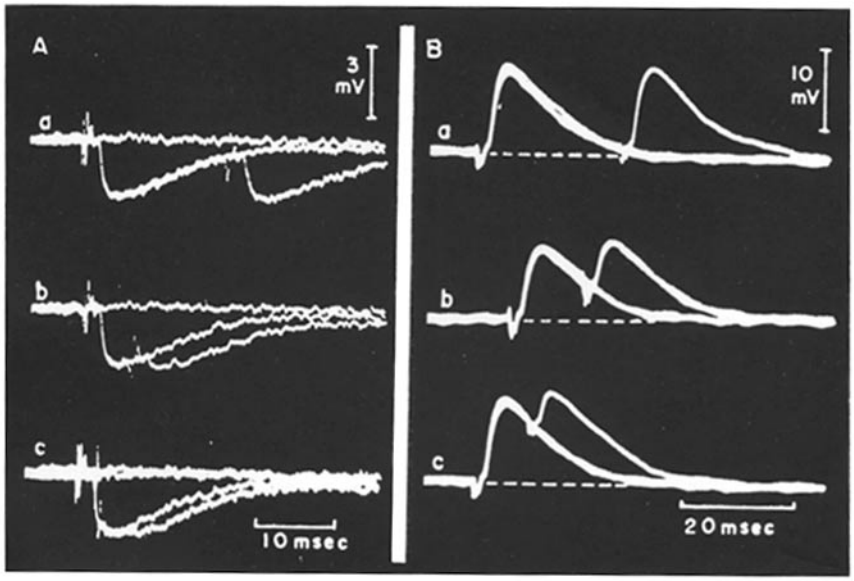

FIG. 7. Time course of *I* potentials and ceiling effect. Fast receptor cell, intracellular recording 22°C. A, under light stretch single *I* impulse sets up polarization potential, rising to a peak in about 2 msec. and decaying to half within 11 msec., total duration near 30 msec. Addition caused by second *I* impulses at intervals of 18.0, and 6.0 msec. is determined by initial potential level, not exceeding first polarization peak. In c, I_2 contribution at an interval of 2.0 msec. is quite small. B, in relaxed fast receptor (resting potential 70 mv.) individual *I* potentials depolarize membrane with a time course similar to that in A. Note different sweep speeds in A and B. Repeated superimposed sweeps in all records.

greatly changed by stretch, although one might perhaps expect an acceleration of the falling phase. For instance, in the experiment plotted in Fig. 5 the time to half-decay at different levels of stretch depolarization fluctuated, unrelated to the amount of stretch, between 12.5 and 15 msec. The time course measurements of small potentials may involve relatively large errors.

Not in all cells was the single *I* potential peak near a ceiling level. With repetitive stimulation the potentials frequently summed, building up to twice (or more) the peak value of individual *I* potentials.

Postinhibitory Polarization (Depression).—In Fig. 8Aa an impaled slow cell

was stretched and made to discharge at a regular frequency of 7/sec. A train of *I* impulses at 45/sec. in Fig. 8Ab inhibited the discharge of the steadily stretched preparation by preventing the prepotential from reaching the firing

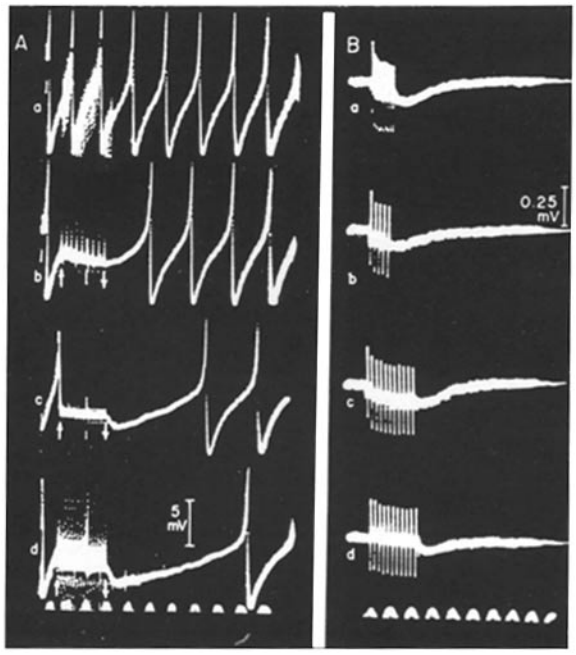

FIG. 8. Postinhibitory polarization. Aa, stretched slow receptor cell from the eighth thoracic segment discharges at 7/sec. Intracellular records. Ab, *I* impulse train at 45/sec. inhibits discharge. Ac, *I* impulse train at 150/sec. causes longer inhibitory period. End of inhibition potential is followed by a delayed polarization phase. Ad, *I* train at 200/sec. The delayed polarization potential is further increased. Ba, extracellular records, another slow receptor cell under light stretch. *I* train at 100/sec. sets up repolarization during stimulation, followed by additional delayed polarization. Bb, stimulation at 50/sec. causes polarization and little postinhibitory effect. Bc, longer *I* train at 50/sec. sets up marked postinhibitory polarization phase. Bd, less stretch on cell. *I* impulses at 50/sec. set up smaller polarization potential during train. Note there is no diminution in postinhibitory polarization. Time, 10 C.P.S.

level of the cell. On cessation of *I* stimulation the full stretch depolarization was able to develop once more, leading to a subsequent orthodromic impulse within 200 msec. A train of impulses at 150/sec. in Fig. 8Ac, although lasting for the same duration and reaching the same ceiling, had a more prolonged inhibitory effect. The last *I* impulse was not followed immediately by a decline of the polarization potential but by a further increase of polarization. The

interval between cessation of *I* stimulation and the first postinhibitory impulse was now increased to 450 msec. This gap was further lengthened, to 625 msec., if the *I* frequency was increased to 200/sec. in Fig. 8Ad. In addition the delayed "positivity" was somewhat greater.

Postinhibitory polarization was seen at various levels of membrane potential. The records of Fig. 8 B were obtained with extracellular leads in a slow cell which was lightly stretched below the discharge level. The postinhibitory effect was dependent on frequency and duration of *I* of stimulation. Thus in Fig. 8 Ba a short train of ten impulses which polarized the cell during stimulation at 100/sec. was followed by an "afterpositivity," while five *I* impulses at 50/sec. in Fig. 8 Bb did not show the effect. If stimulation of the same frequency was prolonged, by only six *I* impulses, the delayed polarization appeared (Fig. 8 Bc). The same train of stimuli was given again in Fig. 8 Bd but with the cell more relaxed. There was practically no polarization potential during stimulation, but the postinhibitory polarization was still present, in fact it was somewhat increased.

Postinhibitory polarization was not seen in all cells and the stimulus frequency which caused it was also variable. For instance, it may not appear even after a few seconds of stimulation at 50/sec., while on occasions there was an indication of its presence even after a single *I* stimulus, suggested by a double phase during the potential decay. The postinhibitory polarization could occur after the *I* potential had started to fall off, showing an independent rise on top of the declining phase. The factors determining these variable latent periods between cessation of *I* stimulation and rise of the polarization are not known. Since the postinhibitory polarization effectively prolongs inhibition it may perhaps be appropriate to call it postinhibitory depression in contrast to the facilitation phenomena (see below). The delayed polarization seems to represent an "active" independent process since it appears in opposition to the depolarizing action of stretch. This phenomenon reminds one of the slow wave which is seen on repetitive stimulation in eserinized neuromuscular junctions (10) or in cat's stellate ganglia (9) when acetylcholine is implicated. That specific mechanism, however, seems unlikely in this preparation.

Postinhibitory Facilitation.—The prepotential which precedes the first orthodromic impulse after postinhibitory polarization is slowed (Fig. 8 Ab to d), compared with the prepotentials of the control discharges in Fig. 8 Aa. Furthermore, the regular rate may not be resumed by several succeeding discharges. The opposite phenomenon is also observed in many preparations. Thus, after inhibition has been stopped, the rate of rise of prepotentials increases and the discharge rate may be transiently accelerated in some slow receptors (see Fig. 3). The phenomenon is seen more strikingly in fast adapting cells which have been stretched near to their firing level. A short burst of inhibitory impulses may initiate one or a group of afferent discharges in a

quiescent receptor cell. In Fig. 9a seven *I* stimuli at 23/sec. (between arrows) were followed by a train of nine afferent impulses in this critically stretched cell. The recording was done with external leads. More detail is seen in Fig. 9b, with intracellular recording, where each of six *I* stimuli caused a repolarization of 13 mv. The last *I* potential was followed by a rapid depolarization,

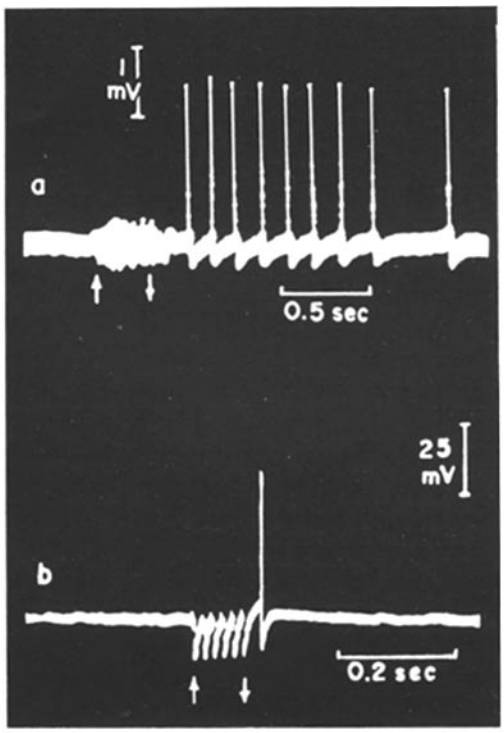

FIG. 9. Postinhibitory excitation. Fast receptor cells stretched to near firing level. *a*, extracellular recording. Between arrows *I* stimuli at 23/sec. are followed by afferent discharge. *b*, intracellular record. Six *I* impulses are followed by one conducted impulse.

reaching several millivolts above the "resting" level and thus setting up one afferent discharge. In the slow cells afferent discharges could not be initiated after cessation of *I* stimulation at near threshold stretch.

One may perhaps think of this inhibitory "rebound" phenomenon in the following manner: Inhibition effectively removes the stretch stimulus from the cell, which becomes then virtually relaxed in terms of membrane change. A quick redevelopment of the former depolarization level when inhibition ceases may be equivalent to application of a quick renewed stretch. Following stretch, an initial discharge acceleration is known to occur in these receptors (12).

Since the firing level at which the first postinhibitory impulse arises is within several millivolts of the membrane level at which the initial stretch depolarization sets up a discharge, the phenomenon may be unrelated to threshold changes.

4. Interaction of Antidromic and Inhibitory Activity

If a sensory stretch receptor axon is stimulated along its course, an antidromic impulse (A) will normally invade the soma-dendrite system. The antidromic potentials may be significantly modified if inhibitory impulses are sent

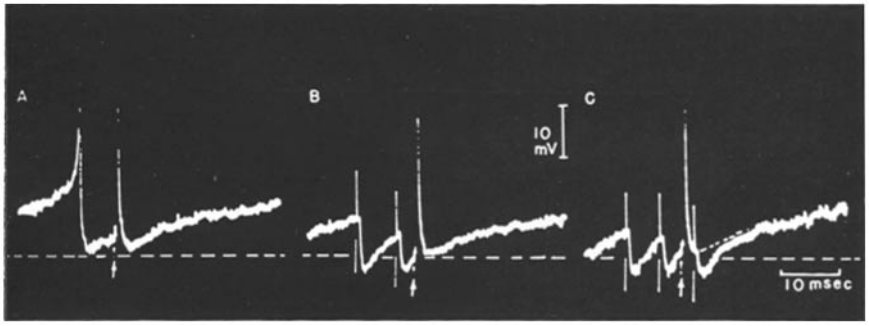

FIG. 10. Persistence of generator potential during antidromic impulse. Intracellular records from a stretched slow receptor of the second abdominal segment. Lower portion only of large impulses is seen. A, an antidromic impulse at arrow sent into the cell shortly after an orthodromic discharge. The repolarization phase of both impulses reaches the same level. B, two *I* impulses precede the antidromic. C, during peak of *A* repolarization phase a third *I* impulse further polarizes, indicating that distal dendrite portions were still depolarized. Dotted line drawn through membrane level to which *A* impulses repolarize.

into the cell at the same time. By this approach it was attempted to gain further information on (*a*) the conditions of excitation spread in the dendrites and in the cell soma, (*b*) the mechanism of inhibitory action.

Persisting Generator Potential in the Dendrites.—In the two preceding studies it was concluded that the generator potential which is set up by stretch in the dendritic terminal region persisted to some extent during the conducted activity in the receptor cell. Supporting evidence for these views is obtained in those cells in which the equilibrium point for *I* action is at a high membrane potential level, near the resting potential which is measured in an unstretched "relaxed" cell. If such a cell is stretched, orthodromic discharges (*O*) are set up when the membrane potential, measured in the cell body, is depolarized by about 12 mv. The repolarization phase of *O* brings the membrane potential back to within several millivolts of the resting potential of the relaxed cell. If an *A* impulse is sent into such a cell, its repolarization phase is similar to that of the *O* im-

pulses. This is shown in Fig. 10 A at high amplification with only the lower portions of the conducted impulses visible. The first deflection is an *O* potential, while at the arrow an *A* impulse was set up. In Fig. 10 *B* taken immediately afterwards, *A* was preceded by two *I* impulses. The repolarization peak of *A* was practically the same as in the exposure in Fig. 10 A (an interrupted line is placed through the records at that level) while the inhibitors repolarized 2.6 mv. below that level. In Fig. 10 C one more *I* impulse was placed at the time of the repolarization peak of *A*, adding to it until the *I* ceiling level was reached. The expected time course of *A* in the absence of inhibition is drawn in (dotted line), taken from Fig. 10 A.

The following interpretation of the results is proposed: If no stretch is applied to a cell the *A* repolarization is complete, ending in an afternegativity. If the cell is stretched, however, the *A* impulse again tends to repolarize to the relaxed resting potential level, but this full repolarization is prevented by the generator potential which persists in the dendrites (for details see reference 13). The evidence for the persisting dendritic generator potential is provided by the two closely related observations that (*a*) *I* impulses alone are able to repolarize the cell further than the recovery phase of the *A* impulse (Fig. 10 B, dotted line) and (*b*) that an *I* impulse, arriving during the height of the *A* recovery phase, virtually "turns off" the remaining generator potential (Fig. 10 C). In these tests the inhibitory potential serves merely as an index, showing that during antidromic invasion of the dendrites there still remained at least a portion of the generator potential. These results supply strong evidence for the thesis advanced in the previous studies that during stretch the dendrites provide a persisting "drive" for the more central portions of the cell. Furthermore, the experiments support the view that since antidromic impulses fail to "wipe out" the potential in the peripheral dendrite region, the usual all-or-none impulses do not occur in the terminals (*cf.* also reference 5).

The Inhibitory Potential during Various Phases of Antidromic Activity.—Antidromic invasion sets up in the entire cell a series of potential fluctuations during which the effect of *I* impulses can be tested, providing some information which otherwise could be obtained only if *I* impulses were examined while conditions of stretch were actually altered (*e.g.* Fig. 6). In Fig. 11, *I* impulses were sent into the dendrites at different phases of the afterpositivity in a lightly stretched slow receptor cell. All records present superimposed sweeps first of *A* alone and then of *A* together with *I*. The largest contribution was made by *I* if its action started during the trough of the after positivity (Fig. 11 B). As the *A* potential returned toward the base line the *I* depolarization decreased (C) and became negligible if the inhibitor acted about 120 msec. after the beginning of antidromic invasion (right hand portion of D, note artefact). In this cell the *I* equilibrium level coincided with the base line. If further stretch depolarization was applied, the *I* potential reversed its polarity (see below). No detectable

change in the conducted antidromic impulse time course was seen (at fast sweep speeds) if *I* action started during the rapid repolarization phase. The first effect became obvious during the early portion of the afterpotential (Fig. 11 A).

The illustration of Fig. 12 is from a fast receptor cell taken at a steady initial stretch (A), and during relaxation (B). Trains of *I* impulses in Aa polarized the cell body by 3.3 mv., while in Ba they built up a depolarization of 3.5 mv. As

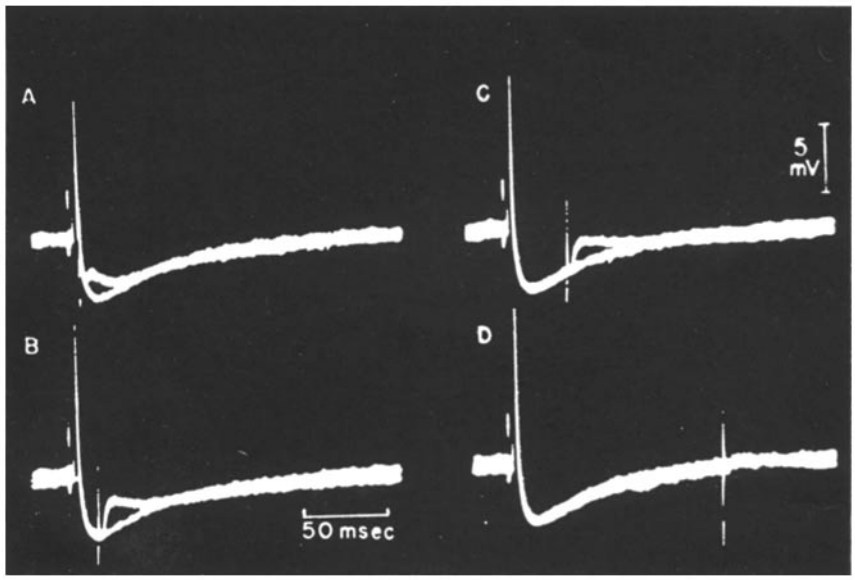

FIG. 11. *I* impulses during afterpositivity. Intracellular records from slow receptor cell, eighth thoracic segment, under light stretch. Resting potential 60 mv., 22°C. Single *I* stimulus sets up depolarization potentials which are largest in B during repolarization peak of an antidromic impulse (only lower impulse portion is seen). In D the *I* impulse sets up a very small potential only (note artefact at right).

expected, the stretched *A* impulse had an afterpositivity (Ab), while in Bb this was absent. If *A* invaded the cell during an *I* train its repolarization phase was practically " cut off" at the equilibrium level of the inhibitor action which merely served as a new base line. Thus in Fig. 12 Ac the *A* impulse could not repolarize appreciably below the level which was set by the inhibitory train. The expected time course of *A* in the absence of inhibition is indicated by the dotted line. Similarly, in the unstretched cell the *A* impulse could not repolarize below the ceiling depolarization (Fig. 12 Bc). The decaying phase of the *I* potential, however, could largely be wiped out and thereby shortened if *A* invaded the cell during that period (not illustrated).

These results merely expand the previous findings that the *I* contribution

depends on the postsynaptic membrane potential level and that mainly those parts of the antidromic impulse which are linked with excitation spread in the dendrites are modified.

One further observation which was, however, exceptional is presented here because it may have implications in respect to inhibitory mechanisms. At the start of the experiment, stimulation of the *I* axon to a slow cell inhibited dis-

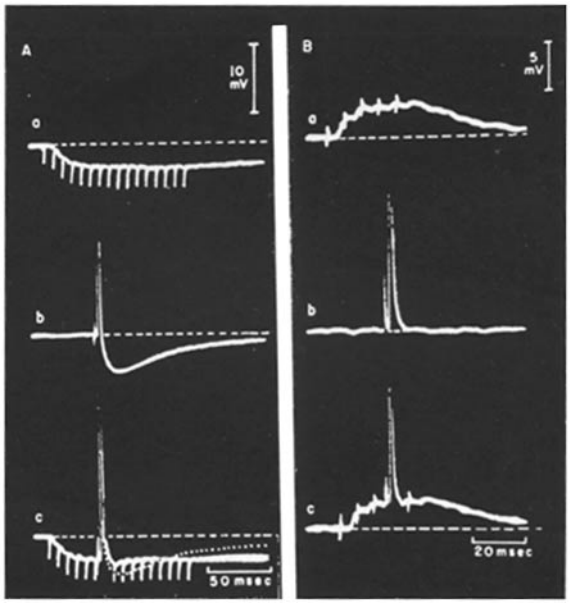

FIG. 12. Slow receptor cell, eighth thoracic segment, 21°C. Resting potential 70 mv. (unstretched). Aa, cell under stretch, train of *I* impulses at 130/sec. polarizes. Ab, antidromic impulse (*A*) alone. Ac, *A* invades cell during inhibitory train which prevents development of complete afterpositivity. Dotted line indicates expected time course. Ba, cell relaxed, train of five *I* impulses at 200/sec. depolarizes. Bb, *A* alone shows no afterpositivity. Bc, *A* during inhibition is not appreciably changed (see text).

charges as usual. Subsequently the *I* depolarization potential increased to 15 millivolts (Fig. 13 A, note slower sweep speed) and eventually to about 17 mv. when it set up an orthodromic impulse (70 mv. peak). Occasionally this *I* impulse even started a short high frequency burst of 2 to 3 orthodromic impulses. If an *A* impulse was sent into the cell (Fig. 13 C), it reached the same peak as the orthodromic impulse set up by inhibition, and if (*A*) was fired into the cell during the *I* potential (not illustrated), it merely substituted for the orthodromic impulse. Just as in Fig. 12 Bc, the *I* depolarization did not "collapse" or was diminished unless the *A* impulse arrived during the later portion

of the potential decay. In two other experiments in which *I* by itself did not cause excitation in an unstretched cell, it could facilitate the setting-up of discharges by adding to the prepotential which is caused by stretch. Over a limited range the cell could be driven by *I* stimulation. Furthermore, in such preparations the *I* potential was rather insensitive to stretch, while the *A* impulse showed the expected changes, such as the appearance of the after-

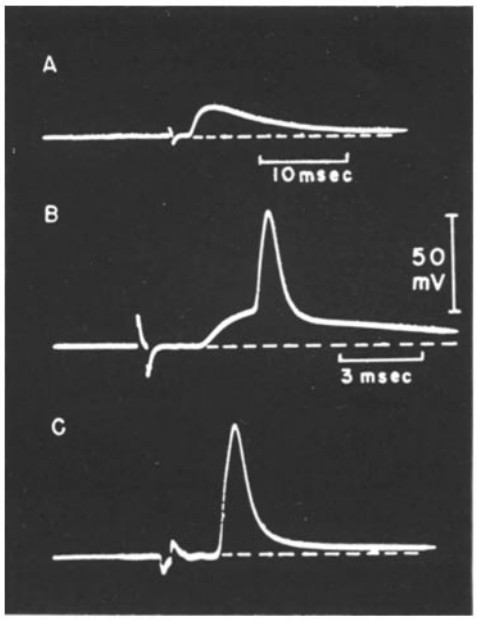

FIG. 13. Excitation by *I* impulse. Intracellular record, slow receptor cell eighth thoracic segment, 22°C. Resting potential 70 mv. A, *I* depolarization potential of 15 mv. B, *I* potential reaches 17 mv. and sets up orthodromic discharge. C, antidromic impulse alone.

positivity. These observations were certainly abnormal, but the cause of the changes is not known.

Modification of Antidromic Invasion by Inhibition.—It was pointed out above that normally inhibition did not alter the *A* impulse rise, its peak, or fast initial falling phase. In some experiments, however, in which the *A* impulse peak had gradually declined to about 50 to 60 mv., the spike could be reduced by several millivolts. In Fig. 14 the *I* axon was excited at 10/sec. and the small depolarization potentials were superimposed in the exposures of the recurring sweep. At the same time an *A* impulse was moved across the sweep and it was clearly seen that the reduction of the *A* peak which occurred, had a time course similar to that of the *I* potential. The effect, although small, was definite and

was greatest (2 mv.) at the height of *I* depolarization. The *A* impulses of Fig. 14 had a small indication of a "delay" near their peak of 60 mv. (see reference 13). This experiment could not be repeated in some of the trials and was seen in five cells only. It led, however, to a closer study of blocked antidromic potentials (see below).

In a fast receptor from the second abdominal segment (Fig. 15) *A* impulses declined to a low value and the potentials consisted largely of the electrotonically conducted component of the axon impulses which had been arrested centrally from the cell body. The records were taken during differing stretches

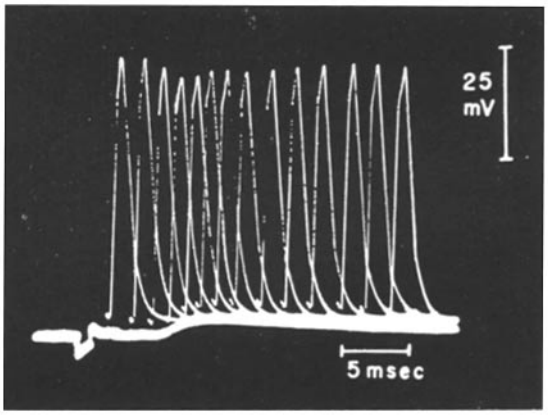

FIG. 14. Slow cell, intracellular recording. Sweeps recurring at 10/sec. are superimposed. *I* impulses set up inhibitory depolarization. Antidromic impulses placed at different times are reduced. Impulse peaks reduced maximally (2 mv.) during initial phase of *I* potential. Resting potential 70 mv. Control size of antidromics given by the potential peaks of the two impulses at the beginning of sweep before *I* potential rise.

of the cell and three phases only are shown here. In Fig. 15 A the cell was relaxed, with *I* stimulation by itself causing depolarization. In B the cell was lightly stretched with *I* alone setting up a negligibly small potential change, while in C the stretch was strong enough so that a train of *I* impulses repolarized the tissue. Under all these conditions a consistent result was obtained; namely, inhibition reduced the size of the blocked *A* potentials and accelerated their decline. In the upper row (Aa, Ba, and Ca) are the small *A* impulses alone, while below in Ab, Bb, and Cb they are given shortly after cessation of several *I* impulses. The dotted lines are drawn in, superimposing the *A* impulse as it appeared in the absence of inhibition, for easier comparison. Allowance was made for the *I* potential shifts caused by *I* trains alone. In Fig. 15 Ab the potential peak was reduced from 17 to 12 mv and the half-time for decay from 3.0 to 1.3 msec. In Fig. 15 Bb the reduction was from 27 to 12 mv. and the

half-time of the potential from 1.5 to 1.2 msec., while in Cb the *A* impulse diminished from 8 to 6 mv. and the decay time from 3.4 to approximately 2.0 msec. The effect of inhibition on *A* increased if the preceding *I* frequency was greater. It had also a rapid temporal decay, which is shown graphically in Fig.

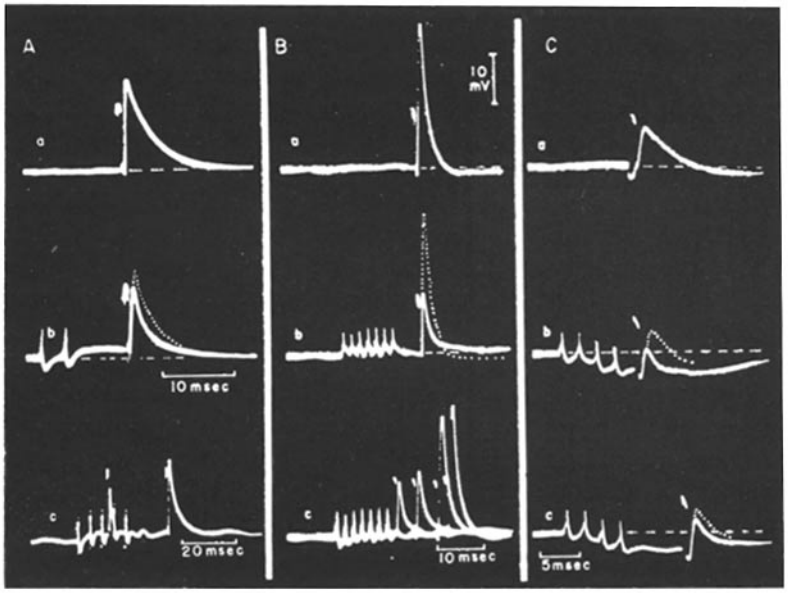

FIG. 15. Effect of inhibition on blocked antidromic (*A*) impulses. Fast cell, second abdominal segment, 21°C. Resting potential 70 mv. (unstretched). Intracellular electrodes, filled with 0.6 M K_2SO_4. Aa, *A* impulse alone (17 mv.), block at the axon-soma boundary region. Ab, two *I* impulses set up depolarization in relaxed cell. *A* is reduced and its time course accelerated. Dotted line indicates control. Ac, two *A* impulses. A_1 during inhibitory train more reduced than A_2 after cessation of *I* stimulation (note slower sweep). Ba and Bb, preparation lightly stretched. *A* impulse again greatly reduced by preceding train of seven *I* impulses at 500/sec. No appreciable potential change was set up by *I* train alone. Bc, *A* impulses placed at different times after *I* train, reveal time course of *I* effect. Ca and Cb, cell stretched, *I* impulses polarize (b) and again reduce *A*. Cc, *A* moved later, is less affected. Voltage calibration same for all records. Large dots preceding *A*'s are artefacts (see text).

15 Bc in which sweeps were superimposed while *A* was moved closer to the *I* train. In Fig. 15 Ac two *A* impulses were sent into the cell (note slower sweep) the first one much reduced (9 mv. against control of 17 mv.) its time course shortened, while the second *A*, 16 msec. after the last *I* in the preceding train, was 14 mv. (control 17 mv.). Similarly it is clear from Fig. 15 Cc that if *A* is moved later, it approaches the control value (dotted line superimposed).

The following alternatives may be proposed in an attempted explanation:

(*a*) Inhibition may alter, by changing the membrane potential, the site to which the *A* impulse penetrates, *i.e.* in the case of Fig. 15 the impulse would be blocked further centrally from the axon–cell body boundary. This in turn would reduce the peak value of the electrotonic potential spread and may accelerate the time course by reducing the local, partially conducted impulses which may be set up in the cell body during conduction block (13). (*b*) The inhibitory action may change the properties of the dendrite region by increasing their conductance and thereby changing conditions of current flow, causing the effects seen in Fig. 15. Alternative (*a*) seems untenable for the following reason: The effect is independent of the direction of the potential change which is set up by the *I* impulses, clearly seen in Fig. 15 Ab, Ac, and Cb. Especially interesting is Fig. 15 Bb since inhibition alone did not cause appreciable electrical changes, yet it strikingly reduced the *A* potential. In any event, only polarization of the cell body would be expected to enhance the blocking of impulses (see below). Proposition (*b*) may be thought of in the following manner: Current will flow between the portion of the axon occupied by a blocked impulse and the soma-dendrite system. Part of the current will traverse the cell body and part will flow through the dendrites. It is this latter portion which will be increased during the postulated conductance change. As a consequence the cell body, in which the intracellular lead is located, will be depolarized to a lesser extent since much of the current will be diverted through the lowered resistance path of the dendrites. This itself will reduce the potential in the cell body and presumably will diminish the chances for "local" partially conducted impulse activity.

Against the explanation suggested above, it may be held that dendritic depolarization as set up in Fig. 15 Ab and Ac, should enhance invasion of the cell body by a partially blocked impulse. This has been demonstrated during stretch (*cf.* reference 13, Fig. 5). Analogous facilitation of antidromic invasion has actually been seen in the same fast cell from which the illustrations of Fig. 15 were taken. After these observations the electrode was taken out of the cell soma and later reinserted. At that time the *A* invasion was more labile. If a single *A* impulse was set up it invaded the soma, but still causing a potential of 55 mv. only. The invasion readily "fatigued" and even at slow repetition rates of 5 to 10/sec. the *A* potential suddenly dropped to a lower value. In Fig. 16 Aa single *I* (10 mv.) and *A* (55 mv.) impulses, each taken separately, are shown. In Fig. 16 Ab two *A*'s (A_1 and A_2) were sent into the cell 20 msec. apart at a repetition rate of 5/sec. The full cell body invasion was promptly blocked and the potential fell suddenly to 24 mv. If now an *I* impulse was added, A_1 was facilitated and suddenly grew to 55 millivolts (the control value) while A_2 at the end of the *I* depolarization potential was little affected (Fig. 16 *A*c). An essentially similar effect is shown in Fig. 16 Bb where A_2 at an interval of 5 msec. (single sweep exposure) failed to conduct fully into the cell soma. If it was

combined with several I stimuli (Fig. 16 Bc) axon-soma transmission for A_2 was largely restored, presumably because the soma-dendrite system was in a partially depolarized state. It is interesting that at the same time the A_1 was actually reduced by 1 to 2 mv. as compared with the control. This was tested repeatedly and presumably is due to the same mechanism which reduced the impulse in Fig. 14, most likely based on a conductance change of dendrites.

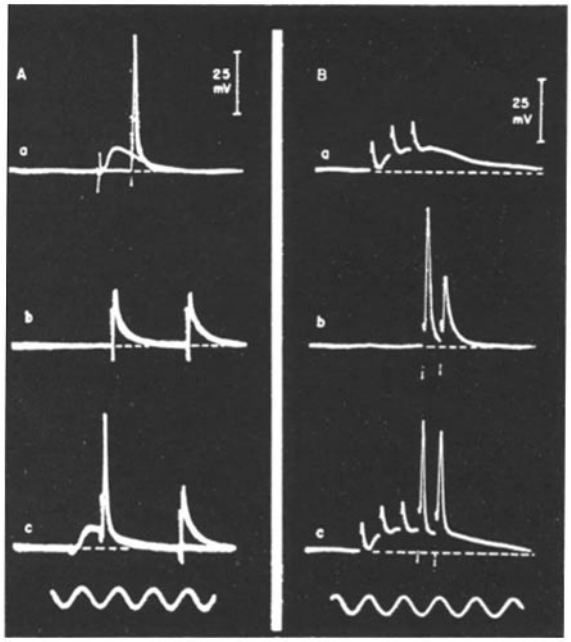

FIG. 16. Facilitation of antidromic invasion by I action. Same fast cell as in Fig 15. Aa, single I and A potential on same sweep (not stimulated together). Ab, A_1A at 20 msec. interval, sweep repetition rate of 5/sec. Full invasion of soma is blocked Ac, I impulse facilitates soma invasion by A_1. Ba, three I impulses alone. Bb, A_1A at 5 msec. interval single sweep, A_2 is blocked. Bc, I stimulation facilitates invasion by previously blocked A_2. Time 100 C.P.S.

In Fig. 16 Ba the I potentials alone, building up to 10 mv. depolarization, are shown.

The experiment of Fig. 16 indicates how the consequences of conductance changes may be masked in a cell if the mechanism for antidromic invasion of the cell body is labile and quite critically poised. The facilitating aspects of the inhibitory potential change may be seen exclusively. Only if the transmission block from the axon to cell body is well advanced can inhibitory action, independently of electrical change, reduce the potentials set up by an arrested axon impulse.

DISCUSSION

The interpretation of the principal results of this study is largely determined by acceptance of the histological and physiological evidence which indicates that the inhibitory (*I*) axons form their synaptic contacts on the dendrites of the stretch receptor cells. The dendrites are thus the seat of origin for both excitatory (generator) and inhibitory action. Since one has to record from the cell body, all the measurements of the primary events in the dendrites are inevitably indirect. The membrane potential change which the intracellular electrode measures during the graded non-propagated dendrite activity, before the setting up of propagated impulses, is the result of current flow. The spatial decrement which must occur along the dendrites is not known, but one may perhaps assume a decay to about half over 0.5 mm. based on determinations on *Carcinus* axons (21). The electrotonically conducted changes from dendrites, recorded in the cell body, may then be reduced by 20 to 80 per cent depending on cell size and configuration. In addition to the diminution in potential size the time course of dendritic events as seen in the cell body will be slowed.

It has consistently been seen that the *I* impulses bring the receptor membrane toward an equilibrium level. If the membrane potential is displaced in either direction from a certain value, inhibition tends to restore that potential. Thus the *I* potential may be either a depolarization or a polarization depending on the state of the receptor cell. There is no evidence that inhibition can increase the membrane potential beyond the level which is found when a cell is unstretched and "at rest." In this sense the term "repolarization" seems at present preferable to hyperpolarization. The equilibrium level, or zero point, at which the *I* potential reverses its sign, has been rather variable and it is not possible to decide which is the "normal" value. The depolarizing action of *I* impulses may perhaps be found in cells which are not entirely normal. It cannot be, however, the result of mechanical injury during cell impalement or of diffusion of materials (for instance chloride) from the capillary electrodes, or the result of the formation of various liquid junction potentials, because it was seen with KCl or K_2SO_4-filled leads, or when recording was done from the outside of cells. Further, such cells could have resting potentials of 70 mv. or more and conduct potentials of 70 to 80 mv. for many hours. Only in one preparation did the *I* depolarization progressively increase during the experiment until it reached the cell's firing level (Fig. 13). The most frequent equilibrium level for *I* action was approximately 5 mv. below the resting potential, *i.e.* if one measured a 75 mv. membrane potential in a relaxed cell, inhibition would reduce it to about 70 mv. The plotting of Fig. 5 shows such a case. This conclusion is tentative, based on studies in ten cells only out of over a hundred preparations in which either the experimental conditions were not comparable or the data were incomplete because a stretch-relaxation series was not obtained. On the whole it

has become clear that the equilibrium of *I* action is near the cell's resting potential level. It is interesting that in contrast the excitatory junctional transmitter at the neuromuscular junction has its equilibrium level near zero potential (7); *i.e.*, it tends to produce complete depolarization.

From a point of view of cell function inhibition is equally effective wherever the equilibrium level is found, as long as it remains below the threshold for excitation of conducted impulses. Essentially, each *I* impulse, whether it initially polarizes or depolarizes, tends to "hold" the membrane transiently (several milliseconds), preventing fluctuations in any direction. If a sufficient number of impulses are given, the membrane potential may virtually be "fixed" or clamped at a given level (Figs. 3 C, 8, 12). Such an action should be able to prevent impulse conduction once it has started. A reduction or blockage by vagus stimulation of propagating muscle impulses set up by "direct" stimulation has actually been observed recently in the pacemaker cells of tortoise hearts (Hutter and Trautwein, unpublished). In the stretch receptor cells, however, blockage of all-or-none conduction may not play a role and does not seem to be necessary, since inhibition is located at a site where it can act in a flexible graded manner, in opposition to the graded processes of excitatory generator action. For instance, even a very small polarization of the membrane potential, if it occurs near the firing level, can prevent a conducted impulse from arising.

Perhaps the most notable results relating to inhibitory mechanisms were obtained from the study of the interaction between antidromic and inhibitory impulses. When evaluating these experiments one has to recall that the inhibitory action, located in the dendrites, can have only an indirect effect on the cell body activity. As the antidromic impulse approaches the cell, current flow results between the axon and the soma-dendrite system. If the dendritic membrane potential is changed during that period by inhibition, current distribution will be altered. The same should occur if the dendritic conductance alone increases without alteration in membrane potential, since an increased portion of the total current will pass through the dendrites, thus reducing the component which traverses the cell body. If the safety margin for axon-soma conduction is great, inhibition does not alter appreciably the soma invasion, the impulse rising phase, impulse height, or the early portion of the repolarization. Only the "afterpotential," linked more closely with dendritic excitation spread (13) is changed, depending on the amount of stretch (Figs. 8, 11, 12). If, however, antidromic invasion is blocked and the soma potential reflects mainly the electrotonic spread from a nerve impulse arrested somewhere in the axon–cell body boundary region, two effects are seen, namely a reduction in potential size and an acceleration in the decay of the potential. Such effects need not be linked with the electric potential changes caused by the inhibitor action, because they are independent of the direction of the *I* potential (Fig. 15).

One may interpret these results as following from an increased conductance in the dendrites. The magnitude of the change could not be estimated since the current distribution is not well known. The results resemble the inhibitory membrane resistance decrease which was observed by Fatt and Katz (15) at the crustacean nerve-muscle junction. Those changes were similarly independent of the electrical potential which may accompany inhibitory activity. The effects due to conductance increase may be masked on occasions by the consequences of inhibitory membrane changes. For instance, when antidromic invasion block is critical, the *I* depolarization will facilitate cell body invasion (Fig. 16) in the same manner as if the dendrites had been depolarized by stretch (*cf.* reference 13, Fig. 5).

There exists no evidence at present in crustacea regarding the manner in which an inhibitory axon impulse sets up the inhibitory synaptic potential change. A possible transmitter substance was suggested by Pantin's (30) results, perfusing crustacean muscles. One's preference on the whole has to be based mainly on analogies from other species, largely on the detailed and impressive evidence which has accumulated on excitatory junctions or on the known "chemical" inhibitory mechanisms in the heart. On the basis of the present experiments one may merely speculate how depolarization, brought about by stretch deformation, is so effectively counteracted. One may suppose, for instance, that during the inhibitory conductance change for which some evidence has been presented, the permeability of the dendrites is altered to ions whose movement normally maintains or restores the resting potential (20). In this connection the earlier results of Howell and Duke (23) and Lehnartz (28) on the potassium-liberating action of vagus stimulation should be recalled. In addition to potassium there are indications that at least one other ion, chloride, is involved (6). To pursue this approach one has to know more about the nature of the mechanism by which stretch deformation acts. It does not seem possible to utilize here the concept of competitive inhibition which has been discussed for the crustacean neuromuscular junctions (15, 26), postulating that the inhibitor competes with the excitatory transmitter for a common receptor molecule. No "transmitter substance" is likely to play a role during stretch. One may think along the following lines: (*a*) Stretch increases the permeability to some ions (perhaps largely sodium) whose movement may be stopped or prevented by the inhibitory transmitter which also increases the movement of other ions (potassium, chloride) through the membrane; (*b*) stretch depolarization actually may not be prevented as much by inhibition as appears, recording being done in the cell body at some distance from the dendrites. The inhibitory conductance change may effectively put a barrier for current spread between cell body and dendrites, by decreasing the space constant, thus at the same time preventing the setting up of conducted impulses.

Some of the analogies which exist between processes of excitation spread in receptor cells and impaled spinal motoneurons of cats have been discussed before (13). It may also be useful to examine the possible connections of the present results with some of the inhibitory phenomena described in the cat (4, 6) and in crustacean neuromuscular junctions (15). First of all, at the nerve-muscle junction *I* impulses produced generally no electrical effects or only small ones and in the motoneuron they set up polarization potentials only. If the membrane potential in both tissues was changed by applied currents, the *I* impulses produced a depolarization when the resting potential was increased and a polarization when it was decreased. Under those conditions their inhibitory reactions resembled the behavior of the stretch receptor cell. The absence of *I* depolarization at motoneurons and at nerve-muscle junctions when no polarizing currents were passed through them, may be explained if the *I* equilibrium level is near the normal resting potential in those tissues. It should also be noted that in the present preparation the amplitude of the *I* potential could be almost as large as the stretch-induced resting potential change in either direction from the equilibrium level (Figs. 4 and 5). In this respect the compensating action of the *I* impulses for membrane potential displacement resembles more the motoneuron (6) than the neuromuscular junction in which the resting potential had to be changed much more before an *I* potential appeared. For instance, a resting potential shift of 20 millivolts was necessary before *I* potentials of 1 or 2 mv. appeared.

In all three tissues discussed above the *I* action resembles in many respects the excitatory synaptic events. First there occurs a brief active phase of several milliseconds during which the postsynaptic membrane becomes displaced. Afterwards the membrane returns approximately exponentially to the level at which it had been set, either by applied currents or by stretch (Fig. 7). The latter phase of the *I* potentials is apparently due to a passive dissipation of membrane charge. An exception to the simple disappearance of inhibitory action is seen during the delayed polarization phase (Fig. 8), which may represent a mechanism by which the relatively short inhibitory action can be extended, dispensing with the need of prolonged high frequency *I* stimulation to suppress excitation. The records perhaps indicate the persistence of a transmitter. Why the delayed polarization should go beyond the equilibrium established by a train of *I* impulses, is puzzling.

The generalized nature of the inhibitory phenomena discussed above is further indicated by recent findings on the heart pacemaker mechanism. In simultaneous independent studies Del Castillo and Katz (8) and Hutter and Trautwein (24) impaled pacemaker cells in the sinus venosus of frog and tortoise. They found prepotentials which rise during the diastole and lead to conducted muscle impulses. Vagus excitation tends to repolarize the membrane, thus preventing development of the pacemaker potential or reducing it after

it had been started. This effect is presumably linked with the hyperpolarization of Gaskell (18). The pacemaker cells in a beating heart resemble discharging stretch receptor cells in much detail, the pacemaker action being analogous to the generator action located in the dendrites. No resting potentials can, however, be obtained in the active heart and depolarizing *I* effects have not been seen.

In addition to inhibitory mechanisms, this study relates to the general problem of sense organ control. The existence of efferent excitatory neural regulation in a sense organ has already been shown and worked out in great detail in the mammalian muscle spindle. In short, the sensory discharge is determined not only by applied external stretch, but in addition by efferent "motor" fibers which can increase the sensitivity of the receptor, can augment the discharge rate, or can start afferent discharges in the absence of passive stretch. The same principle operates in the crustacean stretch receptors (for detailed mechanisms see reference 25). It is therefore of interest that now there has been demonstrated in a sense organ a direct inhibitory control mechanism which can effectively counteract stretch stimulation. The reflex function of this type of organization remains obscure. Quite recently Galambos (17) in a preliminary note described in the cat suppression of auditory nerve activity by stimulation of efferent fibers to the cochlea, thus showing the same principle in the mammal. It is quite likely that the eye also possesses an excitatory and inhibitory efferent innervation for which histological evidence has existed for a long time (for a recent discussion see Granit (19)).

Although only one efferent axon has been found to innervate the sensory cells, the possibility of the existence of an additional nerve, perhaps of excitatory nature, cannot be excluded with certainty, particularly if it is of very small diameter. It should be recalled that a second "accessory" nerve has been described by Alexandrowicz (1, 2) in the lobster.

SUMMARY

Following the preceding studies on the mechanisms of excitation in stretch receptor cells of crayfish, this investigation analyzes inhibitory activity in the synapses formed by two neurons. The cell body of the receptor neuron is located in the periphery and sends dendrites into a fine muscle strand. The dendrites receive innervation through an accessory nerve fiber which has now been established to be inhibitory. There exists a direct peripheral inhibitory control mechanism which can modulate the activity of the stretch receptor. The receptor cell which can be studied in isolation was stimulated by stretch deformation of its dendrites or by antidromic excitation and the effect of inhibitory impulses on its activity was analyzed. Recording was done mainly with intracellular leads inserted into the cell body.

1. Stimulation of the relatively slowly conducting inhibitory nerve fiber either decreases the afferent discharge rate or stops impulses altogether in

stretched receptor cells. The inhibitory action is confined to the dendrites and acts on the generator mechanism which is set up by stretch deformation. By restricting depolarization of the dendrites above a certain level, inhibition prevents the generator potential from attaining the "firing level" of the cell.

2. The same inhibitory impulse may set up a postsynaptic polarization or a depolarization, depending on the resting potential level of the cell. The membrane potential at which the inhibitory synaptic potential reverses its polarity, the equilibrium level, may vary in different preparations. The inhibitory potentials increase as the resting potential is displaced in any direction from the inhibitory equilibrium.

3. The inhibitory potentials usually rise to a peak in about 2 msec. and decay in about 30 msec. After repetitive inhibitory stimulation a delayed secondary polarization phase has frequently been seen, prolonging the inhibitory action. Repetitive inhibitory excitation may also be followed by a period of facilitation. Some examples of "direct" excitation by the depolarizing action of inhibitory impulses are described.

4. The interaction between antidromic and inhibitory impulses was studied. The results support previous conclusions (*a*) that during stretch the dendrites provide a persisting "drive" for the more central portions of the receptor cell, and (*b*) that antidromic all-or-none impulses do not penetrate into the distal portions of stretch-depolarized dendrites. The "after-potentials" of antidromic impulses are modified by inhibition.

5. Evidence is presented that inhibitory synaptic activity increases the conductance of the dendrites. This effect may occur in the absence of inhibitory potential changes.

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