Spontaneous Activity in Isolated Somata of *Aplysia* Pacemaker Neurons*

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ABSTRACT Somata of pacemaker and nonpacemaker neurons were isolated by ligatures tied around the axons between the somata and the synaptic regions, and the transmembrane potentials of the isolated somata were recorded. Isolated somata of pacemaker neurons had a spontaneous discharge while isolated somata of nonpacemaker neurons were quiescent. In addition, the time course of accommodation in isolated somata of pacemaker and nonpacemaker neurons was found to be different. In pacemaker neurons, injection of current produced a change in rate of discharge sustained for the duration of current injection, while in nonpacemakers, current injection produced only a transient change in discharge rate. Evidence is presented that the pacemaker locus and spike trigger zone in the intact pacemaker neuron are located on the soma.

INTRODUCTION

Inherent rhythmicity may be a property of some neurons. Observations which lead to this conclusion are that action potentials in some neurons are preceded by slow gradual depolarizations (Tauc, 1957) similar to the prepotentials preceding action potentials in cardiac pacemaker regions (Hoffman and Cranefield, 1960), that the rate of discharge of these neurons is a function of the transmembrane potential (Arvanitaki and Cardot, 1941; Tauc, 1957; Terzuolo and Bullock, 1956; Otani and Bullock, 1959; Biederman, 1964), and that the pacemaker rhythm can be reset by current injection (Arvanitaki and Chalazonitis, 1961; Strumwasser, 1965). The literature concerning pacemaker activity in molluscan neurons has recently been reviewed by Tauc (1966).

Although these findings strongly suggest that spike initiation in these neurons is the result of pacemaker mechanisms present within the cells,

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the demonstration of neuronal discharge after complete isolation of a neuron provides definitive proof of pacemaker activity. The complexity of neuronal interconnections in all central nervous systems has thus far prevented such isolation.

However, by utilizing the unique anatomy of the ganglia of the marine molluska, *Aplysia*, the somata of single neurons have been isolated from the rest of the nervous system and their spontaneous activity recorded. In the visceral ganglia of *Aplysia* the large somata (100–400 μ) of the unipolar neurons lie just under the connective tissue capsule, with the axons coursing to the

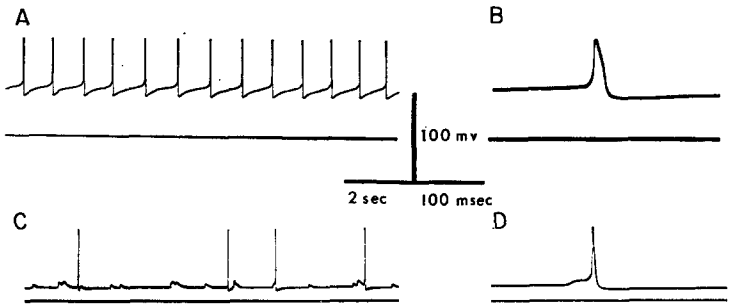

FIGURE 1 A and B. Transmembrane potentials recorded from a pacemaker neuron. In A, note the gradual decrease in membrane potential in the interspike interval. B is an expanded sweep showing configuration of pacemaker spike. C and D, transmembrane potentials recorded from a driven neuron. In C, note the synaptic activity. Due to the compressed time scale in this recording, the EPSP's generating the first and second spikes in the series are not obvious. D is an expanded sweep showing the configuration of the synaptically initiated spike.

interior of the ganglia. As all molluscan synapses are on the axon (Gershenfeld, 1963), isolating the soma from the axon isolates the soma from the influence of all other neurons. This isolation was accomplished by ligation of the axon between the soma and synaptic regions using the technique of Oomura and Maeno (1963). With this preparation, activity endogenous to the soma can be examined and the neuronal pacemaker activity can be demonstrated.

METHODS

The abdominal ganglion of *Aplysia californica* was removed from the animal, pinned in a modified dark-field chamber, and immersed in flowing artificial seawater ("Marine Magic," Lambert Kay, Inc., Los Angeles, Calif.) chilled to 10–16°C. The contents of the chamber were visualized with the aid of a Zeiss otoscope. The connective tissue covering the ganglion was slit, exposing the somata of some neurons.

Transmembrane potentials were recorded with microelectrodes filled with 3 M KCl or 2 M K citrate and having an outer diameter of 1–2 μ. The potential recorded with the microelectrode was connected to the first stage of a unity gain amplifier (Bak, 1958) and the output of the amplifier was led directly to the input amplifier of a Tek-

tronix 565 oscilloscope. The pool was grounded through a Ag-AgCl electrode surrounding the preparation; junction potentials were balanced out with a compensating voltage between the Ag-AgCl indifferent electrode and ground. Current was passed into the cell through a Wheatstone bridge circuit.

In most cases, the activity of an exposed intact neuron was recorded and the neuron classified as a pacemaker or nonpacemaker neuron (see Results). The electrode was then withdrawn from the neuron and the soma of the neuron was dissected free from the adjacent connective tissue. A noose of a fine silk filament was then lowered over the cell body and tightened around the axon hillock (Oomura and Maeno, 1963). The

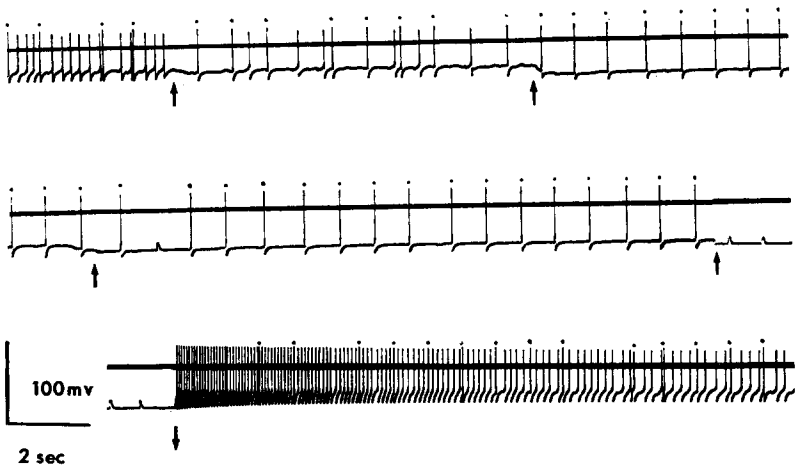

FIGURE 2. Transmembrane potential from a pacemaker neuron receiving a regular excitatory input. The synaptically generated spikes are marked with a dot. Two electrodes were placed in the soma, membrane potential was changed by current passed through one electrode and membrane potential recorded with the other. The arrows indicate an increase in the hyperpolarization of the membrane. The downward arrow indicates the time at which the hyperpolarizing current was turned off. Note the increased firing after the membrane returned to resting potential.

microelectrode was reinserted into the soma, which was now isolated not only from the axon but also from the rest of the ganglion, since all the synapses are on the axon (Gershenfeld, 1963). Certain neurons identifiable in all ganglia by their size, pigmentation, and location always have the same pattern of activity (Coggeshall, Kandel, Kupferman, and Waziri, 1966). In a few cases, these identifiable cells were used and the initial step of determining the activity of the intact neuron was eliminated.

RESULTS

Activity in Intact Neurons

Two types of activity have been found in intracellular recordings from *Aplysia* neurons. Some spikes appear to be initiated by a pacemaking mechanism within the neuron while other spikes are initiated in response to synaptic

excitation. The spontaneously initiated spikes are found only in some cells, pacemaker neurons, while all cells can generate spikes in response to excitatory synaptic input, if present. The discharge from pacemaker neurons can be either very regular discharges of spikes (beating) or intermittent bursts of spikes followed by long periods of hyperpolarization (bursting). Pacemakers may be distinguished from driven neurons (cells generating spikes only in response to excitatory synaptic input) by the effect of polarization of the soma on the discharge frequency (Tauc, 1957) and by the shape of the action potential.

In pacemakers, the firing rate decreases with increasing hyperpolarization until the discharge finally ceases. In driven neurons, small hyperpolarizing currents cause the synaptic potentials (EPSP's) to become more prominent, but do not affect the discharge frequency of the neuron. With larger hyperpolarizations, the EPSP's are still observed but they no longer generate a spike (Tauc, 1957).

There are several differences in the configuration of pacemaker spikes and synaptically generated spikes. The pacemaker spike is preceded by a gradual depolarization similar to the prepotential found in cardiac muscle nodal fibers (Hoffman and Cranefield, 1960) (Fig. 1 A and B), while a synaptically generated spike is always preceded by an EPSP (Fig. 1 D). In these experiments a difference was also observed in the falling phase of the pacemaker and synaptic spikes. In a pacemaker spike, repolarization of the somatic membrane is slow initially and increases abruptly at some point; the spike thus appears similar to that of a Purkinje fiber (Draper and Weidmann, 1951) (Fig. 1 B). In a synaptically generated spike, the falling phase usually does not show the initial slow repolarization (Fig. 1 D).

The differences between spontaneously initiated and synaptically generated spikes were exceptionally well demonstrated in the recordings shown in Fig. 2, from a pacemaker also receiving a large regular EPSP which always produced a spike, except when the PSP occurred during or immediately after a pacemaker spike. As the membrane was hyperpolarized, the frequency of the pacemaker spikes decreased while that of the synaptically generated spikes was unaltered. With further hyperpolarization, the pacemaker discharge of the cell ceases, and only the synaptically generated spikes are observed. With still further hyperpolarization, only the large EPSP is recorded.

Criteria for Successful Ligation

Isolation of the soma was attempted only after determining whether the cell was a pacemaker or a driven cell, except in some experiments when an identified cell (Coggeshall et al., 1966) was ligated; in these cases the activity of the intact neuron was not determined. After ligation of the axon hillock, the

soma was again impaled with a microelectrode and the transmembrane potential recorded.

To ensure that the recorded activity was not due to injury, the criterion was established that the resting potential after ligation be within 5 mv of the value before ligation, except in those few cases in which ligation was attempted without recording activity in the intact neuron. For these neurons, the requirement was made that the resting transmembrane potential be greater than 35 mv. A change of ±5 mv was accepted because it was thought that tonic synaptic bombardment could, in some cells, alter the resting potential to that extent. In the successfully ligated cells presented in Table I there were only

TABLE I

Experiment No.	Spike activity in intact neuron	Antidromic or synaptic potentials in intact neuron	Spike activity in ligated soma	Antidromic or synaptic potentials in ligated soma
13-2	Quiescent	Antidromic	Quiescent	No antidromic
16-4	Driven	EPSP's up to 10 mv	Quiescent	EPSP's<0.1 mv
15-2	Driven	EPSP's up to 10 mv	Quiescent	EPSP's<0.1 mv
84-L_1	Quiescent—occasional spike	EPSP's up to 10–15 mv	Quiescent	EPSP's between 0.1–0.5 mv
15-5	Pacemaker	No PSP's	Pacemaker	No PSP's
19-1	Pacemaker	PSP's 5 mv	Pacemaker	PSP's<0.1 mv
L_4*	Pacemaker	(Usually IPSP's 10–15 mv)	Pacemaker	PSP's<0.1 mv
L_{11}*	Pacemaker	(Usually PSP's up to 10 mv)	Pacemaker	PSP 0.1–0.5 mv
82-5	Pacemaker	PSP's up to 10 mv	Pacemaker	PSP<0.1 mv
R_{15}*	Bursting pacemaker	(Usually EPSP's up to 5 mv)	Pacemaker	PSP<0.1 mv

* In these experiments conducted in the laboratory of Dr. E. R. Kandel, identified cells were used (Coggeshall et al., 1966) and only the activity of the isolated somata was recorded.

two cells, of the seven in which the resting potential was recorded prior to ligation, in which ligation changed the resting potential by more than 1 mv. In these cases, the resting potential of the isolated soma was 3–4 mv greater than the resting potential of the intact neuron. During these experiments, an isolated soma with a resting potential greater than 20–25 mv was always able to generate a spike in response to electrical stimulation; this observation was previously reported by Oomura and Maeno (1963).

All recordings were made from cells in which the resting potential was maintained for some minutes while recording from the soma. It was observed that if a resting potential did not decrease during the first minute of recording, no noticeable decrease was measured for the duration of the recording, which lasted in some cases for over 2 hr. Frequently in both intact and ligated neurons, the membrane potential and spike amplitude increased by as much as 10 mv during the first 10 min of recording; this increase has been interpreted as indicating that the electrode had "sealed in" (i.e., that the hole in the

membrane made by electrode impalement had sealed over, removing a low resistance shunt in parallel with the membrane).

Evaluation of the completeness of isolation was more difficult than evaluation of the extent of injury. During a preliminary series of experiments, three isolated somata were examined under a water immersion lens. No axonal segments were visible above the ligatures. However, "isolation" in these experiments referred to isolation of the soma from changes in axonal membrane potentials; i.e., to an electrical isolation. Therefore, the parameters chosen for measuring isolation were electrical rather than visual. At the onset of the experiments, the criteria established by Oomura and Maeno (1963) were employed; i.e., the lack of change in the somatic transmembrane potential in response to both antidromic and orthodromic stimulation was considered to demonstrate isolation. Prior to ligation, the course of the axon was determined by successive stimulation of the five major nerves from the ganglion (the two connectives, the siphon, pericardial-genital, and the siphon nerves) and the antidromically and orthodromically elicited spikes were recorded. The soma was then ligated, the microelectrode reinserted, and the change in the somatic transmembrane potential caused by neural stimulation was recorded with the highest gain of a Tektronix 2A63 amplifier (1 mv/cm). With this test, no signs of spikes or PSP's were ever recorded in ligated somata.

The lack of response to neural stimulation was not made a routine part of the experimental procedure for two reasons. First, the antidromic spike could be blocked by injury anywhere along the axon and, therefore, was not a specific test for determining whether the soma was effectively isolated from the axonal synaptic region, which is located within a millimeter or less of the soma (Gershenfeld, 1963). Second, the stimulating electrodes hindered the manipulations necessary for ligation. Since most *Aplysia* neurons show spontaneous PSP's, the routine check on the effectiveness of isolation was examination of the somatic transmembrane potential after ligation for any signs of spontaneous PSP's. With the highest gain of the amplifier (at this time a Tektronix 3A3) a PSP of 0.1 mv could have been detected. Since PSP's in the intact neuron can be 10 mv or more, reduction of the PSP's to less than 0.1 mv would indicate ligation had increased the longitudinal resistance between the synaptic region and the soma a minimum of a hundredfold. That this increase was sufficient for isolation was shown by the observation that in two ligated somata small spontaneous fluctuations of 0.1 to 0.5 mv in the transmembrane potential, presumably attenuated PSP's, had no effect on the activity of the somata. Before ligation, one of these neurons, a pacemaker, had shown an irregular discharge with both EPSP's and IPSP's; after ligation, the soma showed a very regular discharge. Before ligation, the other neuron, a nonpacemaker, had generated spikes in response to EPSP's; after ligation the

soma never generated a spike except in response to direct electrical stimulation. For this reason, the two somata (L_{11} and 84-L_1) were included in Table I.

The effectiveness of a ligature in isolating a soma was particularly well demonstrated in one experiment carried out with Dr. E. R. Kandel. After recording from an isolated pacemaker soma for half an hour, the ligature was tightened so that the isolated soma was severed from the axon. After removal of the soma from the ganglion and reinsertion of the microelectrode, the discharge frequency and spike amplitude were the same as those recorded in the ligated soma.

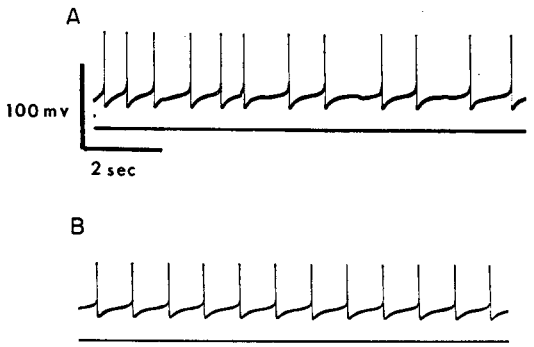

FIGURE 3. A is the transmembrane potential recorded from an intact pacemaker neuron receiving both inhibitory and excitatory synaptic input. B is the transmembrane potential recorded from the same neuron after ligation of the axon hillock. No change in resting potential was recorded.

Activity of Isolated Somata

Over 100 neurons were ligated in this series of experiments, and only 10 neurons met the criteria for successful isolation. Table I presents the results obtained from the 10 cells. The isolated somata of pacemaker neurons showed discharges characteristic of the intact neurons. Typical discharges recorded from a beater pacemaker before (A) and after (B) ligation are shown in Fig. 3. Note that the endogenous rate of discharge remained unchanged after ligation. Fig. 4 A is a recording from a bursting pacemaker (R_{15}, Coggeshall et al., 1966; parabolic burster or cell 3, Strumwasser, 1965). Fig. 4 B is a recording of the discharge from an isolated soma of this identifiable neuron from another ganglion, showing intermittent bursts of spikes. The isolated somata of driven neurons were quiescent, although in all cases, they were able to generate spikes in response to direct electrical excitation. Fig. 5 A is a recording from an intact driven neuron, with EPSP's frequently generating spikes; Fig. 5 B is a recording from the same soma after ligation, showing the complete lack of EPSP's as well as the spike initiated by direct electrical stimulation. The

amplitudes of the spikes generated in the isolated somata were always 10–15 mv (9–15%) lower than that of the spike generated in the intact neuron. The change in spike amplitude between Fig. 3 A and 3 B is typical of the decrease in amplitude after ligation. A possible explanation for this finding is presented in the Discussion.

Recordings were made from many somata which were injured by the ligation, as evidenced by a resting potential lower than that recorded from the intact neuron. The recordings from these somata are of interest because, even in injured somata, the dichotomy in the activity of pacemakers and nonpacemakers was still observed. Injured, isolated somata of pacemakers showed a

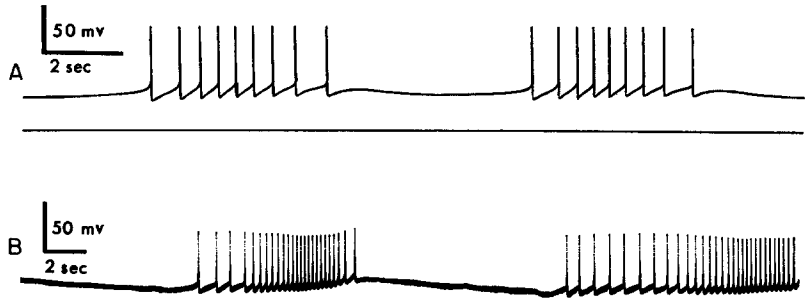

FIGURE 4. A is the transmembrane potential recorded from an intact identified, bursting pacemaker neuron. B is the transmembrane potential recorded from this identified neuron in another ganglion after ligation of the axon hillock. At the temperature at which this experiment was conducted, one may observe initial hyperpolarization before bursting in the intact bursting neuron, and it is not related to synaptic activity. Note the change in scales between A and B.

high frequency discharge of low amplitude spikes. This discharge was maintained until the resting potential decreased to the point where spike generation could no longer be elicited by direct electrical stimulation (approximately 25 mv). Injured, isolated somata of nonpacemakers showed no maintained spontaneous discharge irrespective of resting potential. Usually these injured somata were able to generate low amplitude spikes in response to direct electrical stimulation.

Responses of Isolated Somata to Prolonged Injections of Current

In the intact neuron, it is impossible to separate the effects of membrane polarization on discharge frequency from those of the simultaneous synaptic bombardment. The isolated soma, devoid of synaptic bombardment, provides an excellent preparation for examining the effect of membrane polarization.

By the injection of current, it was demonstrated that in the isolated somata of pacemakers, firing rates were functions of membrane potential. A systematic study was made of accommodation to prolonged injections of current in one

beating pacemaker both before and after ligation. Fig. 6 shows the effect of current on the firing frequency of the isolated soma. With constant hyperpolarizing current, the firing rate decreases considerably at first; then the rate increases slightly to a steady rate. In response to depolarizing current, the firing rate first increases markedly, then decreases slightly to a steady rate, which is maintained for the duration of the depolarization. With the injection of hyperpolarizing current, both the initial and final firing rates are related to the amount of current injected. With small depolarizing currents, less than 1.4×10^{-9} amp, both the initial and final firing rates increase with increasing

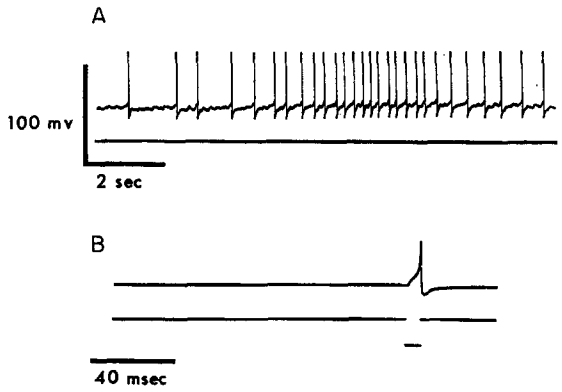

FIGURE 5. A is the transmembrane potential recorded from an intact driven neuron. B is the transmembrane potential recorded from the same neuron after ligation of the axon hillock, as well as the spike elicited by intracellular stimulation. The lower trace shows the current passed through the intracellular electrode.

current. There was no significant difference in the response of the isolated soma to 1.4, 1.8, and 2.2×10^{-9} amp. This lack of effect was probably due to the limitation of firing rate imposed by the refractoriness of the excitable membrane (Katz, 1936; Hodgkin, 1938). Membrane polarization had essentially the same effect on the discharge frequency of the neuron before and after ligation. The major difference was that in the intact neuron, the interspike intervals showed much more variability because of the irregular excitatory synaptic input.

The effect of membrane polarization on the isolated somata of nonpacemakers is entirely different from that on the isolated pacemaker somata. Since the isolated somata of driven neurons are quiescent, the only effect of hyperpolarizing current is to increase the membrane potential. A systematic study of accommodation to prolonged injection of depolarizing current was made in one nonpacemaker both before and after ligation. The firing pattern was a function of depolarizing current (Fig. 7). With the injection of 1.24×10^{-9} amp, only five spikes were elicited, the last four being lower in amplitude

than the first. Note that in this case the initial firing rate seemed to reach a maximum with 1.0×10^{-9} amp. The decrease in firing rate with excessive depolarizing current (1.24×10^{-9} amp) might be due to carrier inactivation

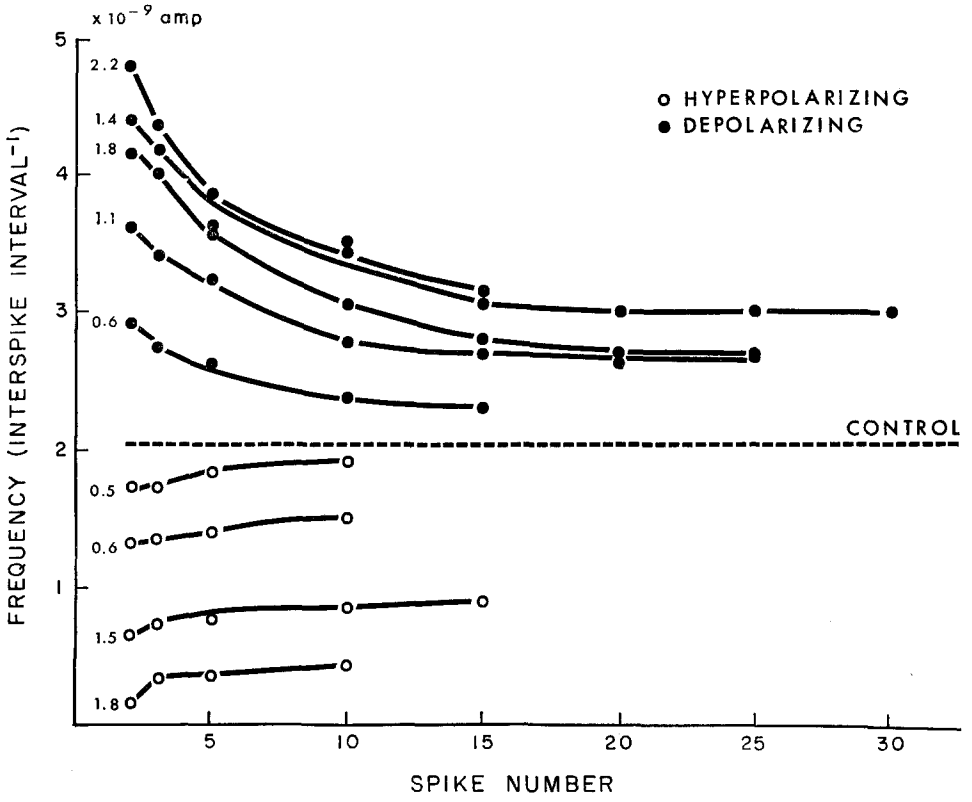

FIGURE 6. This graph shows the effect of constant current injection on the frequency of discharge in an isolated pacemaker soma. The reciprocal of the interval in seconds between a spike and the following spike is plotted as a function of the position of the spike in the train following membrane polarization. Since the nonpacemaker soma does not produce a sustained discharge, comparison of the responses of pacemaker and nonpacemaker somata is made easier when the firing rate is plotted as a function of the position of the spike in the train resulting from polarization of the membrane, instead of as a function of time after polarization. In addition, this method of presentation emphasizes that accommodation to injected currents appears to be related to the number of spikes initiated by the injected current.

resulting from membrane depolarization (Hodgkin and Huxley, 1952), although the limited amount of data does not permit this conclusion to be drawn with any certainty. The effect of current injection in the intact nonpacemaker neuron was quite different from that in the isolated soma. Before ligation, injection of 0.5×10^{-9} amp had elicited a train of 20 spikes, although sustained discharge was never elicited in a driven neuron.

The difference in the responses of pacemakers and of nonpacemakers to membrane polarization was also observed in injured, isolated somata. The firing pattern resulting from passage of constant current was recorded for four injured somata, two pacemaker and two nonpacemaker. In the injured pacemaker somata, the firing rate was found to be a function of membrane potential. In injured somata of driven neurons, depolarizing current elicited one

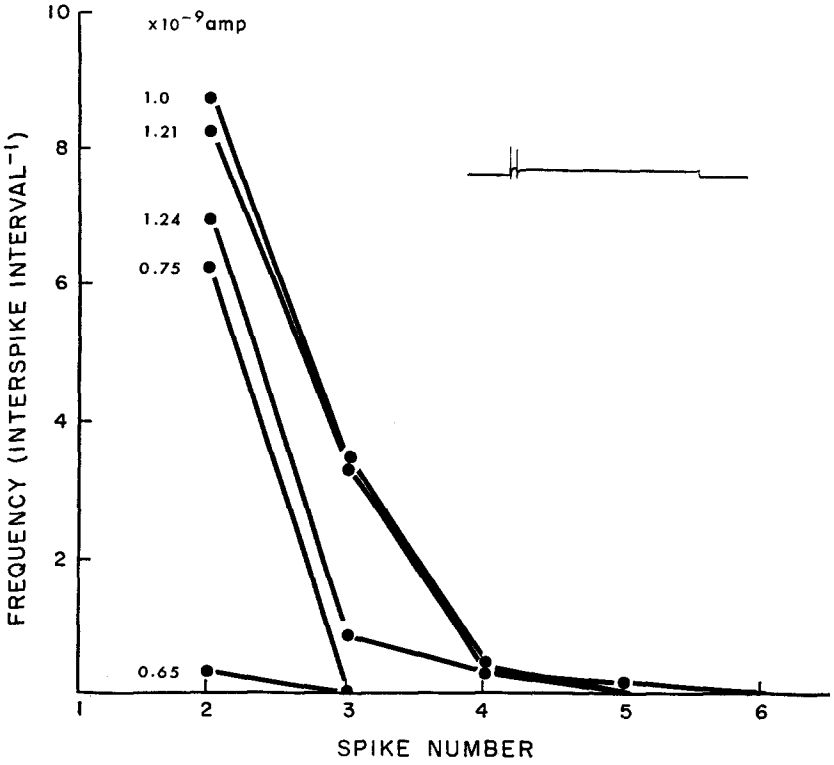

FIGURE 7. This graph shows the effect of constant current injection on the frequency of discharge in an isolated nonpacemaker soma. Note the differences between the ordinate scales for Figs. 7 and 6. The inset is the response of this isolated soma to 0.75×10^{-9} amp.

full-sized spike and, usually, only one abortive spike, irrespective of the magnitude of the current.

Response of Isolated Somata to Acetylcholine

Acetylcholine (ACh) causes hyperpolarization of the chemosensitive membrane of some *Aplysia* neurons (H cells), while it causes depolarization of the chemosensitive membrane in others (D cells) (Tauc and Gershenfeld, 1962; Tauc, 1964). The response of some of the isolated somata to iontophoretically applied acetylcholine was measured. The isolated soma was found to have the same qualitative response to ACh as did the intact neuron. In neither case could pacemaker neurons be distinguished from nonpacemakers by their response to ACh. Even in those somata obviously in-

jured by ligation, response to ACh could be elicited. Since the ACh micropipette had to be removed during ligation, and since the placement of the pipette is a major factor in determining the amount of ACh necessary to produce a response, the relative AChsensitivity before and after ligation could not be determined precisely. However, it is possible to state that, with the ACh pipette in close proximity to the soma at both times, the apparent ACh sensitivities before and after ligation were approximately the same. This observation provides confirmation that the somatic membrane is chemosensitive, even though not innervated (Tauc and Gershenfeld, 1962).

DISCUSSION

Neuronal Pacemaker Activity

For a long time it has been thought that some invertebrate neurons can spontaneously initiate spikes. Recent summaries of the evidence for pacemaker activity have been provided by Bullock and Horridge (1965) and Tauc (1966). This concept of pacemakers was not based on the demonstration that some neurons initiate spikes when electrically isolated from their synapses but rather on more indirect evidence such as the effect of membrane polarization on discharge frequency, resetting of discharge patterns by current injection, and the presence of prepotentials. The results of the experiments described in this paper show conclusively that some neurons possess the capacity to initiate spikes spontaneously. In addition, they show that the criteria previously used to distinguish pacemaker activity from synaptic activity are valid. In all neurons classified as pacemakers by these criteria, the isolated somata showed pacemaker activity. In all neurons classified as driven, the isolated somata were quiescent.

Dichotomy between Pacemaker and Nonpacemaker Neurons

Perhaps the most important conclusion to be drawn from these experiments is that not all neurons can assume pacemaker activity in a normal environment. In the past, it had been assumed that spontaneous firing in molluscan neurons was solely a function of membrane potential (Tauc, 1957). More recently, the effects of changes in the external and internal environment of an *Aplysia* neuron have been shown to influence markedly the presence or absence of pacemaker activity (Strumwasser, 1965; Carpenter, 1967; Carpenter and Alving, unpublished results), but the role which membrane polarization plays in pacemaker activity has not been systematically examined.

The results of the present investigation and many previous observations show that the spontaneous firing rates of pacemakers are determined by membrane potential and that pacemaker activity can be turned off by hyperpolarization of the soma. A driven neuron will also initiate a train of spikes when the somatic membrane is depolarized. However, from the data of both successfully and unsuccessfully ligated somata, it is evident that changes in

membrane potential only modulate the capacity to initiate spikes in neurons which already show pacemaker activity under the prevailing experimental conditions.

The experiments on both intact neurons (Alving, unpublished results) and isolated somata indicate that accommodation to prolonged injections of depolarizing current occurs at different rates in pacemakers and nonpacemakers. Pacemaker neurons and somata responded with an initial high frequency discharge which then decreased slightly to a rate maintained for the duration of the current. On the other hand, the nonpacemakers responded to depolarizing current with a much higher initial frequency (maximum was almost twice as high), but stopped firing before cessation of the applied current. The ability to produce an initial very high frequency discharge and the inability to produce a sustained discharge may be closely related. In relation to this finding, it is interesting to note that the discharge frequency of the bursting pacemakers is much higher than that of the beating pacemakers.

An inherent difference between pacemakers and nonpacemakers is also indicated by the constancy of the activity of the few large cells in the visceral ganglion which can be readily identified by their size, location, and pigmentation; for example, the giant neuron (R_2, Coggeshall et al., 1966) is a nonpacemaker and the six o'clock white cell (parabolic burster, Strumwasser, 1965; R_{15}, Coggeshall et al., 1966) is a bursting pacemaker. This constancy would be remarkable if all neurons possessed equal potential for pacemaker activity. On the other hand, this constancy would be anticipated if there were inherent differences between neurons, and if only certain neurons were capable of spontaneously initiating spikes under normal environmental conditions. In addition, a difference between pacemaking and nonpacemaking neurons has been observed in the responses of the two types of neurons to long duration depolarizations applied under conditions of the voltage clamp (Alving and Frank, 1966). This difference may also be related to the presence or absence of the pacemaker mechanism.

Therefore, it can be concluded that there is some intrinsic difference between pacemaker and nonpacemaker neurons and that only when the pacemaking capacity is present will changes in membrane polarization elicit sustained changes in discharge pattern.

Location of Pacemaker Site and Spike Trigger Zone in the Intact Neuron

Tauc (1962 *a*, 1962 *b*) has shown definitively that in the giant cell, a nonpacemaker, the site of spike generation is always on the axon. (See Tauc and Hughes, 1963.) In the experiments described above, the observation comparing accommodation in a driven neuron before and after ligation supports this conclusion. The isolated soma accommodated to the injected depolarizing current much more rapidly than did the intact neuron and could not generate

a second full-sized spike. This suggests that the site at which spikes were generated in the intact neuron was not on the soma and, therefore, presumably was on the axon.

Several quantitative similarities between the pacemaker locus of the intact neuron and that of the isolated soma have been observed which lead to the conclusion that the pacemaker locus and the spike trigger zone in the intact neuron are on the soma. Very little difference was observed between the time course of accommodation of the intact pacemaker neuron and that of its isolated soma (unpublished results), which suggests that the characteristics of the somatic pacemaker locus are the same as those of the normal pacemaker locus, at least in those somata isolated in this series. The rates of firing of the intact neuron and of the ligated soma were always very similar and, in some cases nearly identical (Fig. 3).

In pacemaker neurons, the smooth gradation between pacemaker prepotential and spike indicates that the soma is the normal pacemaker locus, since in cardiac muscle this gradual transition is seen only in recordings from the actual cardiac pacemaker region, while recordings from the other potential pacemaking loci show an abrupt discontinuity between prepotential and spike. Occasionally, a sharp break between prepotential and spike can be seen in potentials recorded from *Aplysia* pacemaker neurons (for example, Tauc, 1960; Junge and Moore, 1966). Undoubtedly, in these cases the pacemaker loci are far from the somata; however, these discontinuities are usually seen only in injured cells and have never been consistently observed in any identified pacemaker (Alving, unpublished observations; Chalazonitis, personal communication).

The difference in the configuration of the pacemaker and synaptic spike suggests that the trigger zone for spike initiation, as well as the normal pacemaker site, is located on the soma, since it can be demonstrated (Fig. 8) that the amplitude and configuration of the spike recorded in the soma are dependent upon the sequence of axon and soma spike. Fig. 8 shows potentials recorded simultaneously from the axon (top trace) and the soma (bottom trace) of the giant cell (R. Wurtz, unpublished results). Fig. 8 A is a recording of potentials when the cell is stimulated antidromically; in this case the axon spike precedes the soma spike. Fig. 8 B is a recording of potentials when the cell is stimulated synaptically; in this case the axon and soma spikes occur simultaneously. Tauc (1962 *a*) has previously shown similar recordings as well as recordings of potentials when the soma spike slightly precedes the axon spike. From the results of these two investigators, the conclusion can be drawn that the amplitude and configuration of the spike recorded from the soma are partially determined by the temporal relation of the axon and soma spikes. The spike recorded from the soma is largest when the axon spike precedes it, is smaller when the two spikes occur simultaneously, and smallest when the soma

spike precedes the axon spike. The finding that the spike amplitude in an isolated soma is lower than that in an intact neuron is consistent with the observation that the axon spike contributes to the amplitude of the potential recorded from the soma. In addition, the soma spike has a discontinuity in the rising phase when it is preceded by the axon spike, is smooth when they occur simultaneously, and shows delayed repolarization in the falling phase when it is followed by the axon spike. Delayed repolarization was never observed in the ligated somata of the giant cell.

The differences in spike configuration found in the giant cell, a nonpacemaker, can be employed in interpreting the differences in origin of pacemaker

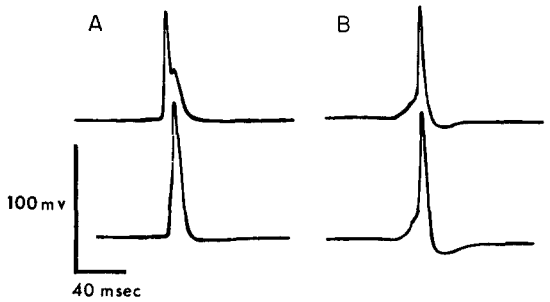

FIGURE 8. Transmembrane potentials recorded from the axon, 1 mm from the soma (upper trace) and from the soma (lower trace) of the giant cell. A shows the potentials recorded when the neuron is stimulated antidromically. B shows the potentials recorded when the neuron is stimulated orthodromically. These recordings are unpublished results of Dr. R. H. Wurtz.

and synaptic spikes. In a neuron having both pacemaker and synaptic spikes, the amplitudes of the pacemaker spikes were lower than those of the synaptic spikes (Fig. 2). In addition, pacemaker spikes from pacemaker neurons usually show delayed repolarization in the falling phase although the extent of this delay is quite variable. Both of these observations indicate that in a pacemaker spike the soma is activated before the axon, which would imply that the spike trigger zone as well as the pacemaker locus is on the soma.

In summary, there is strong evidence that the pacemaker site and the spike trigger zone of the intact pacemaker neuron are located on the soma. (See also Frank and Tauc, 1964.) The somata of pacemaker neurons have been shown to possess the ability to initiate spikes spontaneously. The pacemaker loci on the isolated somata have been shown to have the same intrinsic rhythm as the pacemaker loci on the intact neurons. In addition, the ligated soma and the intact neuron show the same time course of accommodation to polarization of the somatic membrane. Furthermore, location of the pacemaker locus and spike trigger zone on the soma provides an explanation for certain features of the pacemaker spike, such as the smooth gradations be-

tween pacemaker prepotential and spike commonly found only in the pacemaker region, and the delayed repolarization in the falling phase of the spike.

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