# Aplysia Giant Cell: Soma-Axon Voltage Clamp Current Differences

Abstract. *Under voltage clamp, local membrane currents have been measured in several regions of the soma. The early inward current appears to pass largely through the membrane of the axon and of the soma near the axon in normal media. After $10^{-5}$ molar tetrodotoxin (TTX) is added to the bathing medium the larger inward current is found in the somatic membrane away from the axon. The late currents are larger at the soma in both normal and TTX media.*

In the giant neurons of *Aplysia*, two action potentials may be independently elicited and have been identified as axonal and somatic spikes (*1*). The axonal spikes have a lower threshold and shorter time constants than the somatic spikes, and are associated with the site of spike genesis. These cells have subsequently been shown to be able to produce action potentials with bathing media containing either $Na^+$ or $Ca^{2+}$ (*2*) and have been found to have inward currents carried by both these cations under voltage clamp (*3*). It has been suggested that in the snail, which has similar cell properties, action potentials in the axon are mainly due to sodium while those in the soma are mainly produced by calcium (*4*).

This report describes some results of voltage clamp experiments on the left giant cell (*5*) in which the local membrane currents ($I_L$) from different areas of the soma and proximal axon (shown divided into regions in Fig. 1A) were recorded simultaneously with the total clamping current ($I_T$) and membrane potential ($V_C$, $V_M$). For these experiments it was necessary to completely remove the connective tissue from the left pleural ganglion to allow the external placement of a pair of pipette electrodes (marked $I_L$ in Fig. 1A) with tips displaced lengthwise so that one is about 400 $\mu$m behind the other, at different regions of the giant cell. Local current measurements have been made (*6*), but not in such a way that one region could be compared to another on the same cell.

A uniform membrane potential at all regions (total space clamp) could not be obtained for any one placement of the current and voltage electrodes on this cell, especially if it was in good condition, even with current electrodes of low resistance. Therefore, the following method was adopted to ensure that the currents originating in clamped membrane could be measured independently of the total current needed to clamp the region of interest, as well as the rest of the cell in varying degree. The ensemble of electrodes $V_C$, $I_T$, and $I_L$ were moved together from region to region for each series of measurements and at each location, the $I_L$ pair was placed as closely as possible to $V_C$ to record the potential drop in the media due to the currents of the region of membrane near $V_C$. One of the apparent causes for uncontrolled potentials at $V_M$ away from $V_C$ is the intracellular resistance, which becomes appreciable in relation to the membrane resistance during the activation of the early inward current. However, since the $I_L$ electrode pair is placed over the membrane near $V_C$, it is expected that the measured current is from membrane which is regionally space clamped to the potential recorded by $V_C$. Since a common ground was used in the bath some errors may be introduced in the absolute value of the membrane potential because of voltage drop in the media and polarization of the AgCl ground electrode, but they should be small and common to all measurements.

The value of $I_L$ was measured during the clamp interval by recording the potential difference between the tips of the pair with a high-quality differential amplifier. This method measures the ionic currents in the bathing medium as a potential drop between the tips of the pair and is optimally sensitive for currents passing along the line between the tips. Recordings made with the pair rotated on a common axis show only small variations in amplitude, indicating that the currents being measured are largely perpendicular to the surface of the cell and axial with the electrode. The uniformity of the current in this test also indicates a space clamp of the region so that the observed potential drop should be proportional to the current density of this membrane area.

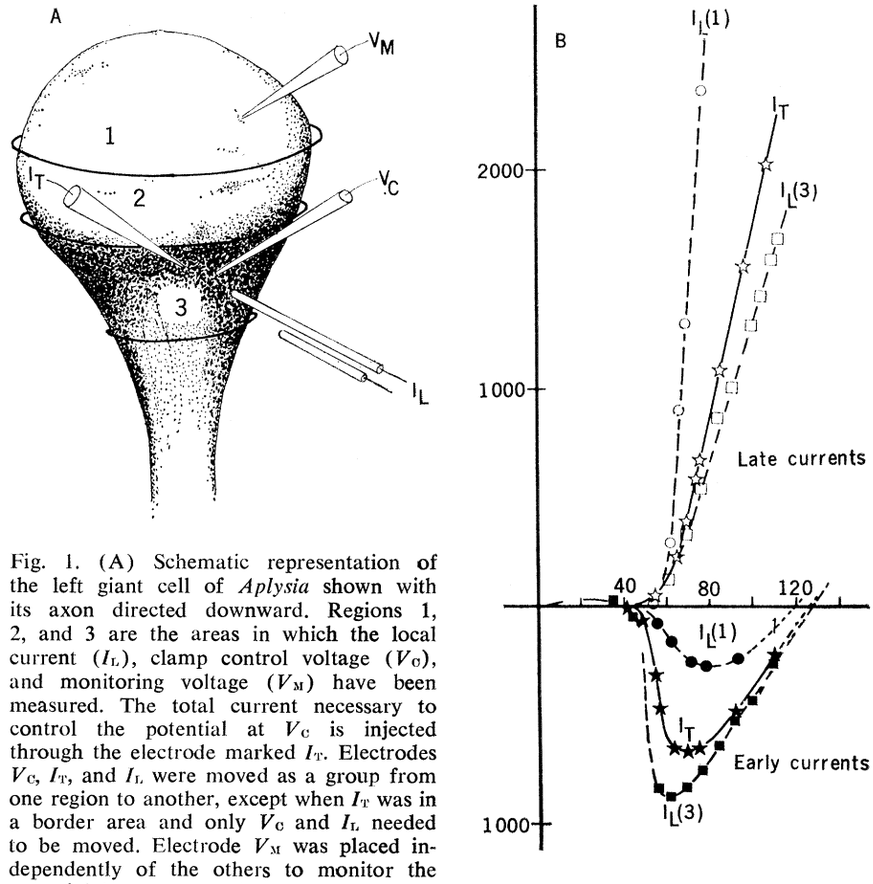


Fig. 1. (A) Schematic representation of the left giant cell of *Aplysia* shown with its axon directed downward. Regions 1, 2, and 3 are the areas in which the local current ($I_L$), clamp control voltage ($V_C$), and monitoring voltage ($V_M$) have been measured. The total current necessary to control the potential at $V_C$ is injected through the electrode marked $I_T$. Electrodes $V_C$, $I_T$, and $I_L$ were moved as a group from one region to another, except when $I_T$ was in a border area and only $V_C$ and $I_L$ needed to be moved. Electrode $V_M$ was placed independently of the others to monitor the potential in remote regions. (B) Current-voltage relations for $I_T$ and $I_L$ in regions 1 and 3. (Closed symbols) Early currents; (open symbols) late currents; (stars) $I_T$; (circles) $I_L$ in region 1; and (squares) $I_L$ in region 3. The membrane potential in millivolts from the holding (resting) potential (60 mv) is given on the abscissa, and $I_T$ ($10^{-8}$ amp) and $I_L$ (microvolts) on the ordinate.

Table 1. Changes in the current ratio $I_L/I_T$ in regions 1, 2, and 3 (Fig. 1A) when $10^{-5}M$ TTX is introduced in the bathing media. The number of experiments used to determine each value is shown in parentheses. Ratios are given as mean ± standard deviation.

| Media | $I_L/I_T$ (microvolts per $10^{-8}$ amp) in region | | |
|---|---|---|---|
| | 1 | 2 | 3 |
| | *Early inward currents* | | |
| Without TTX | (4) 47.6 ± 8 | (5) 40.7 ± 13 | (5) 107.5 ± 10 |
| With TTX | (2) 54.1 ± 0.14 | (2) 19.3 ± 1 | (2) 26.7 ± 9 |
| | *Late outward currents* | | |
| Without TTX | (9) 76.2 ± 30 | (17) 50.8 ± 21 | (4) 12.1 ± 6 |
| With TTX | (2) 154.4 ± 65 | (3) 29.6 ± 7 | (3) 19.0 ± 7 |

The high ($10^{11}$ ohm) input resistance of the amplifier effectively eliminates these electrodes as a possible return path for $I_T$, which could contaminate the $I_L$ measurements. The same pair of electrodes was used for all measurements on a particular cell. Microelectrodes for injecting current and sensing voltage were made on a microforge for resistance values of about 300 kilohms and 1 megohm, respectively, and were filled with and measured in 3*M* KCl. Voltage monitoring in remote regions ($V_M$) was done with electrodes having resistances of 5 to 10 megohms except when the monitoring electrode was to be used also for voltage sensing and control, in which case its resistance also had to be about 1 megohm. In each region studied, local current electrodes, filled with the bathing medium because of their large tip diameters (20 $\mu$m), were placed as close as possible to the voltage-sensing electrode. All experiments were done with natural seawater or filtered *Aplysia* blood as the bathing media.

A complete series of depolarizing step pulses was made in each region with all the electrodes placed in that region, and curves of current against voltage were plotted for each series. Values of $I_L$ are plotted as measured microvolts instead of milliamperes per square centimeter since such a conversion is not warranted for the present measurements. In this work, only the relative values obtained as the same electrode pair is moved from region to region on the same cell are considered to be valid. Whenever possible the electrodes were placed more than one time in the same region on the same cell. Table 1 is a summary of the results from four cells in normal media, two of which were then bathed in media containing $10^{-5}M$ tetrodotoxin (TTX). Regions are compared by taking the ratio $I_L/I_T$ (expressed as microvolts per $10^{-8}$ amp) and averaging the values obtained for each placement in that region. The early inward $I_L$ and $I_T$ values are taken at their respective maxima on the current-voltage curves, which may not occur at the same membrane potential (see Fig. 1B). The late outward current ratios are obtained from $I_L$ and $I_T$ values obtained at the same membrane potentials, but more than 50 to 60 mv from the resting potential (*7*). It has been found that the total current $I_T$ varies in magnitude with the location of the voltage-sensing electrode on the cell and its relation to the current-injecting electrode. This is not surprising since $I_T$ is determined not only by the membrane conductances near $V_C$, but also by the currents necessary to compensate for potential changes resistively coupled from other regions, both clamped and unclamped.

In normal media, the early inward current ratio is twice as large at region 3 as it is at region 1, with graded values between the two. With the introduction of TTX there is a drastic reduction in the inward $I_T$ and $I_L$ for all regions. However, the ratio in region 1 is now greater than it was in normal media, whereas the ratio in region 3 has fallen to a fourth of its initial value. The resulting inversion of the ratios in this condition implies that the blockade of conductances by TTX has been more effective in regions 2 and 3 than in region 1 and that the remaining ion (or ions) carrying the inward current is predominantly passing through the membrane of region 1.

Since TTX is known to selectively block conductance by $Na^+$ (*2–4*), these results may mean that the predominant ion carrying the early inward current in regions 2 and 3 is $Na^+$, while at the soma a conductance not blocked by TTX, namely that of $Ca^{2+}$, remains. The increased ratio in region 1 after TTX is added indicates that an increased proportion of the total current now passes through this region. However, since the ratio is not greatly increased, and $I_L$ was found to be decreased with TTX, it must be concluded that this region is not exclusively permeable to $Ca^{2+}$. With the data reported here, the relative proportions of these conductances cannot be estimated. However, the contribution of conductance by $Ca^{2+}$ to the overshoot of the action potential in the giant cells of *Limnaea stagnalis* has been described as being about four times greater than that of $Mg^{2+}$ and eight times greater than that of $Na^+$ (*8*).

The early currents shown in the current-voltage relations of Fig. 1B are consistent with these results. In region 1 the voltage dependence of the early $I_L$ is quite different from that of the early $I_T$. In general, $I_L$ in region 1 tends to peak at 5 to 10 mv more positive potentials than $I_T$, to have smaller negative slopes, and to cross zero at lower potentials. These results imply a higher threshold of excitation and a smaller amplitude for the soma spike, which is consistent with earlier findings from stimulation studies in this giant cell (*1*). The early $I_L$ in region 3, however, shows a marked similarity to $I_T$ in rate of onset and zero crossing, the peak occurring at slightly lower depolarization potentials or the same as for $I_T$. These results for the three regions are consistent with the findings (*2*) that the spikes recorded in the soma have a lower threshold and peak at higher potentials in normal bathing media than in bathing media containing TTX or free of sodium.

The late outward $I_L$ are greater in regions 1 and 2 than in region 3, both directly (Fig. 1B) and as ratios (Table 1). If we assume that the late current is due to $K^+$, the graded distribution is interpretable in at least four ways. (i) Extracellular accumulation of $K^+$ in region 3 may decrease the electrochemical driving force and thus decrease the outward current (*9*). However, these measurements have been made 100 msec after the onset of the depolarizing pulse with a minimum time between pulses of 10 seconds, which indicates that accumulation would have to be very rapid and long-lasting. (ii) It has been reported that injection of $Ca^{2+}$ in this cell induces an increased $K^+$ conductance (*10*); therefore, if $Ca^{2+}$ is preferentially moving into regions 1 and 2 a larger late current might be expected in these regions. (iii) An early outward current was described in *Onchidium* and identified as due to $K^+$ by Hagiwara *et al.* (*11*). More recently, it has been reported to exist in many cell types (*12*).

This early outward current might occur predominantly in the soma and have a persistent component which could augment the late outward current. (iv) There may be differences in these membranes which are not interpretable on the basis of currently known mechanisms. Although the late current ratios are disturbed by the addition of TTX, their order of distribution remains practically the same. Individual variations may be attributed to variations in the measurements, but the possibility that TTX is affecting some current component during the late phase is not excluded.

The results appear to support the view that the axon spike is due largely to sodium ions and the soma spike largely to calcium ions. Additionally, it appears that the soma of the giant cell cannot be considered to be a homogeneous membrane throughout.

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## Predatory Attack, Grooming, and Consummatory Behaviors Evoked by Electrical Stimulation of Cat Cerebellar Nuclei

Abstract. *Electrical stimulation at single sites in the rostral fastigial nucleus elicits hypertension, grooming, feeding, and attack behaviors in the cat. The stimulus intensity and availability of suitable goal objects determines the behavior. Bilateral lesions of the area fail to produce motor deficits. The rostral fastigial nucleus may be a cerebellar area for behavioral and autonomic regulation.*

Traditionally, the fastigial nucleus has been viewed as sharing in the general function of the whole cerebellum in the regulation of movement and posture (*1*). However, there have been reports that electrical stimulation in or near the fastigial nuclei may produce cardiovascular (*2*) and even behavioral (*3*) responses. Several questions have been raised by these observations. Does the fastigial nucleus participate in the regulation of visceral and behavioral activities? If so, are such activities independent of the motor function of the nucleus? And, finally, are there anatomically distinct areas of the nucleus that mediate these activities?

Electrical stimulation of the ventromedial portion of the rostral pole of the fastigial nucleus in several species elicits a highly reproducible, stereotyped, and differentiated activation of the sympathetic nervous system (*4–6*). The response, termed the fastigial pressor response (*5*), is characterized by a marked elevation of blood pressure, heart rate acceleration, and vasoconstriction, an autonomic pattern simulating the reflex cardiovascular responses to assumption of an upright posture (*6*). The fact that such stimulation fails to produce evident changes in motor activity (*4, 5*) raises the possibility that this region of the fastigial nucleus may influence visceral activity apart from its participation in somatomotor regulation.

To further evaluate the function of this area of the fastigial nucleus, we examined the effect of electrical stimulation and small lesions in unanesthetized cats on behavioral and motor performance and the relation of these responses to evoked activity. Our results suggest that a restricted portion of the fastigial nucleus may be primarily involved in visceral and behavioral control.

Nine adult mongrel cats of both sexes were studied; it had been shown before surgery that these animals did not spontaneously kill rats. The animals were anesthetized with $\alpha$-chloralose (50 to 60 mg per kilogram of body weight, intravenously). Under sterile conditions, a Silastic rubber cannula was inserted into the common carotid artery, brought out through a stab wound in the back of the neck, and connected to a strain gauge transducer for blood pressure and heart rate recording by standard methods. Stainless steel electrodes, insulated to within 0.5 mm of the tip, were then inserted stereotaxically into regions of the fastigial nucleus from which blood pressure responses could be elicited (*5, 6*). After the electrodes were implanted, the incision was closed and the cannula was plugged.

Several days later, when fully recovered from anesthesia and surgery, the animals were placed in an observation cage. The arterial cannula and wires from the stimulation electrodes were attached to one end of a swivel device located on top of the cage. The strain gauge transducer and wires from a constant current stimulator were connected to the other end of the swivel. In each session, we examined the effects of graded electrical stimuli on behavioral and cardiovascular responses. Various combinations of animal chow, water, and live or dead rats were used for goal objects in the behavioral tests. Animals were observed for several weeks. After a suitable number of testing sessions, a lesion was made at each electrode site by passage of an anodal constant current (150 $\mu$a for 40 seconds). Over the ensuing days, the animals were tested for changes in the evoked autonomic and behavioral responses. In addition, each animal was carefully examined for abnormalities of posture and gait, disturbances of visual and tactile placing, hopping and deep tendon reflexes, and changes in the defensive responses to tail pinch or attack by another cat. After 1 to 2 weeks of testing, the animals were anesthetized with pentobarbital (60 mg/kg, intravenously) and perfused through the aorta with 10 percent formalin for subsequent histological identification of lesion sites.

Electrical stimulation of the rostral fastigial nucleus in these unanesthetized cats produced a prompt elevation of the systolic and diastolic arterial blood