related to queen attendance and brood care.

In addition to its prevalence in the ants, temporal polyethism has evolved in other social insect groups and is most elaborate in the honey bee, where the development of glands involved in certain tasks accompanies the sequence of worker activity. However, in the semisocial and primitively eusocial bees, and in the wasps, age polyethism is far less rigorous and patterns of age-related activity are variable (*8, 9*). The imprecise division of labor in *Amblyopone* and other primitively social species suggests that the elaboration of temporal activity patterns complemented the development of advanced social behavior.

Temporal caste systems are marked by a reduction in the repertory size of each age group (*6*), but in *Amblyopone* no such reduction has occurred and workers show a remarkable degree of behavioral plasticity. This lack of a well-defined division of labor in *Amblyopone* appears to be an extremely primitive character and may provide insight into the basic theme of sociality in ancestral ants. However, the biology of *A. pallipes* is a curious, ambiguous blend of primitive and advanced social traits, and it is difficult to accurately distinguish whether these characters are truly conservative or secondarily derived.

JAMES F. A. TRANIELLO

Department of Biology,
Museum of Comparative Zoology
Laboratories, Harvard University,
Cambridge, Massachusetts 02138

References and Notes

1. D. Otto, *Wiss. Abh. Dtsch. Akad. Landwirtschaftswiss. Berlin* **30**, 1-169 (1958); G. Schmidt, Ed., *Sozialpolymorphismus bei Insekten* (Wissenchaftlichen Verlagsgesellschaft, Stuttgart, 1975); E. O. Wilson, *Behav. Ecol. Sociobiol.* **1**, 141 (1976).
2. Size-correlated behavior has been recorded in *Myrmecia*, but age polyethism remains undocumented in this genus; see C. P. Haskins and E. F. Haskins, *Ann. Entomol. Soc. Am.* **43**, 461 (1950).
3. W. L. Brown, *Bull. Mus. Comp. Zool. Harv. Univ.* **122** (4), 1-230 (1960).
4. A detailed account of the population ecology and sociobiology of *A. pallipes* is in preparation. For general references see W. M. Wheeler [*Biol. Bull. (Woods Hole, Mass.)* **2**, 43 (1900)] and C. P. Haskins [*J. N.Y. Entomol. Soc.* **36**, 179 (1928)].
5. Pupal grooming and transport, the dominant acts of most workers, were not significantly related ($r = .36$, $.8 > P < .9$), nor were any other acts.
6. G. Oster and E. O. Wilson, *Caste and Ecology in the Social Insects* (Princeton Univ. Press, Princeton, N.J., in press), chap. 4.
7. A. B. Forsyth, thesis, Harvard University (1978).
8. C. D. Michener, *The Social Behavior of the Bees* (Belknap, Cambridge, Mass., 1974), chap. 12.
9. J. P. Spradbery, *Wasps* (Univ. of Washington Press, Seattle, 1973).
10. I thank E. O. Wilson, B. Hölldobler, A. Forsyth, and S. Greenberg for critically reading the manuscript. Supported by NSF grant BNS 77-03884 (B. Hölldobler, sponsor) and the Richmond Fund of Harvard University.

23 May 1978; revised 7 August 1978

Prolonged Inhibition in Burst Firing Neurons: Synaptic Inactivation of the Slow Regenerative Inward Current

Abstract. *By using voltage clamping and microiontophoretic techniques, it has been found that the prolonged cholinergic and dopaminergic inhibition seen in* Aplysia *burst firing neurons occludes the inward current on which slow oscillations depend. It also mimics the temperature and ionic sensitivity of that inward current. This prolonged inhibition, which cannot be inverted and is insensitive to extracellular potassium changes, thus appears to result from a synaptically produced inactivation of the regenerative slow inward current underlying bursting.*

In some situations a postsynaptic effect far outlasts the presynaptic firing burst that initiated it. A particularly well documented example of this is the prolonged inhibition produced in burst firing neurons L_2 to L_6 of the left upper quadrant of the abdominal ganglion of *Aplysia californica* (*1, 2*). These cells each receive a monosynaptic inhibitory connection from the cholinergic cell L_{10}.

In most of the follower cells of L_{10} the unitary postsynaptic potential (PSP) is relatively brief (100 to 200 msec), but in the burst firing neurons of the left upper quadrant the L_{10}-mediated inhibitory postsynaptic potential (IPSP) often has an additional phase lasting several seconds. Previous studies have shown that the short phase of the these IPSP's is easily inverted by hyperpolarization and represents an increase in the membrane conductance to chloride (*3*). On the other hand, Pinsker and Kandel (*2*) reported that the long IPSP was not reversible by hyperpolarization and that it was insensitive to changes in chloride and potassium equilibrium potentials. The inability of the long IPSP to invert, together with its high sensitivity to temperature and to ouabain, led Pinsker and Kandel (*2*) to suggest that it resulted from a synaptically mediated increase in the activity of an electrogenic pump. This was disputed by Kehoe and Ascher (*4*) and by Kunze and Brown (*5*), who showed that iontophoretic application of acetylcholine (ACh) to the neuronal soma produced long responses that were inverted at membrane potentials beyond the potassium equilibrium potential. They concluded that the long IPSP in these cells was due largely to an increase in potassium conductance occurring in a "remote area" of the neuron (*4*).

To examine whether the long IPSP in the left upper quadrant cells represents a remote increase in potassium conductance, as well as to explore alternative explanations, we combined voltage clamp and iontophoretic techniques. As a part of this study we also examined a similar prolonged inhibitory response to dopamine exhibited by burst firing neuron R_{15}. Ganglia were bathed in artificial seawater at *p*H 8.0. Voltage clamping was carried out with either single (*6*) or dual microelectrodes placed in the soma. Acetylcholine or dopamine was iontophoresed from the tip of a microelectrode, using a constant-current circuit (*1-4*). For ion substitution experiments potassium was added or removed without compensation, whereas sodium was replaced by sucrose.

Figure 1A illustrates the results of a voltage clamp experiment designed to measure the short and long inhibitory postsynaptic current (IPSC) components. Cell L_6 was voltage clamped and interneuron L_{10} was fired by passing current through an intracellular microelectrode. The postsynaptic current is composed of a short phase of outward current (approximately 100 msec) and a long outward current lasting at least 5 seconds (Fig. $1A_1$). The cell was next clamped to a more negative potential and L_{10} was fired more rapidly. Here the short IPSC's are inverted (inward) but the long IPSC continues to be an outward current (Fig. $1A_2$).

We have explored the voltage sensitivity of the long and short IPSC's by clamping follower cells to various potentials ranging from -30 to -120 mV and measuring the size of both components at each potential. We found that the short IPSC inverted slightly below -50 mV, but the long IPSC approached zero asymptotically and never inverted despite prolonged, high-frequency firing of L_{10}. Similar results were obtained in more than 50 cells clamped at potentials up to -150 mV. We have never seen the long IPSC invert. [Details of these experiments will be reported elsewhere (*7*).]

This unusual voltage sensitivity suggested to us that the long IPSP in bursting cells might result from some mechanism other than the simple increase in potassium conductance (*4*). Since these long IPSP's are capable of modifying the slow oscillatory rhythms of the burst firing neurons (*8*), we explored the possibility that they result from a mechanism that is intimately related to the slow oscillation generator.

Using voltage clamp techniques, it

0036-8075/78/1117-0772$00.50/0

was previously shown that the slow oscillations in bursting cells are dependent on a slow regenerative inward current (mediated by sodium or calcium, or both) that produces a negative resistance region in the current-voltage (*I-V*) curves of these cells (*9-11*). In the work reported here we used voltage clamping to determine the *I-V* curves of bursting cells in the presence and in the absence of prolonged inhibition. We also compared the effects of cooling and of ionic changes on the bursting process and the prolonged inhibition.

To study the effect of the long IPSP on the *I-V* curve of a burst firing cell, two microelectrodes were inserted into the postsynaptic cell and a third electrode used to control the firing of interneuron L_{10}. With L_{10} held silent, a hyperpolarizing voltage command was given to the follower cell (Fig. 1B, inset). This resulted in an incremental outward current, indicative of the presence of a negative resistance characteristic (*9-11*). After the cell was returned to the holding potential, L_{10} was fired rapidly, giving rise to a prolonged inhibitory (outward) current. When a second hyperpolarizing command was given during the long IPSC, the response became an incremental inward current, indicating loss of the negative resistance region. When this procedure was repeated, using a number of different voltage commands, we obtained data from which the *I-V* characteristics of the follower cell could be plotted in the presence and in the absence of the prolonged synaptic activity (Fig. 1B). The control curve obtained before firing the long IPSC shows the typical negative resistance characteristic associated with these burst firing cells; however, the curve obtained during the prolonged IPSC is quite different. Although it coincides with the control curve between −100 and −60 mV, for points less negative than −60 mV the negative resistance region is eliminated and the entire curve thus assumes a positive, relatively constant slope. It appears, therefore, that the regenerative inward current underlying the negative resistance region is occluded by the synaptic activity.

Since the long IPSP mediated by L_{10} is known to be cholinergic (*1-3*), iontophoretic application of ACh should give a response that mimics the synaptic response. In a related study (*7*) we did find that ACh, when iontophoresed on the axon—but not the soma—of left upper quadrant bursting neurons, gave a slow response very much like the long IPSP (that is, it was noninverting) (*12*).

The axonal ACh response also occludes the negative resistance region without altering the membrane conductance at more negative potentials. Figure 1C shows the *I-V* curve of cell L_4 before and after the application of ACh to the axon. The control curve has a pronounced negative resistance region at potentials less negative than −60 mV, but the curve obtained during the long inhibition shows no negative resistance, as in the previous experiment with interneuron L_{10}.

The same type of experiment can be done with burst firing cell R_{15}, which shows prolonged inhibition in response to the application of dopamine. Figure 1D shows that the *I-V* curve of R_{15} obtained before the application of dopamine has a region of negative resistance. However, during the prolonged inhibition the negative resistance region is eliminated without altering the *I-V* curve in the more negative regions (beyond −80 mV).

In all three cases of occlusion (exemplified in Fig. 1, B, C, and D) only the negative resistance region of the *I-V* curve is altered by the prolonged inhibition. This argues against the idea that this inhibition results simply from increased conductance to potassium. If there were an increased potassium conductance, then the *I-V* curves should show increased conductance at voltages outside the negative resistance region, but they do not. Thus it seems reasonable to suggest that the prolonged inhibition results specifically from an inactivation of the inward current that underlies the negative resistance region. If this is the case, then the long inhibition should be insensitive to changes in the extracellular potassium ion concentration. On the other hand, it should be sensitive to the same factors that are known to attenuate this inward current, such as reduced temperature and depletion of external sodium ions.

To compare the ionic sensitivity of the prolonged IPSP with that of the negative resistance characteristic, we extended the voltage clamp experiments to various ionic conditions. Ionic substitution experiments using the synaptic input from L_{10} are difficult to interpret because changes in the ionic milieu alter presynaptic as well as postsynaptic processes. To avoid this problem we utilized the

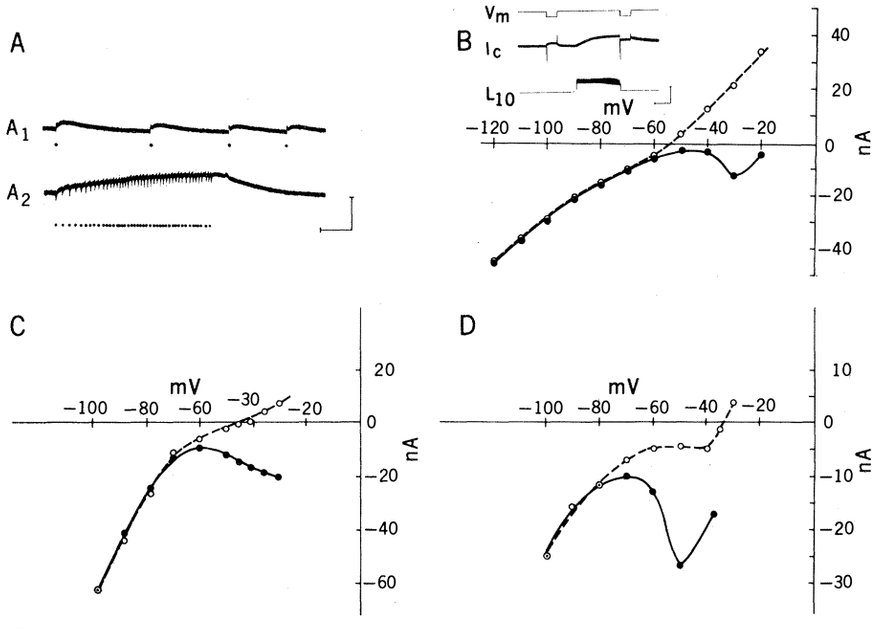

Fig. 1. Voltage clamp study of prolonged inhibition and its occlusive effect on negative resistance. (A) Postsynaptic current recorded from L_6 as L_{10} is stimulated. In A_1, L_6 is clamped at a potential less negative than reversal for the short IPSC and L_{10} is fired four times (indicated by dots). Short and long components are distinctly seen. In A_2, the cell was clamped at a potential more negative than reversal for the short IPSC and L_{10} was rapidly fired (dots). The long IPSC is, however, still present as an outward current. Calibrations, 3 seconds and 15 nA. (B) Synaptic occlusion of negative resistance. (Inset) Cell L_3 was voltage clamped to −35 mV and 10-mV hyperpolarizing commands were given before and then after the firing of interneuron L_{10}. The first command results in an outward-going current, indicating the presence of a negative resistance region. The second command, given just as L_{10} is silenced but during the long IPSP, results in an inward-going current, indicating the loss of negative resistance. Horizontal calibration, 8 seconds; vertical calibration, 30 mV for V_m (membrane potential), 20 nA for I_c (voltage clamp current), and 100 mV for L_{10}. The *I-V* plots are for L_3 before prolonged inhibition (●) and during the long IPSP (○). (C) Current-voltage curves from cell L_3 before (●) and during (○) the long inhibition produced by iontophoresis of ACh. (D) Current-voltage curves from cell R_{15} before (●) and during (○) the long inhibition produced by iontophoresis of dopamine.

axonal iontophoretic responses rather than the IPSP's themselves. Figure 2A shows an experiment in which ACh was iontophoresed onto cell L_3 with the cell bathed in normal (10 m*M*) and then in high (30 m*M*) extracellular potassium. As an example, Fig. $2A_1$ shows each response at a holding potential of −30 mV. The plot of response amplitude against voltage indicates that the long component does not invert with hyperpolarization and that raising external potassium to 30 m*M* had no effect on the ACh response (*13*). In contrast, Fig. 2B shows that removal of sodium from the extracellular fluid virtually eliminates the long response. In this case the cell was voltage clamped to −60 mV, a point more negative than the reversal potential for the short response, and thus the brief response is present as an inward current. When sucrose is substituted for sodium the long component is eliminated, leaving only a brief component (*14*). Thus the prolonged inhibitory response to ACh is depressed by removal of sodium, whereas it is insensitive to changes in the potassium concentration; in these respects it exactly parallels the slow inward current on which the bursting pattern is dependent (*15*).

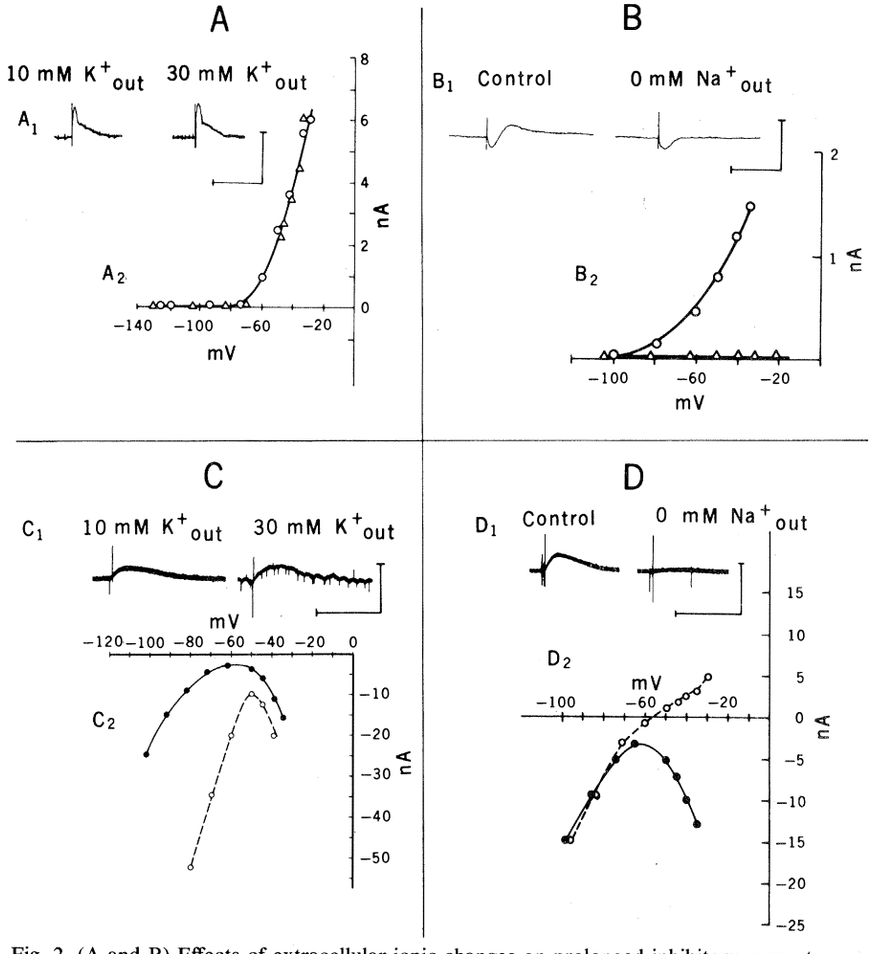

Fig. 2. (A and B) Effects of extracellular ionic changes on prolonged inhibitory currents produced by iontophoresis of ACh onto left upper quadrant bursting cells. (A) Iontophoresis of ACh onto cell L_4 produces a two-component outward current unaffected by potassium. (A_1) Current responses when the cell is held at −30 mV in normal (10 m*M*) and high (30 m*M*) extracellular potassium. The high potassium does not change either the short or the long component. (A_2) Size of the long component from −130 to −30 mV in normal (○) and high (△) potassium. The long component does not reverse and is not altered by the high potassium. (B) Experiment similar to that in (A), except that sodium is replaced. (B_1) Two-component response in L_3 to iontophoresis of ACh. The cell was voltage clamped to −60 mV. The short component is reversed at this potential, but the long component remains outward. In low-sodium seawater, the short component remains but the long component disappears. (B_2) Long current component plotted against voltage in normal (○) and low-sodium (△) seawater. (C and D) Comparison of ionic effects on dopaminergic inhibition and *I-V* characteristics of cell R_{15}. (C) Iontophoresis of dopamine onto R_{15} in normal and high-potassium seawater. In C_1 the cell was voltage clamped to −30 mV. The prolonged outward current response to an iontophoretic pulse of dopamine is unchanged by high-potassium seawater. In C_2 the *I-V* curve of the same cell is plotted for normal (●) and high-potassium (○) seawater. The high potassium does not significantly alter the negative resistance region of the cell. (D) Sodium sensitivity of prolonged inhibitory current responses to dopamine in R_{15}. In control seawater, a dopamine pulse produces an outward current that is eliminated by removal of sodium. Examples are shown in D_1, with R_{15} clamped to −30 mV. In D_2 the *I-V* curve of the same cell is plotted for normal (●) and low-sodium (○) seawater. Removal of sodium eliminates the negative resistance region. Horizontal calibrations, 15 seconds in (A) and (B), 60 seconds in (C) and (D); vertical calibrations, 20 nA in (A), 1.66 nA in (B), and 10 nA in (C) and (D).

The same pattern of ionic sensitivity was exhibited by the prolonged inhibitory response to dopamine in R_{15}. Figure $2C_1$ shows prolonged outward currents produced by iontophoresis of dopamine on this cell in normal and then in high-potassium solutions. Figure $2C_2$ shows the *I-V* curve for the same cell. Elevated potassium does not alter the negative resistance characteristic or the dopamine responses. By contrast, removal of extracellular sodium eliminates the negative resistance characteristic and simultaneously reduces the dopamine response (Fig. 2D).

We previously reported (*9*) that cooling the bursting cells from 22° to 10°C blocks the negative resistance region, and Pinsker and Kandel (*2*) showed that cooling over this same range attenuates the cholinergic inhibition (produced synaptically or iontophoretically). In the course of this study we confirmed these temperature effects, and also found in R_{15} a similar temperature sensitivity of the negative resistance and the prolonged dopaminergic inhibition.

In this study we showed that the negative resistance characteristic of *Aplysia* burst firing neurons is absent during long inhibition and that the sensitivities of these two phenomena to membrane potential, temperature, and ionic manipulations are very similar. We therefore conclude that the prolonged synaptic inhibition results from a reduction of the regenerative inward current underlying the slow oscillations of membrane potential in these burst firing neurons.

Using this model we can explain the changes seen in the long IPSP at different points in the oscillatory cycle. When the burst firing neuron is in its most hyperpolarized phase (just after the spike burst) the slow regenerative inward current is at a minimum and thus synaptic inactivation of it has little effect. Therefore the long IPSP is small or absent. However, as the neuron depolarizes, this inward current increases (regeneratively) and is thus vulnerable to synaptic inactivation. Consequently, the long IPSC is much larger if it occurs during the more depolarized phases of the

oscillatory cycle. In this respect the long IPSP seen in these bursting neurons is quite different from those seen in other systems, where it is attributable to the reduction of a steady (resting) inward sodium current (*16*).

This model also explains why the long IPSP can be reduced in size, but cannot be inverted, as the bursting neuron is hyperpolarized by current injection. If the cell is hyperpolarized to the point where the slow inward current is absent (that is, beyond the negative conductance region), then no long inhibition will be seen and further hyperpolarization has no effect. Our findings that the slow IPSP cannot be inverted and that it is insensitive to potassium changes confirm earlier observations by Pinsker and Kandel (*2*). However, they do not support the suggestion that the long IPSP's might be due to electrogenic pump activation. The conclusion of other workers (*4*, *5*) that the prolonged inhibition in these burst firing neurons is due to a "remote" potassium conductance change is contradicted by our findings (*17*).

The mechanism we have described for the long IPSP in these *Aplysia* burst firing neurons is novel in that it involves synaptic modulation of a regenerative ionic conductance channel. Usually, synaptic transmitters are thought to activate [or, in a few cases (*16*), inactivate] ionic channels that are separate from those involved in excitation processes. In this case, however, the ionic channels being inactivated appear to be the very ones underlying the generation of the oscillating potentials leading to burst firing.

It is especially interesting that synaptic activity as well as the axonal application of transmitter can eliminate the negative resistance characteristic measured for the entire cell. Alving (*18*) showed that the neuronal soma could exhibit burst firing even when ligated from the axon, implying that some slow inward current channels are present at the soma. Although we do not yet understand how events at the axonal site could block the slow inward current for the whole cell, two possibilities seem reasonable. A large proportion of the inward current channels may be at the subsynaptic site. Inactivation of these channels could reduce the slow inward current to a level such that the overall *I-V* curve loses its negative resistance region. Alternatively, the neurotransmitter or some intracellular "messenger" may spread from the synaptic region to other areas of the cell. This would be consistent with the slow time course of the inhibition.

The ability of this synaptic inhibition to directly suppress a voltage-sensitive ionic channel and thereby radically change the *I-V* characteristics of the entire neuron very likely underlies its powerful influence on the burst firing pattern.

WILKIE A. WILSON
Epilepsy Center, Veterans Administration Hospital, and *Department of Pharmacology, Duke University Medical Center, Durham, North Carolina 27705*
HOWARD WACHTEL
Departments of Physiology and *Biomedical Engineering, Duke University, Durham, North Carolina 27706*

References and Notes

1. W. T. Frazier, E. R. Kandel, J. Kupfermann, R. Waziri, R. E. Coggeshall, *J. Neurophysiol.* **30**, 1288 (1967); E. R. Kandel, W. T. Frazier, R. Waziri, R. E. Coggeshall, *ibid.*, p. 1352; J. Kehoe, *J. Physiol. (London)* **225**, 35 (1972).
2. H. Pinsker and E. R. Kandel, *Science* **163**, 931 (1969).
3. J. E. Blankenship, H. Wachtel, E. R. Kandel, *J. Neurophysiol.* **34**, 76 (1971).
4. J. Kehoe and P. Ascher, *Nature (London)* **225**, 820 (1970).
5. D. L. Kunze and A. M. Brown, *Nature (London) New Biol.* **229**, 229 (1971).
6. W. A. Wilson and M. Goldner, *J. Neurobiol.* **6**, 411 (1975).
7. W. A. WIlson and H. Wachtel, in preparation.
8. H. M. Pinsker, *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **36**, 2045 (1977); E. R. Kandel, W. T. Frazier, H. Wachtel, *J. Neurophysiol.* **32**, 496 (1969).
9. W. A. Wilson and H. Wachtel, *Science* **186**, 932 (1974).
10. T. G. Smith, J. L. Barker, H. Gainer, *Nature (London)* **253**, 450 (1975); N. T. Carnevale, thesis, Duke University (1973).
11. R. Eckert and H. D. Lux, *J. Physiol. (London)* **254**, 129 (1976).
12. Traditionally the chemoreceptive properties of the somatic membrane (the most convenient point for microiontophoresis of transmitter agents onto gastropod neurons) have been considered to exemplify those of the entire neuron, and when we iontophoretically applied ACh to the somas of these cells, we confirmed earlier findings that the slow phase of the response could be reversed at −80 mV. On the other hand, when we applied ACh to the proximal axon (about 100 μm from the soma) and the neuron was voltage clamped to increasingly negative potentials, the slow phase of the axonal ACh response asymptotically approached zero, but no reversal of the axonal slow ACh response was seen, even at a potential of −150 mV. This result was obtained in more than 15 cells.
13. In several other experiments we tested the sensitivity of the L_{10}-produced long IPSP to changes in the extracellular potassium ion concentration and simultaneously monitored the size of the short IPSP. In each case we found that high extracellular potassium reduced both IPSP's by the same percentage, and that low extracellular potassium increased both components by the same percentage. The long IPSP did not reverse under any circumstances. We conclude that the variations in extracellular potassium were altering only transmitter release and had no postsynaptic effect on the long IPSP.
14. Sucrose substitution also reduces extracellular chloride, but previous studies have shown that the long IPSP's are insensitive to changes in the extracellular chloride ion concentration. As expected, the brief chloride-dependent component showed a positive-going shift in its reversal potential. However, the effect of this at the −60 mV point was offset by a small reduction in the conductance of the brief response.
15. It has been demonstrated that calcium also contributes to the slow inward current in invertebrate burst firing neurons and that sodium manipulations can alter intracellular calcium [Eckert and Lux (*11*); D. Johnston, *Brain Res.* **107**, 418 (1976)]. We used low-calcium seawater in some experiments and observed a reduction in the slow inward current and the prolonged inhibition. However, since the low-calcium seawater made the cells unstable, we regard those experiments as inconclusive. On balance, it seems reasonable to infer that some calcium component participates in the production of the long IPSP just as it does in the slow inward current.
16. P. A. Smith and F. F. Weight, *Nature (London)* **267**, 68 (1977). These authors propose that a slow IPSP in bullfrog ganglia results from synaptic inactivation of a resting sodium conductance.
17. We do not question the existence of potassium-dependent inhibition in other nonbursting cells reported by Kehoe and Ascher [(*4*); see also P. Ascher, *J. Physiol. (London)* **225**, 173 (1972)]. Nor do we question the potassium sensitivity of somatic ACh responses in left upper quadrant bursting cells.
18. B. O. Alving, *J. Gen. Physiol.* **51**, 29 (1968).
19. Supported by the Veterans Administration, Durham, N.C., and by NIH grant NS 08476. All correspondence should be addressed to W.A.W.

8 June 1978; revised 21 August 1978

Decrease in Adrenergic Axon Sprouting in the Senescent Rat

Abstract. *When the septal area in young adult rats is denervated by a lesion of the fimbria-fornix, adrenergic fibers proliferate within the denervated area. The same operation performed on aged animals gives rise to a qualitatively similar but quantitatively less pronounced response. This reduction in reactive growth may reflect a decreased capacity of the aged brain to remodel its circuitry and restore lost function.*

The adult mammalian brain is capable of axonal growth in response to various forms of damage including loss of neurons. When neurons are destroyed by lesions, their target cells lose some of their synaptic input. In many cases the remaining projections to those cells form new connections and replace, in the morphological sense, those that were lost. This process, termed reactive synaptogenesis, has been well documented in many areas of immature and adult central nervous systems (*1*). Such responses have not, however, been well investigated in the aged brain.

The plasticity of central neurons in aged animals is a particularly critical issue because the aged brain is most susceptible to neuronal loss. Neuronal loss is a normal consequence of the aging process, as well as the most prominent effect of such common disorders of the aging nervous system as stroke, tumors, and senile dementia. Since reactive sy-

0036-8075/78/1117-0775$00.50/0