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The overall goal of this research is to provide insights into the adaptive capabilities of individual neurons, which will lead to the development of machines having some of the information processing capabilities of the nervous system. During the period between 01 August 1987 and 31 July 1988, significant progress has been in three major directions. First, a previous mathematical model of sensory neurons that exhibit adaptive plasticity has been extended to include more detailed descriptions of critical cellular processes. Second, a single-cell neuronal model for classical conditioning has been incorporated into a network and the capabilities of the network to simulate higher-order features of associative learning has been examined and analyzed. Third, experimental studies have been performed on individual neurons in order to examine the modulation of membrane currents and critical second messengers that are believed to contribute to neuronal plasticity and classical conditioning.

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Annual Technical Report

Grant Number: AFOSR 87-0274

Grant Title: Analysis and Synthesis of Adaptive Neural Elements and Assemblies

Period of Report: 1 August 1987 through 31 July 1988

I. Summary

The overall goal of this research is to provide insights into the adaptive capabilities of individual neurons, which will lead to the development of machines having some of the information processing capabilities of the nervous system. During the period between 01 August 1987 and 31 July 1988, significant progress has been made in three major directions. First, a previous mathematical model of sensory neurons that exhibit adaptive plasticity has been extended to include more detailed descriptions of critical cellular processes. Second, the single-cell neuronal model for associative plasticity has been incorporated into neuronal networks and the capacity of the networks to simulate higher-order features of associative learning has been examined and analyzed. Third, the modulation of specific membrane currents and critical second messengers involved in the adaptive plasticity of the sensory neurons has been examined.

II. Research Objectives

The proposed research is designed to examine the adaptive cellular components of a simple biological system that displays basic attributes of intelligence. The objectives are to identify the subcellular processes that underlie the capability of a single neuron for information processing, long-term memory, and goal-seeking behavior. Single identified sensory and interneurons of the mollusc Aplysia which have demonstrated capacities for associative conditioning are being investigated. Cellular neurophysiological techniques are being applied to identify the particular ionic conductances and second messenger systems causally involved in adaptive cellular behavior. Formalisms of the subcellular modifications are being developed and incorporated into a quantitative model of the adaptive neural element. In addition, the adaptive neural element is being incorporated into simple neural networks. Both the single cell and network models are being simulated on a digital computer to assess their ability to fit the experimental data and predict features of nonassociative and associative conditioning in other animals (including humans).

III. Status of Research (Progress Report)

Progess during the past year has been in three areas. The first has been the development of mathematical models that simulate aspects of transmitter release at the single-cell level in neurons that are believed to contribute to neuronal plasticitiy and classical conditioning. The second area has been the development of networks that simulate higher-order features of classical conditioning. The third area has been the analysis of the modulation of membrane properties and second messengers involved in the adaptive plasticity of the sensory neurons.

A. Mathematical Model of Cellular Mechanism Contributing to Presynaptic Facilitation

The neuronal mechanisms contributing to nonassociative and associative learning have been analyzed extensively in two defensive behaviors of the

marine mollusc Aplysia; the siphon withdrawal and the tail withdrawal reflexes. For example, sensitization (the nonspecific enhancement of responses elicited by one stimulus, following stimulation of another pathway) is associated with an increase in the amplitude of the postsynaptic potentials (PSPs) at the sensory to motor neuron synapse. The enhancement of the PSP is paralleled by an increased influx of Ca^{2+} into the sensory neurons, which results indirectly from a reduction in the K^+ currents. The reduction in K^+ currents prolongs the duration of the action potential. Similarly, dishabituation (the restoration of a previously habituated response by a novel stimulus) also results from an enchancement of the PSP at the sensory to motor neuron synapse. Previously, it was believed that sensitization and dishabituation involved a common mechanism (increases in the duration of presynaptic potentials). Recently, however it has become clear that multiple processes and mechanisms are involved (Gingrich and Byrne, 1985, 1987, Hochner et al, 1986). For example, increases in the duration of presynaptic potentials alone can not account fully for presynaptic facilitation of PSPs at depressed ("habituated") sensory synapses.

The current understanding of the various biochemical and biophysical mechanisms that contirbute to synaptic plasticity has allowed us to develop formal descriptions of these processes (Gingrich and Byrne, 1985, 1987). The approach was to transform the processes into mathematical formalisms; assign values to the parameters, which agree with published data, and fit the components together to create a model of transmitter release. Our initial models relying solely on inactivation and enhancement of the Ca^{2+} current (as suggested by earlier experimental data) were incapable of simulating data describing the features of synaptic depression and presynaptic facilitation. In order to simulate the data successfully, we found it necessary to construct a model that employed multiple pools of transmitter that undergo depletion and mobilization. The three basic components of the model are a Ca^{2+} current (I_{Ca}), regulation of intracellular Ca^{2+} levels, and processes of transmitter storage and release. Perhaps the most significant conclusions of this earlier model were the findings that inactivation of I_{Ca} seemed unlikely to account fully for synaptic depression and that mechanisms in addition to spike broadening contributed to presynaptic facilitation (e.g. mobilization of transmitter).

In order to further investigate the potential contribution of spike broadening and mobilization to presynaptic facilitation, the previous model (Gingrich and Byrne, 1985, 1987) was extended by inclusion of more detailed formulations of several components of the model. Specifically, the earlier model was extended to include more detailed descriptions of: i) the kinetics of the Ca^{2+} channel, ii) the diffusion of Ca^{2+} through the cytoplasm, iii) the process of transmitter release, and iv) the postsynatpic potential. The extended quantitative model (Gingrich, Baxter, and Byrne, 1988) provides and accurate description of the input-output relationship for synapses of sensory neurons, and predicts changes in the shape of postsynaptic potentials as a function of mobilization and spike broadening. The results confirm and extend previous experimental studies and indicate that cellular analogs of sensitization (facilitation of nondecremented responses) is mediated primarily by spike broadening; whereas, analogs of dishabituation (facilitation of depressed responses) requires mobilization of transmitter.

B. Simulation of Higher-Order Features of Associative Learning

A novel cellular mechnaism for associative learning, activity-dependent neuromodulation, has been identified in sensory neurons mediating the gill and tail withdrawal reflexes of Aplysia (Hawkins et al., 1983, Walters and Byrne, 1983). This mechanism may explain associative learning on behavioral level. Previously, subcellular events that may underlie this mechanism were mathematichally modeled and the ability of the model to fit available empirical data was examined. In this associative model, the reinforcing or unconditioned stimulus (US) leads to nonspecific enhancement of transmitter release from sensory neurons by activating a cAMP cascade. Spike activity in sensory neurons, the conditioned stimulus (CS), transiently elevates intercellular Ca^{2+}. The CS-triggered increases of intracellular Ca^{2+} 'primes' the cyclase and amplifies the US-mediated cAMP synthesis. As a result of the pairing specific amplification of cAMP levels, transmitter release is enhanced beyond that produced by unpaired stimuli or by application of the US alone. The model was capable of fitting empirical data on activity-dependent neuromodulation and predicted a characteristic interstimulus interval (ISI) curve (Gingrich and Byrne, 1987). At the subcellular level, the model's ISI function is related to the time-course of the buffering of intracellular Ca^{2+}. The magnitude and duration of the pairing specific enchancement of transmitter release is related to the levels and time-course of intracellular cAMP stimulation.

By incorporating information obtained from recent cellular studies in Aplysia and emerging cell biological principles and mechanisms in other systems, this model demonstrates that the same subcellular mechanisms contributing to non-associative learning (sensitization) can be utilized through the addition of a single biochemical step (Ca^{2+}-modulation of adenylate cyclase) to produce an associative neuronal mechanism that may contribute to associative learning Aplysia. At the subcellular level the formation of associations is dependent upon an interaction of two well-established intracellular second messengers, Ca^{2+} and cAMP.

The single-cell model (Gingrich and Byrne, 1987a) accurately simulated many aspects of empirically observed neuronal plasticity that are believed to be cellular correlates of simple forms of nonassociative and associative learning. During the past year the analysis was extended by incorporating the single-cell model into small networks of adaptive elements which reflect the neuronal properties and connectivity patterns in Aplysia (Byrne et al., 1988). An initial network contained two sensory neurons (SNs) both of which excited a single facilitatory interneuron (FN) that feeds back onto the SNs. An assumed property of the facilitatory neuron was that its output accommodated as a result of its activation (Hawkins & Kandel, 1984). Simulations of this network exhibited 2nd order conditioning but not asymptotic blocking when the original model of the sensory neuron (Gingrich and Byrne, 1987) was used. Both 2nd order conditioning and asymptotic blocking could be simulated, however, by modifying the model such that: 1) the synaptic strength of the conditioned sensory neuron (CS+ cell) was at least twice as large as an unconditioned sensory neuron (CS- cell), 2) the time required for complete accommondation of the facilitatory neuron was less than the minimum interstimulus interval necessary for conditioning in the sensory neurons, and 3) the Ca^{2+} levels within the sensory neuron first must surpass a threshold value before associative plasticity can occur. We

also investigated how the incorporation of additional interneurons that recieve excitatory input from, and feed back to inhibit the sensory neurons, would alter the above constraints and possibly make the network more robust. Various models proved functional, but each required specific assumptions (Byrne et al., 1988).

C. Experimental Analysis of Cellular Mechanism Underlying Learning

Voltage-clamp techniques have been applied to individual tail sensory neurons to examine the regulation of ionic conductances in response to the type of modulatory inputs that occur during sensitization and classical conditioning.

In the sensory neurons that mediate the tail withdrawl reflex in Aplysia the neurotransmitter serotonin (5-HT) produces a depolarization that is associated with a decrease in their input conductance. This mechanism is thought to contribute to one type of non-associative learning, sensitization, and one type of associative learning, classical conditioning. Previous studies have shown that 5-HT increases the levels of cAMP in these cells and that injection of cAMP produces decreases in membrance conductance similar to those produced by 5-HT.

Previously it was found that in the sensory neurons, serotonin, modulates not only the novel serotonin-sensitive K^+ current($I_{K,S}$), but also the delayed K^+ current ($I_{K,V}$) (Baxter & Byrne, 1986). In order to determine whether modulation of both of these currents by 5-HT is mediated by cAMP, two-electrode voltage- and current-clamp techniques were used to compare the effects of application of 5-HT, 8-bromo-cAMP and 8-4-chlorophenylthio-cAMP on memebrane currents, spike duration and excitability in isolated clusters of sensory neuron somata.

Computer isolation of membrane currents modulated by 5-HT revealed two affected currents (Baxter & Byrne, 1986). One current had properties consistent with $I_{K,S}$. It was relatively voltage-independent, noninactivating, not blocked by 4-AP and relatively insensitive to TEA. The second current had properties consistent with $I_{K,V}$. It was highly voltage-dependent and was blocked by 4-AP and TEA. Computer isolation of the membrane currents modulated by the cAMP analogues, however, revealed only one prominent current, $I_{K,S}$. The cAMP analogues occluded further modulation of $I_{K,S}$ by 5-HT but did not occlude the modulation of $I_{K,V}$ by 5-HT. Thus, application of the cAMP analogues mimicked the action of 5-HT on $I_{K,S}$, but did not mimic the action of 5-HT on $I_{K,V}$ (Baxter and Byrne, 1987).

To complement the voltage clamp studies described above, the effects of 5-HT and cAMP analogs on spike broadening and excitability were also examined. Sensory neurons were held at -45 mV, and spikes were elicited by short (3 ms, 5 nA) depolarizing pluses to elicit single action potentials and measure spike duration or long (1 s, 0.5 to 3 nA) depolarizing current pulses to elicit trains of action potentials and measure excitability. The brief pulses produced single spikes that had an average duration of 7 ms. The long pulses usually produced brief bursts of no more than 5 spikes. Application of 5-HT broadened the spikes to an average duration of 23 ms and doubled the number of spikes produced during

long pulses. Application of cAMP analogues produced similar increases in the number of spikes to long pulses, but only modestly broadened the spikes to an average duration of 9 ms. The cAMP analogues occluded further increases in spike number during subsequent 5-HT application, but did not occlude 5-HT induced spike broadening.

These results suggest that in pleural sensory neurons only one of the two currents modulated by 5-HT is sensitive to elevated intracellular levels of cAMP. This current ($I_{K,S}$) appears to be critical for membrane excitability, with modest effects on spike duration. In contrast, modulation of $I_{K,V}$ by 5-HT dramatically broadened the spike. This action of 5-HT may require an as yet unidentified second messenger system.

The use of the method of computer subtractions has allowed, for the first time, a detail investigation of the serotonin-sensitive membrane currents. The kinetics and voltage-dependence of the responses indicated that the serotonin-modulated currents had several components rather than a single component as was first believed. The magnitude and time-course of the second component ($I_{K,V}$) suggest that this current may play a significant role in modifying the waveform of the action potential. The first, slow current ($I_{K,S}$), may be important in regulating cell excitability and cell adaptation to prolonged stimulation. By incorporating these currents into the computer simulations of these neurons it will be possible to determine the functional significance of each current.

As part of our investigation on the role of cAMP in the adaptive plasticity of the sensory neurons we examined the effects of the adenylate cyclase activator forskolin. Forskolin (FSK) is often used in studies of the modulation of ion channels to specifically activate adenylate cyclase. Previously, it was found that application of forskolin reduced the transient ($I_{K,A}$) and voltage-dependent ($I_{K,V}$) K^+ currents in sensory neurons isolated from pleural ganglia of Aplysia (Baxter & Byrne, 1985). However, it was found that application of cAMP analogues reduced only the serotonin-sensitive ($I_{K,S}$) K^+ current (Baxter & Byrne, 1987); suggesting that forskolin has actions unrelated to elevation of cAMP. During the past year we confirmed that forskolin reduces the amplitudes of $I_{K,A}$ and $I_{K,V}$ in a dose-dependent manner (10-300 uM); apparently without altering the kinetics of the currents (Baxter and Byrne, 1988). This action of forskolin is not mimicked by cAMP, which reduces only $I_{K,S}$. Furthermore, 1,9-dideoxyforskolin, an analogue which does not stimulate the cyclase, also reduces voltage-activated K^+ currents. In contrast, Modified-FSK (Calbiochem), an analogue which stimulates the cyclase, reduces only $I_{K,S}$. While the nature of interaction between forskolin and ion channels is not known, the simplest interpretation of these results is that forskolin can act as a K^+ channel blocker. In addition to activating adenylate cyclase, forskolin may have a direct affect on ion channels that is not mediated via cAMP. Consequently, forskolin is not suitable for use as an agent to examine the cAMP-dependence of the modulation of ion channels.

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14. Baxter, D.A. and Byrne, J.H. Analysis of two K^+-currents modulated by serotonin in the pleural sensory neurons of Aplysia, in preparation.

15. Baxter, D.A. and Byrne, J.H. Differential effects of serotonin and cAMP on spike broadening and excitability in pleural sensory neurons of Aplysia, in preparation.

V. Professional Personnel

Baxter, Douglas, Ph.D.
Buonomano, Dean (graduate student)
Byrne, John, Ph.D.
Gingrich, Kevin, M.D.
Susswein, Abraham, Ph.D

VI. Interactions

Aspects of the work were presented at the previous Society for Neuroscience meeting and two abstracts will be presented at the Society for Neuroscience meeting in Toronto this November. The work was also presented at the Review Meeting for Air Force sponsored Basic Research in Neurosciences, Brooks Air Force Base, November 30 to December 2, 1987, at the American Association for Artificial Intelligence Symposium on Parallel Models of Intelligence, Stanford University, March 22 to March 24, 1988 and at the Bat-Sheva De Rothschild Foundation Seminar on Neural Networks Models and their Relevance to Biology, Jerusalem, Israel, May 24 to June 3, 1988.

VII. New Discoveries and Specific Applications

The most notable achievements were the discovery of novel outward currents modulated by serotonin but not cAMP and the further extension of neural models for nonassociative and associative learning. It is too early in the research to comment on specific applications of this research but eventually the results will be relevent to aspects of artificial intelligence. No inventions were made.