# 13

# The Logic of *Limax* Learning

ALAN GELPERIN, J. J. HOPFIELD, AND D. W. TANK

## 1. Introduction

We wish to understand the neuronal computations performed on sensory inputs that result in the categorization of those inputs, their storage as memory states, and their associative combination. Our experimental and theoretical work is focused on the neuronal computations performed on odor and taste inputs in the CNS of *Limax maximus,* a terrestrial mollusk convenient for behavioral, neurophysiological, and neurochemical experiments (Gelperin, 1983). The questions posed in this specific system are designed to illuminate issues of learning and memory storage with panphyletic generality.

Several aspects of the learning behavior of *Limax* have encouraged us to attempt a unique synthesis of the approaches of behavioral biology, neurobiology, and neural modeling. While *Limax* displays many of the learning phenomena of higher organisms, including primates, it accomplishes these learning tasks using only about 10,000 cells in its CNS. We are using behavioral experiments to define the major types of learning exhibited by *Limax* and for each type of learning to establish the critical interevent timing relations that allow learning to occur. Neurophysiological and neurochemical experiments delimit the areas of CNS necessary for learning and describe the types of cellular elements and synaptic interactions available for modification during learning. The neural modeling asks how a collection of relatively simple neurons might interact synaptically to collectively accomplish the learning tasks. The model avoids assumptions about precise anatomical details, instead asking questions about the computational consequences of a simple set of rules governing synaptic interactions.

ALAN GELPERIN • AT & T Bell Laboratories, Murray Hill, New Jersey 07974 and Department of Biology, Princeton University, Princeton, New Jersey 08544. J. J. HOPFIELD • AT & T Bell Laboratories, Murray Hill, New Jersey 07974 and Divisions of Chemistry and Biology, California Institute of Technology, Pasadena, California 91125. D. W. TANK • AT & T Bell Laboratories, Murray Hill, New Jersey 07974.



A. I. Selverston (ed.), *Model Neural Networks and Behavior*

We will first describe the behavioral and neurophysiological attributes of *Limax* learning. Then a detailed model of the *Limax* learning that incorporates a memory storage network is described, with clear indication of how the model relates to present data and raises questions amenable to experimental answer.

## 2. *Limax* Learning: Intact Animals and Isolated Brains

### 2.1. Associative Conditioning of Intact Animal

*Limax* is a generalist herbivore. It employs a variety of mechanisms to optimize its food choices when confronted with plant prey containing bitter and/or toxic chemical defenses. One such optimization mechanism involves learning to avoid plant odors associated with toxicosis (Gelperin, 1975) or a bitter taste (Sahley *et al.*, 1981a; see also Whelan, 1982; Gouyon *et al.*, 1983). The training procedure by which an attractive plant odor (e.g., carrot, potato, mushroom) is paired with a bitter taste (e.g., quinidine sulfate) is formally identical to a Pavlovian conditioning paradigm, where the attractive plant odor is the conditioned stimulus (CS) and the bitter taste is the unconditioned stimulus (US). Before training, the CS is attractive, eliciting both approach and ingestion. After training, the CS is repellent, eliciting avoidance and rejection. These changes in response to the CS odor due to conditioning are easily and quantifiably measured in an odor-choice chamber described elsewhere (Sahley *et al.*, 1981a).

The *Limax* CNS *in situ* can perform several associative logic operations on taste and odor inputs as revealed by a series of first-order and higher-order conditioning experiments (Sahley *et al.*, 1981b). The essence of these neural computations can be extracted by positing that before training there are several innately attractive food odors (A+, B+, and C+) and innately bitter and repellent tastes (e.g., Q−) available. First-odor conditioning is obtained by paired presentation of A+ and Q−. The result is that A (but not B) is now avoided (A−). The control procedures that demonstrate that this result depends on the association of A and Q are fully described in Sahley *et al.* (1981a).

Describing the conditioning results at this level of abstraction facilitates presentation of the higher-order conditioning data of Sahley *et al.* (1981b), as shown in Fig. 1. Second-order conditioning involves a two-phase training procedure wherein A− acts as a US during the second phase of conditioning. During compound conditioning, the animal learns to avoid both A and B in one training trial when A and B are presented together as a compound CS. The demonstration of blocking (Kamin, 1969) involves a two-phase training procedure with first-order conditioning in phase one and compound conditioning in phase two. The unexpected outcome is that animals do not acquire an aversion to the second CS introduced in phase two of conditioning.

Another striking parallel between results of *Limax* and vertebrate conditioning experiments has emerged from studies on the effect of extinguishing the aversion to food A after second-order conditioning of food B. Theoretically, the experiment can distinguish whether or not, after second-order conditioning, the aversion reac-

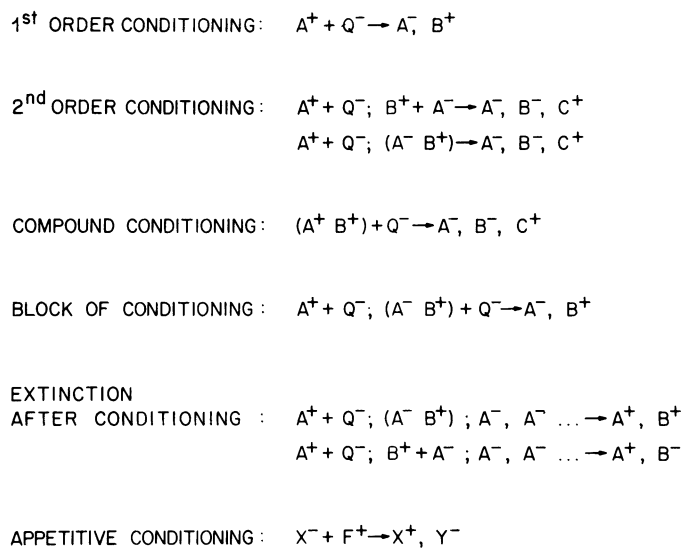



FIGURE 1. Summary of the logic operations performed by the *Limax* CNS as revealed by conditioning experiments. Stimuli in parenthesis are presented simultaneously.

tion to food B is mediated via the internal representation of food A. If extinguishing the aversive reaction to food A alone also results in the disappearance of the aversive reaction to food B, then the conditioned aversion to food B must depend on the integrity of the internal representation of food A. Conversely, if extinguishing the conditioned aversion to food A after using food A to produce second-order conditioned aversion to food B *does not* diminish the aversion response to food B, then clearly the conditioning to food B does not depend on the internal representation of food A. Rescorla (1984) has pointed out that either outcome can be produced depending on the timing of stimulus presentation during phase two of the second-order conditioning procedure. If A and B are presented simultaneously during phase two of the second-order conditioning procedure, extinction of A also extinguishes B. If A and B are presented sequentially during phase two of the second-order conditioning procedure, then extinction of A does not extinguish B.

The *Limax* data obtained using the postconditioning extinction procedure are just what one would predict from the precedents in the vertebrate conditioning literature (Sahley *et al.*, 1984). If odor A and odor B are given simultaneously during phase two of a second-order conditioning experiment and then the aversive response to odor A is extinguished by repeated presentations of odor A alone, the aversive response to odor B is also eliminated (Fig. 1). Conversely, if odor A and odor B are given sequentially during phase two of the second-order conditioning procedure, then extinction of the aversive response to odor A does not diminish the aversive response to odor B.

*Limax* also can acquire appetitive approach responses to neutral or weakly

aversive odors if a very attractive taste (0.5 M fructose) is paired with exposure to the neutral odor (Sahley *et al.*, 1982). We do not know yet whether the higher-order phenomena documented with aversive conditioning also obtain with appetitive conditioning. Delaney has recently shown that *Limax* can learn to avoid diets deficient in a single essential amino acid (Delaney and Gelperin, 1983) using an as yet unidentified postingestive consequence of eating the deficient diet. This learning occurs after one day's intake of the deficient diet and is retained for at least three weeks. The list of neuronal computations probably is not complete as yet.

## 2.2. Training of Isolated Brain

The reliability of the associative learning combined with the robustness of molluscan neurons and synapses (Reingold and Gelperin, 1980) led to the first experiments aimed at training the isolated central nervous system of *Limax* (Chang and Gelperin, 1980). Rather than odor–taste pairings, taste–taste pairings were applied to the normal taste input pathway via the lip chemoreceptors. The taste stimuli used were standardized extracts of the foodplants used in the whole animal training. The output used to assess learning was the neural substrate of ingestion, termed feeding motor program (FMP). Feeding motor program is a pattern of cyclic, coordinated motoneuron activity recorded from buccal ganglion nerve roots which can be recognized unambiguously by a naive observer given the buccal nerve recordings and a measuring algorithm (Gelperin, Chang, and Reingold, 1978). Isolated lip–brain preparations were differentially conditioned to supress FMP responses to a food extract that initially triggered FMP by pairing lip application of the food extract with lip application of a bitter-tasting substance such as colchicine, tannic acid, or quinidine. The evidence for associative learning derives from the findings that the FMP response decrement is selective to the food extract paired with the bitter taste and that there is a critical time interval within which the food extract and bitter taste must be applied for response suppression to occur (Culligan and Gelperin, 1983). Changes at the taste receptors themselves as a causative mechanism are made less likely by the finding that the lip–brain preparation can learn after being trained using one lip and tested using the other, naive lip.

Another approach to studying the mechanism of memory storage in the isolated brain is to train the animal, prepare its brain for neurophysiological recording, and assess *in vitro* the retention of the memory that had been entered *in vivo* (Gelperin and Culligan, 1984). The intact animals were given taste–taste training using the same food extracts and quinidine solution later used to stimulate their lip chemoreceptors after reducing the animal to a lip–brain preparation. Cold anesthesia of both the animal and brain was used to protect the synapses from alteration and depletion during dissection. One half of the animals given paired training yielded lip–brain preparations showing a selective FMP response suppression to the food extract paired with quinidine. None of the animals given unpaired training yielded lip–brain preparations showing a selective FMP response suppression (Gelperin and Culligan, 1984). These results make it likely that the whole ani-

mal and isolated brain training procedures are accessing the same stimulus representation and associative learning mechanisms. Further tests of this idea are possible using a two-phase training procedure, with phase one presented to the intact animal and phase two applied to the isolated lip–brain preparation.

## 2.3. Training Cultured Brains

The memory retention times shown by the isolated lip–brain preparations (8–12 hr) are much shorter than the retention times shown by the intact animal (2–3 weeks). To test whether this difference results from the impoverished biochemical environment experienced by the isolated brain living only in saline, we developed techniques for dissecting the animal under semisterile conditions and setting up the lip–brain preparation in a sterile culture medium. Under these conditions, FMP responses can be elicited for several days in response to taste stimuli applied to the lips. Test solutions are conveyed to and removed from the lip chambers by tubing connected to a peristalic pump. The preparation is shown diagrammatically in Fig. 2.

Before training, the responses of the preparation to two different attractive food extracts (A+, B+) are assessed. Stimulus A+ is applied to the lips for 30 sec and the FMP response recorded. Stimulus B+ is applied 1 hr later for 30 sec and the FMP response recorded. After another hour, A+ is again applied for 30 sec, followed immediately by application of a concentrated solution of quinidine sulphate to the lips for 10 min. The preparation is then rested for several hours before testing.

Testing is started with a 30-sec application of B+, repeated every 30 min until

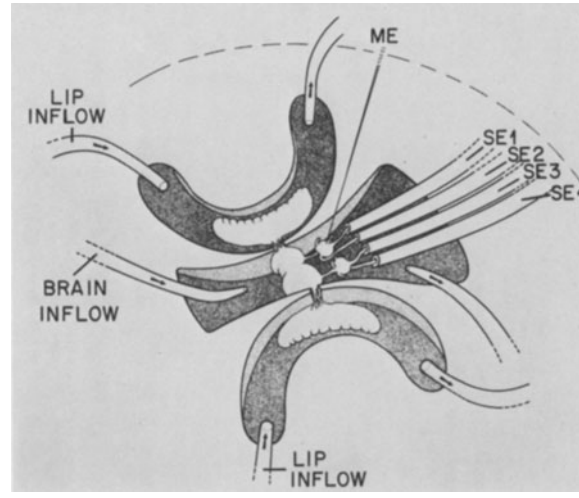



FIGURE 2. Schematic diagram of the isolated nervous system of *Limax maximus*. Cerebral ganglia, buccal ganglia, and the lip chemosensory regions are left attached to one another via nerves. The two lip halves are placed in separate chambers that allow independent perfusion with food-derived stimuli. SE 1, 2, 3, 4 = Suction electrodes 1, 2, 3, 4; ME = microelectrode.

a reproducible level of FMP response is obtained. Then the response to A is determined leaving at least 30 min after the preceding B+ application.

An example of a result obtained with this training and testing procedure is shown in Fig. 3. Initially, at t = 0 and t = 1 hr, the responses of the preparation to 30-sec applications of rat chow extract (A+) and apple juice (B+) were determined. Both stimuli elicited strong FMP responses from the preparation. At t = 2 hr, a 30-sec application of A+ was followed immediately by application of quinidine sulfate solution to the lips for 10 min. For unknown reasons, the FMP evoked in response to A+ was potentiated by the subsequent quinidine exposure.

The preparation was then rested for several hours before its responsiveness to B+ was determined. At t = 10 hr, a 30-sec application of B+ elicited a short but clear bout of FMP. A 30-sec application of A at t = 11 hr did not elicit any FMP. The complete suppression of the FMP response to A was retained for almost two days, even though the preparation received several tests of its response to A which contributed to extinction of the learned response suppression. At t = 45 hr, a weak FMP response consisting of two bites was evoked by A. This response was much weaker than the immediately preceding response to B+ at t = 44 hr. The magnitude of the response to A increased gradually over the next two days. The result indicates that it should be possible to study the transition of the memory trace from short-term to long-term form in the isolated brain preparation, as well as the effect of selective biochemical manipulation of the culture medium to enhance or retard synthetic events thought to be important in memory function.

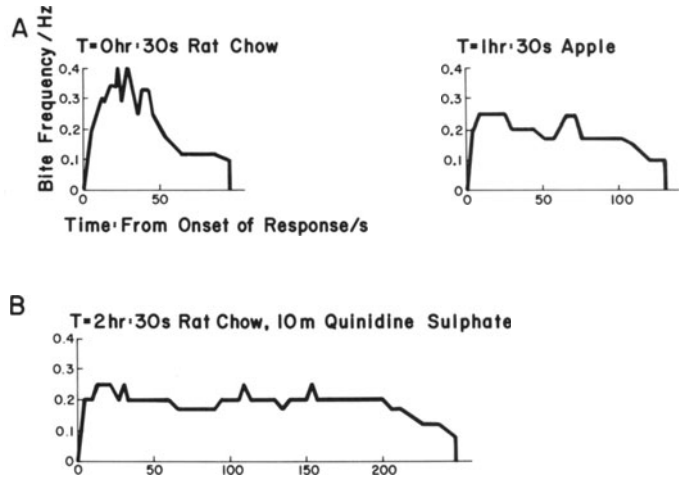
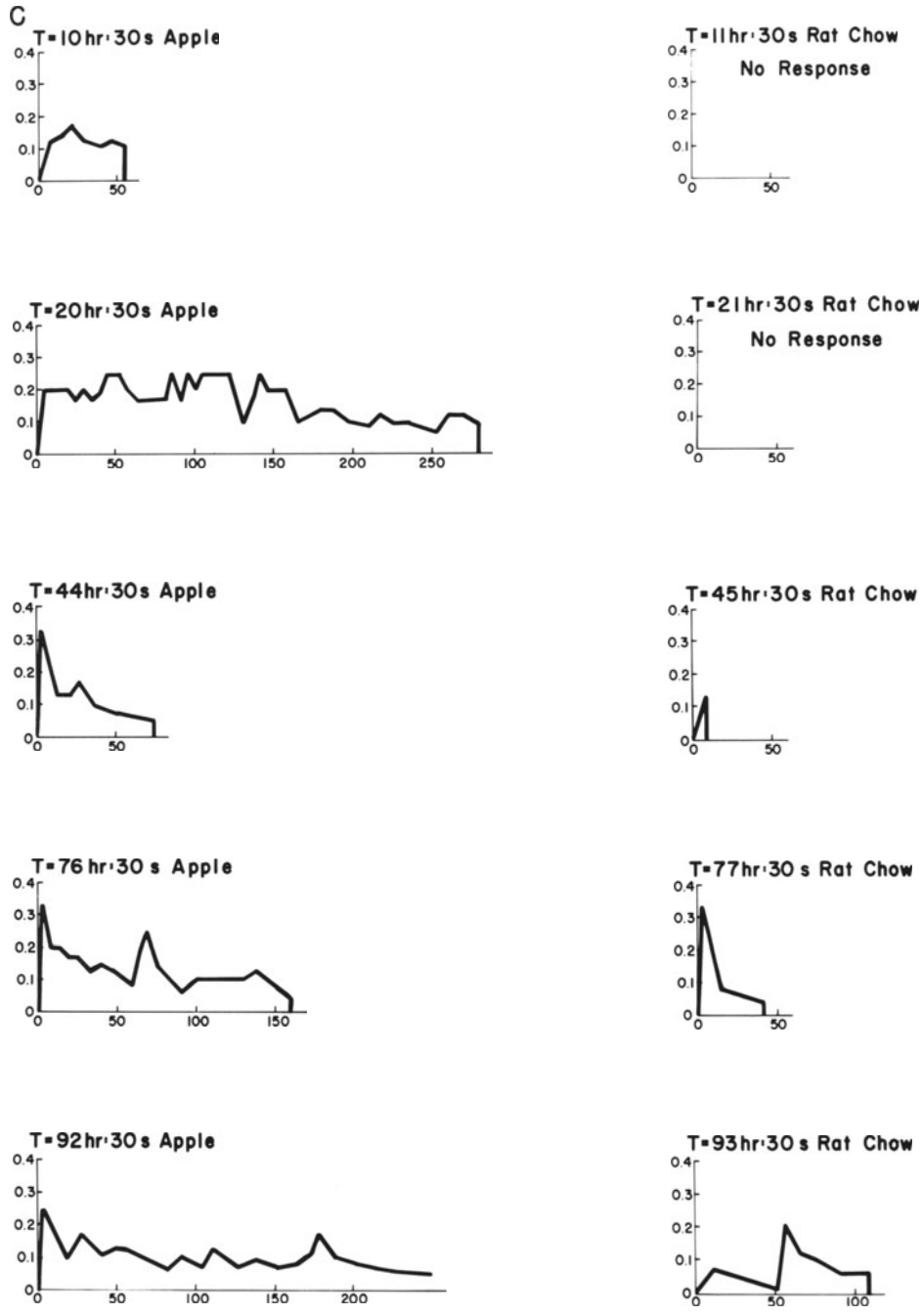



FIGURE 3. Four-day retention of a learned taste aversion by an isolated lip–brain–buccal ganglia preparation maintained in an enriched culture medium. In each graph the instantaneous frequency (in Hz) of bites in the feeding motor program produced in response to a stimulus applied to the lips is plotted against the time (in seconds) elapsed from the start of that response. The time elapsed from the start of the experiment and the type of stimulus applied are shown above each graph. (A) Control responses of the preparation to rat chow (CS1) and apple (CS2) prior to training. (B) Response during the training




procedure in which a 30-sec application of rat chow (CS1) was followed immediately by a 10-min application of quinidine sulfate (US). (C) Responses to apple and rat chow, applied at intervals following the training procedure. The response of the preparation to rat chow was suppressed completely for at least 19 hr following training. It remained suppressed relative to the response to apple for the duration of the experiment.

## 2.4. Interneurons

The primary sensory neurons for taste and smell are numerous, small, and located peripherally in the lips (Benedeczky, 1977) and nose (Gelperin, 1974; Kataoka, 1976; Chase and Kamil, 1983). Rather than start by examining the sensory neurons directly (see review by Croll, 1983), we studied interneurons that receive and integrate synaptic input from peripheral chemoreceptors. Our initial study focused on the metacerebral giant cells (MGCs), a pair of serotonergic interneurons known to modulate the expression of FMP (Gelperin, 1981). A preparation of the noses, olfactory nerves, and cerebral and buccal ganglia was developed that allowed odor puffs to be delivered to the olfactory epithelium while an *en passant* electrode recorded from the olfactory nerve and an intracellular record was obtained from an MGC (Egan and Gelperin, 1981). These experiments demonstrated that olfactory afference can provide synaptic excitation to the MGC sufficient to elicit spikes from a quiescent cell or increase spike rate in an active cell. We have not yet determined whether this input from the olfactory nerve monosynaptically excites the MGC, although fiber tracts from the olfactory nerve were shown to arborize near the MGC (Egan and Gelperin, 1981). Input from one olfactory nerve can affect both the ipsilateral and contralateral MGCs. Further work is necessary to determine if the functional effect of the synaptic input to the MGCs differs when attractive or repellent odors are applied to the nose. Activity of the MGC homologue in *Aplysia* is correlated directly with the level of food arousal (Kupfermann and Weiss, 1982) and bilateral lesion of the *Aplysia* MGCs leads to a specific change in the biting response with no change in other aspects of feeding or other behaviors (Rosen *et al.*, 1983).

Kemenes *et al.* (1982) studied the responses of an identified cerebral neuron (C2) to input from lip chemoreceptors in *Helix*. The C2 neuron increased its rate of action potential production by 2.5 to 3 times in response to lip application of 3% sucrose or apple juice, but was only weakly activated by distilled water or mechanical stimulation. It will be important in further work of this type to monitor FMP output from the buccal ganglion and test the effect of imposed interneuron activity (e.g., driven activity in C2) on the feeding control system. The burgeoning collection of such feeding interneurons (Benjamin, 1983; Rosen *et al.*, 1982; Weiss *et al.*, manuscript submitted) provides ample material for studying changes in chemosensory integration due to learning (Davis *et al.*, 1983; compare with Chapter 14, this volume).

A large population of chemosensory interneurons is located in the procerebral lobes of the cerebral ganglia in *Limax* (Veratti, 1900; Zs-Nagy and Sakharov, 1970). These interneurons are intrinsic to the procerebral lobe and are remarkable both for the complexity of their arborizations and the existence of axosomatic as well as axoaxonic synapses (Zs-Nagy and Sakharov, 1970). Anatomical studies indicate that the olfactory nerves provide input to the procerebral lobes, but the three lip nerves do not (Chetail, 1963). Although there are no monoamine-containing somata in the procerebral lobe, monoaminergic fibers containing both serotonin and dopamine innervate the intrinsic interneurons. This suggests that monoaminergic neurons extrinsic to the procerebral lobe might modulate the processing of olfactory

information by the intrinsic interneurons. Consistent with this anatomical picture is the finding that the taste input–FMP output reflex pathway does not require the procerebral lobes for its operation and that a brain conditioned *in vitro* retains its taste–taste association after procerebral lobe amputation (I. Cooke and K. Delaney, unpublished observations). It will be very interesting to test the effect of procerebral lobe amputation on odor-elicited synaptic events as recorded from the MGC using the nose–brain preparation.

Another approach to the identification and mapping of interneurons in the feeding control system is the use of immunohistochemical staining, most easily implemented using the whole-mount procedure of Beltz and Kravitz (1982). The entire population of neurons using a particular transmitter can be revealed if an antibody to that transmitter or its synthetic enzyme is available. Work in a variety of molluscan systems has implicated serotonin, dopamine, acetylcholine, gamma-aminobutyric acid, histamine, small cardioactive peptide B, FMRFamide, cholecystokinin, and metenkephalin as involved in some aspect of the neural control system for feeding, An example of *Limax* buccal neurons showing immunoreactivity for a FMRFamidelike substance is shown in Fig. 4. The large neuron is cell B1, which sends a very large axon into the cerebrobuccal connective, through the cerebral ganglion to the abdominal ganglion, and out a peripheral nerve to the heart and kidney region (D. J. Prior and A. Gelperin, unpublished observations). The cerebral ganglia also contained scattered, small immunoreactive neurons. Nerve fibers with FMRFamidelike immunoreactivity were observed in buccal ganglion nerve roots, the cerebrobuccal connectives, and some cerebral ganglion nerve roots (Cooke and Gelperin, 1984). This result immediately suggests experiments testing the family of FMRFamidelike peptides (Greenberg *et al.*, 1983) for a controlling or modulatory function on the FMP reflex and its associatively conditioned modification.

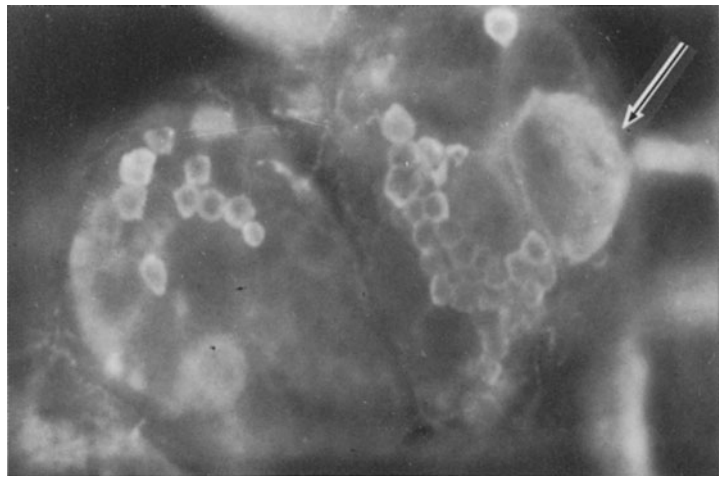


FIGURE 4. A *Limax* buccal ganglion stained with fluorescent-labeled FMRFamide antibody as a whole mount. The arrow indicates cell B1 in the lateral lobe of the ganglion.

## 2.5. Neurochemical Approaches

A neurochemical analysis of the cerebral and buccal ganglia of *Limax* using the technique of high-pressure liquid chromatography indicated the presence of substantial amounts of dopamine and serotonin (Wieland and Gelperin, 1983). Addition of exogenous dopamine to the isolated cerebral and buccal ganglia resulted in the generation of FMP, while addition of the dopamine blocker ergonovine blocked the ability of lip chemostimuli to trigger FMP. There appears to be a set of dopaminergic synapses whose function is obligatory for operation of the taste input–FMP output reflex, and whose modulation may play a role in learned alterations of the reflex. To examine the effects of altering dopaminergic synapses on the FMP reflex and learning ability of the isolated lip–brain preparation, dopamine synthesis is blocked by applying alpha-methyl-*p*-tyrosine (Wieland *et al.*, 1983). Since measurement of total dopamine in the ganglia is a very insensitive measure of depletion caused by alpha-methyl-*p*-tyrosine, we are now examining dopamine released into the medium bathing the ganglia in response to a short pulse of high (50 mM) potassium medium (Wieland *et al.*, 1984). After removal of the outer connective tissue sheath, a pair of cerebral and buccal ganglia can liberate up to 5 pmole of dopamine in 30 min of high-potassium stimulation, an effect blocked in low-calcium saline containing 5 mM cobalt. Now the effect of alpha-methyl-*p*-tyrosine on the synaptically releasable pool of dopamine can be assessed, first neurochemically and then physiologically. Lip–brain preparations are also being prepared with altered dopaminergic transmission by prior injection of the neurotoxin 6-hydroxydopamine.

In addition to studies aimed at directly manipulating the dopamine pool available for synaptic release, other transmitters known or suspected of modulating dopaminergic transmission are also of direct relevance. Met-enkephalin is known to cause dopamine release in several molluscan ganglia (Stefano, 1982), hence its effect on the feeding system in *Limax* is of particular interest.

The effect of serotonin on the lip–brain preparation parallels the effect of intracellular stimulation of the MGCs, namely, modulation of the intensity of the output. Exogenous serotonin application does not trigger FMP, but will speed up and intensify motor output elicited by lip input (Wieland and Gelperin, 1983). Potassium-stimulated release of serotonin from the isolated lip–brain preparation has also been measured (2.5 pmole per 30 min) so as to have an internal control for the specificity of treatments aimed at the selective alteration of dopamine.

A quite different approach to altering chemistry before studying the learning ability of the isolated brain is to use dietary supplements of neurotransmitter precursors given to the animal for several weeks before testing its brain physiologically. This strategy works well for those transmitters whose synthesis is limited by precursor availability, such as acetylcholine (Blusztajn and Wurtman, 1983). Slugs raised on a high choline diet (1.14% wt/wt) have a significantly higher level of choline in their blood than slugs raised on a low choline diet (0.03% wt/wt) (Barry and Gelperin, 1982a). Transmission at an identified cholinergic synapse is augmented by an increase of only 1.5 $\mu$m choline in the medium bathing the synapse, an increase in choline concentration well within the range caused by ingestion of the

high-choline diet (Barry and Gelperin, 1983b). Several lines of evidence indicate that the synaptic augmentation caused by increased choline availability in this concentration range is due to effects on acetylcholine synthesis in the presynaptic neuron (Barry and Gelperin, 1984). These observations relate directly to the search for learning and memory storage mechanisms because the slugs fed the high-choline diet showed much better retention of the conditioned odor–taste association than did the slugs fed the low-choline diet (Sahley *et al.,* 1985). Since both high-choline and low-choline slugs showed the learning after one day, the effect appears to be on retention.

## 3. LIMAX Learning: The Neural Model

### 3.1. Components of the Model

The neural model of associative learning attempts to simulate the experimental results of associative learning experiments done with the real mollusk. The model uses neural elements whose properties and interactions are realistic abstractions of real neurons (Hopfield, 1982, 1983). The model is currently embodied in a computer simulation, the LIMAX simulation program. The relationships between the components of the model (LIMAX), both neural hardware and processing algorithms, and the components of the real animal *(Limax)* will be discussed after presenting the model and its operation. LIMAX has three major parts (Fig. 5): (1) an array of lip chemoreceptors, (2) a taste categorizer/taste memory network, and (3) a learning control and motor output network. These three components operate as follows.

The lip chemoreceptors are taken to be 100 sensory neurons, each responding to several components of the food-plant extracts used as chemostimuli in the behavioral experiments. The result of presenting a food extract to the array of sensory neurons is to drive some of the receptors strongly and others less so. The sensory code for a particular food is thus described as a set of 100 random numbers, each lying between zero and unity, with an average of 20 values greater than 0.5. This sensory signal was then "clipped": sensory neurons with large outputs produced maximum outputs (value of 1), while the neurons with small outputs generated no output (value of 0). This procedure transforms the sensory input from a particular food from continuous numbers into a list of 100 1s and 0s, with about 20 1s. A taste input from a mixture of foods is produced in an analogous way as the input from a single food, hence the sensory signal will represent an admixture of the two foods in which the total number of maximally active sensory cells was maintained near 20.

The aversive taste input pathway for quinine is represented in a special way in LIMAX in that inputs on this pathway are innately aversive and do not have to be learned to be recognized. The aversive input can vary in strength, but does not become less aversive due to paired application with an attractive taste input. This aversive input pathway is represented in Fig. 5 as a single quinine-recognition sensory cell. This is convenient and rational because the ultimate logic of LIMAX will

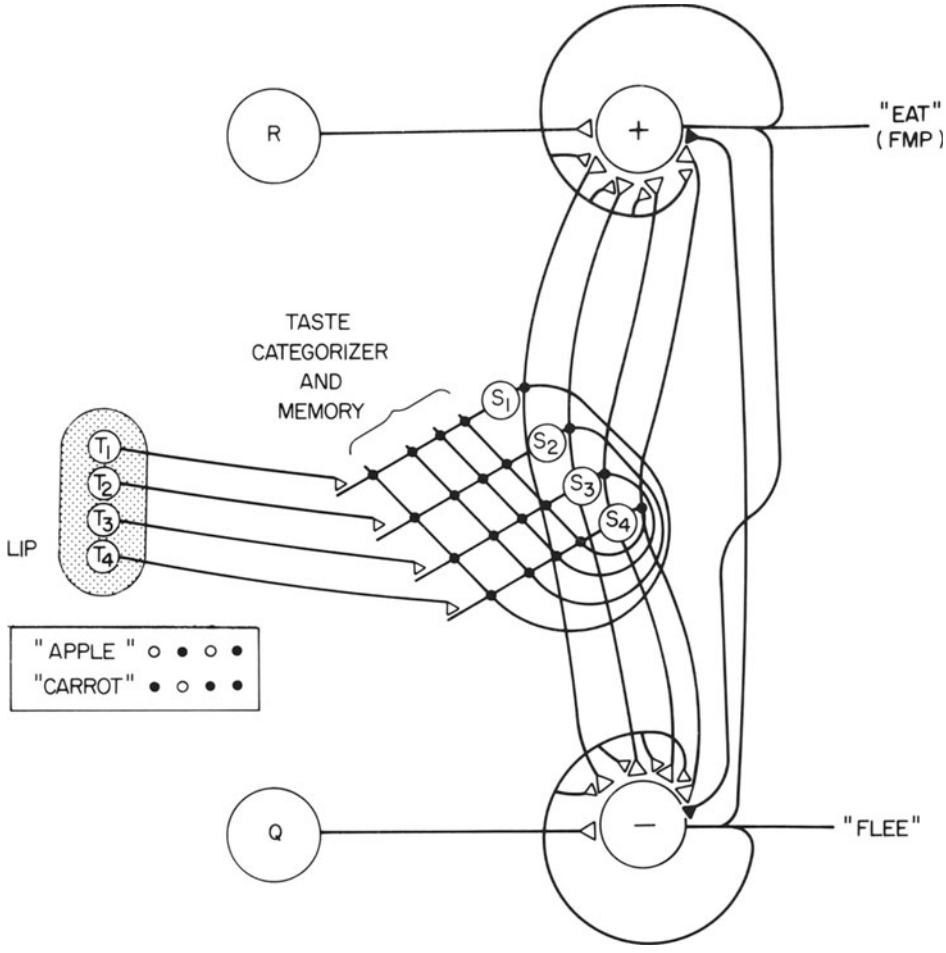



FIGURE 5. A diagram of the components of the LIMAX simulation program.

not depend on any particular attributes of quinine. Any innately aversive stimulus (quinine, sodium chloride, electric shock) could be used to activate this pathway. An innately rewarding taste input pathway is also available.

The taste categorizer/taste memory network receives its input from the chemoreceptor array and has the ability to modify the strengths of the synaptic connections between its elements to produce a set of stable activity patterns. A particular stable activity pattern in the taste categorizer network is an internal representation of the taste input from a food class. A detailed description of how this system works is given in Appendix 1.

The taste categorizer network performs several functions that are crucial to the capability of the LIMAX simulation to learn appropriately. First, internal representations of foods that LIMAX has been exposed to in the past are contained in the organization of the synapses in this network. When presented with a particular

food that elicits a pattern of activity in the taste receptors that is close to, but not necessarily exactly the same as, on of the familiar food classes, the categorizer network "recognizes" the similarity. It does so by assuming a firing pattern of its constituent neurons that represents the known food class. The ability of the categorizer network to converge to a unique stable activity state from similar but different activity states is an inherent feature of its organization (see Appendix 1) and is the reason for its description as a "categorizer." Second, when presented with a novel food that is not rapidly or easily categorized as a known familiar food, the network will, if presented with the new food a sufficient number of times, build an internal representation of this new food class and include it as a new category of its known foods.

The learning control/motor output network determines the response of the animal when presented with a food: will it "eat" or "flee." We consider these possibilities mutually exclusive and have modeled their neural hardware with cross-inhibition between a command neuron for "eating" and a command neuron for "fleeing." These command neurons, called the "plus" (+) and "minus" (−) cells, are meant to represent the observed motor responses of the animal, but not the central pattern generators responsible for the control of particular muscles. Note that only one of the two command neurons can be active at any time.

The learning control network is functionally located in the synapses between the taste categorizer and the motor center and controls the learned association of taste categories with one of the two possible motor responses. Every neuron in the categorizer network synapses on the "plus" and "minus" cells. How the firing pattern of the neurons in the categorizer influences the activity of the "plus" and "minus" cells determines the ultimate activity of the organism because it is the pair of "plus" and "minus" cells that coordinate in a mutually exclusive way whether LIMAX is in the "eat" or "flee" state. Hence when food A is presented to LIMAX, it will elicit a "flee" response if the synapses from the neurons in the categorizer that are active in the internal representation of food A are strongly excitatory on the "minus" cell and weakly excitatory on the "plus" cell. It is actually the summed averages of these synaptic influences from the active categorizer neurons onto the "plus" and "minus" cells that determine the actual motor output.

Now one can see what synaptic modification is required when an initially attractive food A is presented to LIMAX paired with an aversive input from quinine. If food A is attractive, this means that the firing pattern of the categorizer that is the internal representation of food A provides strong excitatory drive to the "plus" cell and weak (or at least less strong) synaptic drive to the "minus" cell. In order to make food A aversive, this pattern of synaptic drive must be reversed: The active categorizer neurons for food A must provide stronger synaptic drive to the "minus" cell than the "plus" cell. This change in synaptic strengths between categorizer neurons and the "plus" and "minus" neurons is the essential step in the conditioning.

Special care must be taken to insure that the conditioning shows the temporal specificity observed in real experiments. We only want LIMAX to learn that food A is aversive if food A is presented before or concurrent with the presentation of an aversive stimulus. An aversive stimulus, whether it is quinine or a previously aver-

sively conditioned food, is one which, when presented alone, leads the organism into the "flee" state. Hence if we want LIMAX to learn that attractive food A is to be aversive, in a way consistent with the observed temporal specificity of the presentation of CS and US, we want to change the synaptic strengths between the active categorizer neurons representing food A and the "plus" and "minus" cells only if food A occurs before or during a transition of the state of the organism from the "eat" to the "flee" state. In LIMAX, this temporal specificity is embodied in the logic of the synapse modification rule. Synapse modification occurs only when there is a well-defined temporal relationship between activity in the presynaptic terminal of a "categorizer" cell and the postsynaptic cell (+ or − cell). This rule is illustrated in Fig. 6. Although synapse modification requires the presence of presynaptic activity, the sensitivity to synaptic change is not uniform with time after a presynaptic action potential. The time course of synapse change sensitivity has the form shown in Fig. 6B as the consequence of a typical timecourse of excitation of the "minus" cell illustrated in Fig. 6A. This sensitivity defines a time window: if the presentation of the CS precedes this window as in forward conditioning (Fig. 6C), a maximal increase occurs in the synaptic strength. If the CS is presented after this window as in backward conditioning (Fig. 6E), the synapse modification is greatly reduced, or if there is sufficient delay, completely eliminated.

Although this modeling appears to involve the use of Hebbian synapses, this is strictly true only in the sense of an operational definition. The operational definition of a Hebbian snyapse is that if the presynaptic and postsynaptic elements are active at the same time, then the strength of the synapse is increased. From the point of view of cellular and biochemical hardware, this learning element is usually considered as a two-terminal device consisting solely of the presynaptic and postsynaptic terminals. In the gill withdrawal conditioning system of *Aplysia,* a three-terminal synapse has been suggested as the basic learning element (Kandel and

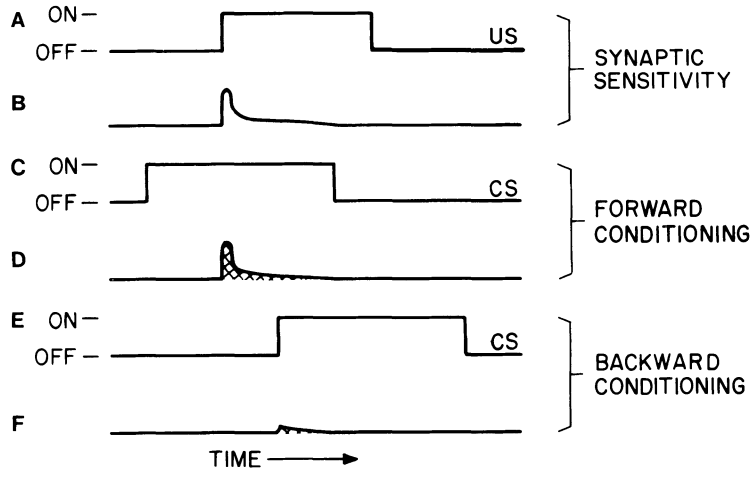



FIGURE 6. Diagram of the temporal specificity of the synapse modification algorithm operating on the synapses between the taste categorizer network and the plus and minus neurons.

Schwartz, 1982; Chapter 12, this volume). In addition to the presynaptic and postsynaptic cells, the third terminal is provided by the facilitator synapse on the presynaptic terminal. The efficacy of transmission between the presynaptic and postsynaptic cell is increased when activation of the presynaptic terminal briefly precedes activation of the facilitator. From our operational point of view, it is possible to think of the Hebbian synapse as a special case of a three-terminal learning element. If the postsynaptic cell synapses on the presynaptic terminal as a facilitator synapse, then the strength of the synapse between the presynaptic and postsynaptic cell will increase with simultaneous activation of the presynaptic cell. In this special case, the postsynaptic cell also plays the role of the facilitator (Hawkins and and Kandel, 1984). The time-order dependence we have described and use in our model, not present in the sample Hebb description, can likewise be described with either two- or three-terminal hardware.

## 3.2. Learning with LIMAX

A typical learning experiment with the LIMAX simulation involves the following steps: LIMAX is presented with a new food A, which may or may not resemble one of the known foods already in its memory. The taste neurons responding to A drive the categorizer network, which converges either to a previously established category or to a new one for food A. Let food A initially be either neutral or attractive. This means that the synaptic drive from the active neurons in the categorizer representing food A to the "plus" and "minus" cells is either unbiased or stronger to the "plus" cell. The state of LIMAX is now either neutral (i.e., not in either the eat or flee state) or eat. Now LIMAX is presented with quinine, which drives it into the "flee" state. As illustrated in Fig. 6, this is the correct time sequence for learning and LIMAX modifies the synapses from the categorizer network to the "minus" cell so that the active inputs to the "minus" cell are strengthened. The synapses from the categorizer network to the "plus" cell are also modified so that active synapses are weakened. After a few such pairings of food A and quinine, presentation of food A alone causes LIMAX to "flee," i.e., food A has become aversive.

For the system to work well, two additional features are necessary. First, when an association has been learned well, further learning of the same association should cease. Without this control, one association presented often and strongly would obliterate all other memories. This control can be achieved by limiting the synapse-strengthening algorithm so that the synaptic strength between any two neural elements cannot exceed a fixed value. Second, we implemented an algorithm whose effect is to hold constant the total synaptic strength of all the inputs to each neural element. When one synapse to an element was set to unity, another synapse chosen at random was set to zero. As a result, older memories will become weakened and slowly destabilized as new ones are learned. A food learned a long time ago and not tasted again will not have its memory degraded with time per se, but only as a result of learning many new tastes afterward.

The simulation of first-order conditioning just described effectively models the learning experiments with the real mollusk. Second-order conditioning is accom-

plished by the learning algorithm in a way which emphasizes one of the important computational properties of the categorizer network. Take the case in which LIMAX is presented with food B. As before, after the categorization process, there is an internal representation of the category into which food B is placed that is manifest in the particular firing pattern of the neurons of the categorizer network. If food B is unbiased, the state of LIMAX is neutral, whereas if food B is attractive, the state of LIMAX will be "eat." Now LIMAX is presented with food A, which has been aversively conditioned previously by pairing A and quinine. The effect of driving the categorizer with the sensory neuron pattern that codes for food A while the state of the categorizer is still food B is to change the firing pattern of the categorizer neurons so that it partially resembles both A and B. Since food A has been strongly aversively conditioned, the categorizer inputs to the "minus" cell will be strongly driven, causing the state of LIMAX to change to "flee" while the categorizer firing pattern still partially resembles the representation of food B. Hence, the conditions for the learning algorithm are satisfied, and the neurons that are firing in the categorizer network will have their synapses with the "minus" cell strengthened and their synapses with the "plus" cell weakened. The internal representation of the category that includes food B will be changed so that it is more aversive. After a few trials, food B will achieve an aversive status equal to food A, that of a food aversively conditioned in a first-order learning paradigm.

Note that any system that uses the same neural hardware for input of both the CS1 and the aversively conditioned CS2 in a second-order learning experiment will face the question as to how the information in CS1 is stored while the new information representing CS2 is input. In LIMAX, partial information about CS1 is retained in the firing pattern of the categorizer which is, however, progressively being influenced by CS2. This approach is in contrast to registerlike memories where the representation of CS1 is stored physically separate from the representation of CS2, or the simpler case (Hawkins and Kandel, 1984) where CS1 and CS2 are represented by physically distinct input pathways.

What is the effect of extinguishing the aversiveness of food A after using food A to produce second-order aversive conditioning of food B? Our system does not show extinction, but we can achieve the same effect in a different way by presenting food A followed by a rewarding taste (0.5 M fructose) a few times, after which A will be attractive. Recall that foood B is aversive because of the strong synaptic connections between the categorizer neurons that are on in the representation of B and the "flee" neuron. Since the categorizer neurons that are active in the B representation are generally not the same neurons as are on in the food A representation (although a few of them may be in common), extinguishing food A has little effect on the aversive behavior to food B.

Second-order conditioning can be done in a different way. Once again, we begin by establishing a first-order conditioned aversive response to food A, and then present a 50–50 mixture of foods A and B. This mixture is neither A nor B, but similar to each. Within the present modeling, this food would drive about five neurons that are on in both A and B, five that are on in A but not B, five that are on in B and not A, and five that are on in neither A nor B. While this food mixture AB is present, the synapse modifications appropriate to it are made in the cate-

gorizer network and AB itself becomes a new food category, which is neither A nor B. At the same time "flee" will come on during this exposure because the food AB contains a very significant half of its active neurons that are common with A and which therefore are excitatorily connected to the minus cell. When the state changes to "flee," some connections to "flee" are strengthened from neurons that are active in state AB but not in state A. If this conditioning is only done once and the period of time of the AB stimulus presentation substantial, AB will become a known category in which the dominant connections responsible for the behavior "flee" are from neurons active in state A to the "minus" cell. Notice that the system does not know category B; it knows only categories A and AB. Now when LIMAX is presented with food B, its initial response will be through using the known food category that is closest to B, namely the category AB, since B is not familiar. Its initial behavior will then be "flee," and therefore classified as second-order conditioning.

The effect of extinction of A (again carried out by learning a positive association with A) will now be quite different. The extinction of A will succeed in eliminating the connections between the neurons that are active in state A and "flee," and will replace them by excitatory connections between these same neurons and "eat." When next food B (unfamiliar) is presented and invokes the similar category AB, there will be little tendency to flee because the excitatory connections between neurons active in A, and which were responsible for the aversive behavior to category AB, have been chiefly eliminated and replaced by excitation of "eat."

## 3.3. Relation of LIMAX and *Limax*

The way we have modeled the primary chemosensory input represents an explicit choice between two alternatives: the labeled-line theory and the across-fiber pattern hypothesis. Although these theories are not strictly mutually exclusive, many primary sense cells in the gustatory and olfactory pathways each respond to several components of a biologically relevant, complex food stimulus (Croll, 1983; Derby and Ache, 1984; Dethier and Crnjar, 1982). The much smaller body of data on response properties of chemosensory interneurons likewise shows cells with broad response properties such as those used in the modeling rather than food feature detectors. Given our choice of modeling the sensory input using the across-fiber pattern to represent a particular food taste or odor, the choice of 100 cells in the categorizer provides about 15 distinguishable food categories. Although systematic studies to determine how many different distinguishable food categories *Limax* possesses have not been done, more than 30 different food plants are acceptable to *Limax* (Frömming, 1952; Gain, 1891).

The input pathway for quinine has special properties deriving from its use to channel several types of aversive stimuli that cause food aversion learning into the LIMAX neural network. Toxicosis (Gelperin, 1975), bitter taste (Sahley *et al.*, 1981a), and shock (Delaney and Gelperin, 1984) can all promote rapid learning by real *Limax* so a multimodal aversive input pathway is required. One implication of directing the aversive input directly to the learning control circuit is that the aver-

sive properties of quinine should not decrease no matter how many times quinine is paired with an attractive food. This is a testable notion.

Our use of the taste categorizer network to process the CS inputs (food A and B) provides a natural solution to the need for an internal representation of the first CS (food A) which can interact with a second CS arriving later during second-order conditioning. Additional evidence for an enduring "taste memory" is that first-order conditioning can occur with times of 5 to 30 min intervening between the CS (food A) and US (quinidine) (Chang and Gelperin, 1980; Culligan and Gelperin, 1983).

The model has three possible behavioral outputs: feed, flee, and neutral. The neutral state is more accurately described as the absence of activation of either feed of flee. Recent recordings of anterior pedal nerve output before and after *in vitro* conditioning of the isolated *Limax* lip–brain preparation indicate that a neural correlate of foot withdrawal appears due to associative conditioning as FMP disappears (Delaney and Gelperin, 1984). This will allow more explicit measurement of the appearance of the flee state as the feed state is suppressed during learning.

### 3.4. Questions Raised by the Model

Within this simple model, there are different ways in which second-order conditioning can be represented. Different training protocols can result in different physical representations of the synaptic changes responsible for second-order conditioning. Such differences can then show up in behavioral differences, which make sense only when described in terms of what the hardware is doing.

This model of the sensory processing of *Limax* captures the essence of many of the learning experiments that have been carried out. It emphasizes what simple collective systems can do with a minimum of detailed prewiring and without feature detectors (grandmother cells). The model raises many questions to which answers are not yet known. To begin, are the categories in *Limax* food learning plastic, or are the categories fixed (as in immunology)? Does a learning experience involve merely assigning a food to one of these categories and labeling that category? Are the categories A, B, and AB indeed all different? Is the essential memory of foods electrical reverberation (as we have assumed) or chemical? Is this memory the same in first-order conditioning (where time delays can be very long) and in second-order conditioning (where time delays must be short)? How close together in "taste" can two foods be and still be distinguished? Does food memory gradually fade with time, or is it an "all or none" effect? The attempt to model the system generates a set of behavioral and electrophysiological questions whose answers are essential to carrying such modeling further.

## 4. Future Directions

### 4.1. *Limax* Learning

There are a variety of questions best answered by behavioral experiments on intact animals. The existence of higher-order appetitive conditioning needs to be

determined by applying the paradigms used to produce higher-order aversive conditioning, but with neutral or aversive odors as the CS and 0.5 M fructose as the US. Behavioral experiments can explore the nature of the stimulus categories formed during exposure to food odors and tastes, and are the most effective way to accurately determine the time course of the synaptic sensitivity to associative modification depicted graphically in Fig. 6. Finally, the transition of the memory trace from a short-term form that is labile to a long-term form that is resistant to disruption can be studied by cooling animals to 2°C for a brief period at different times after the conditioning is established.

Neurophysiological experiments can capitalize on the ability to produce robust conditioning of isolated brains while recording from interneurons in the feeding control circuit. The metacerebral giant cell and the cerebral dopamine neurons are logical loci from which to record changes in synaptic drive due to conditioning. Electrical recording can also be used to localize the neurons that store the taste or odor memory (CS trace). To determine whether some part of the *Limax* CNS stores information in the way that LIMAX does, multichannel electrical or optical recording methods will be used to record action potentials from tens of neurons simultaneously before and after memory storage.

Neurochemical experiments have been started to determine the number and prevalence of phosphoproteins recovered from extracts of *Limax* cerebral ganglia (B. Oestreicher, T. Yamane, and A. Gelperin, in preparation). As these studies attain cellular resolution and focus on phosphoproteins related to ion channel function, they can contribute in an important way to the elucidation of memory storage machinery.

## 4.2. LIMAX Learning

The LIMAX program is being developed toward a more nearly complete representation of the *Limax* associative learning behavior and to better approximation of both the electrophysiology and biochemistry of real neurons.

The first major aspect of such a development is to model the cellular electrophysiology by "neurons" having a graded response (not off–on) and communicating by means of action potentials (Hopfield, 1984). This will enable us to view the spiking behavior of any LIMAX "neuron" during processing, and thus to see the electrophysiological correlates of information processing in LIMAX. Our understanding of the general principles of these networks suggests that the relevant behaviors will persist and become easier to work with in the system with graded neuronal responses.

Within the present model, it appears that relatively simple changes in the synapse modification algorithm and the cellular anatomy will be able to produce both extinction and blocking. This issue is also being pursued with neurons that spike and have graded responses.

The model so far considered has only a short-term memory (through a combination of synapse change and continued electrical activity) and long-term memory (through synapse change). The addition of an intermediate form of memory, as in a temporary modification of synapses that can later either be made permanent

or allowed to decay, seems likely to be of importance both for the long-term associations made in food learning and for a description of memory consolidation.

Such models will always be incorrect in many details. Nevertheless, the importance of having a complete model is to see how cellular rules of neuron response and synapse modification result in detailed behaviors. Any particular complete model predicts the outcome of scores of behavioral experiments on the basis of cellular suppositions. A few of these will have been used to construct the model. Others are obvious "correct" behaviors, not yet studied experimentally, and for which such a model makes detailed predictions. Finally and perhaps most interestingly, any given model will also have contrived situations for which the model predicts peculiar behavior (the associative learning analogue of perceptual illusions). The comparison of LIMAX with *Limax* in such situations should be particularly informative.

## 4.3. Conclusion

It is an exciting time in the study of memory storage mechanisms. Three specific and detailed models are available (Chapter 12, this volume; Acosta-Urquidi *et al.*, 1984; Lynch and Baudry, 1984) with different biochemical mechanisms mediating the transduction of critically timed patterns of electrical activity into enduring changes of transmitter action. The biophysical and biochemical measurements of *Limax* memory mechanisms can both test the generality of the existing models and perhaps provide important extensions of them needed to attain the more complex conditioning displayed by *Limax*.

The logic of *Limax* learning is apparent at several levels. At the behavioral level, the adaptive value of the learning mechanism is easy to visualize (Whelan, 1982; Gouyon *et al.*, 1983). In some sense *Limax* may be genetically programed to learn reliably about odors and tastes as predictors of positively or negatively rewarding consequences. The operational logic of the learning is summarized in Fig. 1, although further work may well significantly extend this list. The actual synaptic logic of *Limax* is yet to be revealed, but the power of the simple synaptic alteration rules used by LIMAX to accomplish the same tasks with fewer neurons encourages us in our search.

ACKNOWLEDGMENTS. We thank I. Cooke and K. Delaney for critical comments on the manuscript. Work done at Princeton University was supported by NIH Grant MH39160. Work done at the California Institute of Technology was supported by NSF Grant DMR-8107494.

## Appendix 1. A Neural Network for Categorization and Memory Storage

This model results from a search for emergent properties of neural networks that depend on the collective interactions of the neurons rather than localized details of their interactions. It explores what neurons having relatively simple prop-

erties and interacting according to relatively simple rules can accomplish collectively. In particular, a neural network and the algorithm for storing memories within it are described, first using neurons with only two activity states, on and off. This network also categorizes inputs to it based on its past experiences. Finally, it is shown that a network of neurons having graded activity states retains the same collective ability to form categories and store memories.

Consider a network of two-state neurons, each of which is either firing as rapidly as possible ($V_i = 1$) or is inactive ($V_i = 0$). At any time the state of the network of N such neurons can be described by a list on N 1s and 0s, representing the instantaneous activity state of each of the N neurons.

Each neuron is connected to every other neuron in the network. Each neuron $i$ receives inputs from other neurons $j$ through synapses of efficacy $T_{ij}$. The input to neuron $i$ at time $t$ is thus

$$I_i(t) = \sum_j T_{ij} V_j(t) + E_i(t)$$

where $E_i(t)$ is the input to neuron $i$ from sources external to the network, such as direct inputs from sensory neurons. The connection $T_{ij}$ can be either excitatory ($T_{ij} > 0$) or inhibitory ($T_{ij} < 0$).

Each neuron may change its activity state repeatedly as time goes on. The model ascribes to each neuron a behavior that tends to turn neuron $i$ on if its input has been above threshold ($H$) for a while and to turn it off if it has been below threshold. The detailed description of this process lets each neuron inspect its inputs at random times with a specified mean inspection rate. At each such inspection time the *ith* neuron sets

$$\begin{aligned} V_i &\rightarrow 1 \qquad \text{if } I_i > H \\ V_i &\rightarrow 0 \qquad \text{if } I_i \leq H \end{aligned}$$

Since each neuron integrates itself at random times, there is no synchrony in the times at which different neurons change state. Unlike computer processing and much neural modeling, the processing described here is asynchronous. The lag between changes in the inputs to neuron $i$ and the change of state of neuron $i$ incorporates the effects of membrane charging times, propagation delays, and synaptic delays.

Stable activity patterns (states) of the neural network can be specified by the initial values of synaptic strengths between all of the elements of the network. The appropriate values of the synaptic strengths needed to make a set of states ($V^s$; $s$ = 1,2,3, . . .) the stable states of the network are calculated using the following storage prescription

$$T_{ij} = \sum_s (2V_i^s - 1)(2V_j^s - 1)$$

The storage prescription is used to calculate the synaptic strengths to each neuron ($i$) from every other neuron ($j$) in the network necessary to made the network activ-

ity patterns embodied in states $V^1$, $V^2$, $V^3$, . . ., the stable states of the network. Having entered the appropriate matrix of synaptic strengths into the network to specify stable states $V^1$, $V^2$, $V^3$, etc., the network, started with its neurons in a random state, will converge to one of the specified stable states, specifically the stable state that most closely resembles the starting state (Hopfield, 1982). If the network is started with partial information about one of the stable states, i.e., an activity pattern that resembles but is not identical to one of the prespecified stable states, the network will converge to the stable state most like the starting state. After setting the initial synaptic strengths, the only process operating during the convergence to a stable state is the simple asynchronous input-evaluation algorithm just described. It is an intrinsic, emergent property of a network operating in this way that the network in fact converges to a stable state.

The neural network can come prewired with a matrix of synaptic strengths specifying a set of stable activity patterns, analogous to genetically specified memory states. Alternatively, the network can be equipped with an interface to the real world in the form of sensory receptors and a synapse modification algorithm that causes activity states of the network imposed by activity in the sensory receptors to become inherent stable states of the (categorizer) network.

Computer simulations of the operation of such neural networks have revealed that optimum stability requires a certain amount of global inhibition distributed throughout the network without regard to the specified stable states. Also, as more and more stable states (memories) are specified, the performance of the network during recall begins to deteriorate. About 0.15 N memories can be simultaneously stored in the network before the error in recall is severe. Finally, it was found that 30 neurons was the lower size limit for a network able to store stable activity patterns in this way.

Note that the memories in this network are represented in the collected set of synapse strengths and activity patterns of the constituent neurons. Each neuron is active in several memory states, and memory states will have some active neurons in common. Thus particular neurons and synapses cannot be identified with particular memories.

The neural network simulation has recently been extended to make the neurons more realistic and to show that the basic computational abilities of the two-state networks are not lost. Rather than two-state neurons, we used neurons with a graded (sigmoidal) input–output curve. Spike output was taken to be virtually zero with membrane potential (Vm) below a threshold and output was maximum at highly depolarized values of Vm. At intermediate values of Vm, the output was a steeply graded function of the input. Also, the neurons were endowed with membrane capacitance with which they temporally summed their synaptic inputs. When neuron $j$ in the network fires an action potential, its effect on other cells in the network is to dump charge on their input capacitance. The amount of charge (and its sign) that is placed on the input capacitance of neuron $i$ is determined by the value of $T_{ij}$. Hence the magnitude of a given $T_{ij}$ is still to be considered as a measure of synaptic strength, while the sign of $T_{ij}$ indicates if the synapse is excitatory or inhibitory. The effect of the change in the cell's charge is to change the input voltage (membrane potential). However, this change is transitory: the membrane potential decays exponentially back to its resting state with a certain time constant.

Our simulations of networks containing spiking neurons have shown that they retain the ability of the two-state networks previously described to categorize input patterns.

## References


Acosta-Urquidi, J., Alkon, D. L., and Neary, J. T., 1984, $Ca^{2+}$-dependent protein kinase injection in a photoreceptor mimics biophysical effects of associative learning, *Science* **224:**1254–1257.

Barry, S. R., and Gelperin, A., 1982a, Dietary choline augments blood choline and cholinergic transmission in the terrestrial mollusc, *Limax maximus, J. Neurophysiol.* **48:**541–547.

Barry, S. R., and Gelperin, A., 1982b, Exogenous choline augments transmission at an identified cholinergic synapse in the terrestrial mollusc *Limax maximus, J. Neurophysiol.* **48:**431–450.

Barry, S. R., and Gelperin, A., 1984, Acetylcholine turnover in an autoactive molluscan neuron, *Cell. Molec. Neurobiol.* **4:**15–29.

Beltz, B., and Kravitz, E. A., 1982, Mapping of serotonin-like immunoreactivity in the lobster nervous system, *J. Neurosci.* **3:**585–602.

Benedeczky, I., 1977, Ultrastructure of the epithelial sensory region of the lip in the snail *Helix pomatia* L, *Neuroscience* **2:**781–789.

Benjamin, P. R., 1983, Gastropod feeding: Behavioral and neural analysis of a complex multicomponent system, in: *Neural Control of Rhythmic Movements* (A. Roberts and B. L. Roberts eds.), Cambridge University Press, New York, pp. 159–193.

Blusztajn, J. K., and Wurtman, R. J., 1983, Choline and cholinergic neurons, *Science* **221:**614–620.

Chang, J. J., and Gelperin, A., 1980, Rapid taste aversion learning by an isolated molluscan central nervous system, *Proc. Natl. Acad. Sci. U.S.A.* **77:**6204–6206.

Chase, R., and Kamil, R., 1983, Neuronal elements in snail tentacles as revealed by horseradish peroxidase backfilling, *J. Neurobiol.* **14:**29–47.

Chetail, M., 1963, Etude de la regeneration du tentacule oculaire chez un arionidae *(Arion rufus)* et un Limacidae *(Agriolimax agrestis), Arch. Anat. Microsc. Morphol. Exp.* **52:**129–203.

Cooke, I., and Gelperin, A., 1984, Immunocytochemical mapping of the neural control system for feeding in *Limax maximus, Soc. Neurosci. Abstr.* **10:**691.

Croll, R., 1983, Gastropod chemoreception, Biol. Rev. **58:**293–319.

Culligan N., and Gelperin, A., 1983, One-trial associative learning by an isolated molluscan CNS: Use of different chemoreceptors for training and testing, *Brain Res.* **266:**319–327.

Davis, W. J., Gillette, R., Kovac, M. P., Croll, R. P., and Matera, E. M., 1983, Organization of synaptic inputs to paracerebral feeding command interneurons of *Pleurobranchaea californica* III. Modifications induced by experience, *J. Neurophysiol.* **49:**1557–1572.

Delaney, K., and Gelperin, A., 1983, The slug *Limax maximus* shows postingestive food aversion learning to amino acid deficient diets, *Soc. Neurosci. Abstr.* **9:**914.

Delaney, K., and Gelperin, A., 1984, Rapid food-aversion learning with shock as UCS in *Limax maximus, Soc. Neurosci. Abstr.* **10:**509.

Derby, C. D., and Ache, B. W., 1984, Quality coding of a complex odorant in an invertebrate, *J. Neurophysiol.* **51:**906–924.

Dethier, V. G., and Crnjar, R. M., 1982, Candidate codes in the gustatory system of caterpillars, *J. Gen. Physiol.* **79:**549–570.

Egan, M., and Gelperin, A., 1981, Olfactory inputs to a bursting serotonergic interneuron in a terrestrial mollusc, *J. Molluscan Stud.* **47:**80–88.

Frömming, E., 1952, Uber die Nahrung von *Limax maximus, Anz Schadlingskde* **25:**41–43.

Gain, W. A., 1891, Notes on the food of some of the British mollusks, *J. Conchol.* **6:**349–361.

Gelperin, A., 1974, Olfactory basis of homing behavior in the giant garden slug, *Limax maximus, Proc. Natl. Acad. Sci. U.S.A.* **71:**966–970.

Gelperin, A., 1975, Rapid food-aversion learning by a terrestrial mollusk, *Science* **189:**567–570.

Gelperin, A, 1981, Synaptic modulation by identified serotonin neurons, in: *Serotonin Neurotransmission and Behavior* (B. Jacobs and A. Gelperin, eds.), MIT Press, Cambridge, pp. 288–304.

Gelperin, A., 1983, Neuroethological studies of associative learning in feeding control systems, in: *Neuroethology and Behavioral Physiology* (F. Huber and H. Markl, eds.), Springer-Verlag, Berlin, pp. 189–205.

Gelperin, A., and Culligan, N., 1984, *In vitro* expression of *in vivo* learning by an isolated molluscan CNS, *Brain Res.* **304:**207–213.

Gelperin, A., Chang, J. J., and Reingold, S. C., 1978, Feeding motor program in *Limax*. I. Neuromuscular correlates and control by chemosensory input, *J. Neurobiol.* **9:**295–300.

Gouyon, P. H., Fort, Ph., and Caraux, G., 1983, Selection of seedlings of *Thymus vulgaris* by grazing slugs, *J. Ecol.* **71:**299–306.

Greenberg, M. J., Painter, S. D., Doble, K. E., Nagle, G. T., Price, D. A., and Lehman, H. K., 1983, The molluscan neurosecretory peptide FMRFamide: Comparative pharmacology and relationship to the enkephalins, *Fed. Proc.* **42:**82–86.

Hawkins, R. D., and Kandel, E. R., 1984, Is there a cell biological alphabet for learning? *Psycho. Rev.* **91:**375–391.

Hopfield, J. J., 1982, Neural networks and physical systems with emergent collective computational abilities, *Proc. Natl. Acad. Sci. USA* **79:**2554–2558.

Hopfield, J. J., 1984, Neurons with graded response have collective computational properties like those of two state neurons, *Proc. Natl. Acad. Sci. USA* **81:**3088–3092.

Hopfield, J. J., Feinstein, D. I., and Palmer, R. G., 1983, "Unlearning" has a stabilizing effect in collective memories, *Nature (London)* **304:**158–159.

Kamin, L. J., 1969, Predictability, surprise, attention, and conditioning, in: *Punishment and Aversive Behavior* (R. Church and B. A. Campbell, eds.), Appleton-Century-Crofts, New York pp. 279–296.

Kandel, E. R., and Schwartz, J. H., 1982, Molecular biology of learning: Modulation of transmitter release, *Science* **218:**433–443.

Kataoka, S., 1976, Fine structure of the epidermis of the optic tentacle in a slug, *Limax flavus* L, *Tissue Cell* **8:**47–60.

Kemenes, G., Hernádi, L., and Salánki, J., 1982, Identification of cerebral moto-neuron responding to lip stimulation in *Helix pomatia, Acta. Biol. Acad. Sci. Hung.* **33:**215–229.

Kupfermann, I., and Weiss, K. R., 1982, Activity of an identified serotonergic neuron in free moving *Aplysia* correlates with behavioral arousal, *Brain Res.* **241:**334–337.

Lynch, G., and Baudry, M., 1984, The biochemistry of memory: A new and specific hypothesis, *Science* **224:**1057–1063.

Reingold, S. C., and Gelperin, A., 1980, Feeding motor program in *Limax*. II. Modulation by sensory inputs in intact animals and isolated central nervous system, *J. Exp. Biol.* **85:**1–19.

Rescorla, R. A., 1984, Comments on three Pavlovian paradigms, in: *Primary Neural Substrates of Learning and Behavioral Change* (D. Alkon and J. Farley, eds.) Cambridge University Press, New York, pp. 25–45.

Rosen, S. C., Weiss, K. R., Cohen, J. L., and Kupfermann, I., 1982, Interganglionic cerebral-buccal mechanoafferents of *Aplysia:* Receptive fields and synaptic connections to different classes of neurons involved in feeding behavior, *J. Neurophysiol* **48:**271–288.

Rosen, S. C., Kupfermann, I., Goldstein, R. S., and Weiss, K. R., 1983, Lesion of a serotonergic modulatory neuron in *Aplysia* produces a specific defect in feeding behavior, *Brain Res.* **260:**151–155.

Sahley, C. L., Gelperin, A., and Rudy, J. W., 1981a, One-trial associative learning modifies food odor preferences of a terrestrial mollusc, *Proc. Natl. Acad. Sci. U.S.A.* **78:**640–642.

Sahley, C. L., Rudy, J. W., and Gelperin, A., 1981b, An analysis of associative learning in a terrestrial mollusc: Higher-order conditioning, blocking and a transient US pre-exposure effect, *J. Comp. Physiol.* **144:**1–8.

Sahley, C. L., Hardison, P., Hsuan, A., and Gelperin, A., 1982, Appetitively reinforced odor-conditioning modulates feeding in *Limax maximus, Soc. Neurosci. Abstr.* **8:**823.

Sahley, C. L., Rudy, J. W., and Gelperin, A., 1984, Associative learning in a mollusc: A comparative analysis, in: *Primary Neural Substrates of Learning and Behavioral Change* (D. Alkon and J. Farley, eds.) Cambridge University Press, New York, pp. 243–258.

Sahley, C. L., Barry, S. R., and Gelperin, A., 1985, Dietary choline augments associative memory function in *Limax maximus, J. Neurobiol.,* in press.

Stefano, G. B., 1982, Comparative aspects of opioid-dopamine interaction, *Cell. Mol. Neurobiol.* **2:**167–178.

Veratti, E., 1900, Ricerche sul sistema nervoso dei *Limax, Memorie Reale Instituto Lombardo Scienze Lettere* **18:**9.

Whelan, R. J., 1982, Response of slugs to unacceptable food items, *J. Appl. Ecol.* **19:**79–87.

Wieland, S. J., and Gelperin, A., 1983, Dopamine elicits feeding motor program in *Limax maximus, J. Neurosci.* **3:**1735–1745.

Wieland, S. J., Zaininger, H., Jahn, E. G., and Gelperin, A., 1983, Dopamine and serotonin in *Limax* feeding: Distribution and metabolism, *Soc. Neurosci. Abstr.* **9:**75.

Wieland, S. J., Jahn, E. G., and Gelperin, A., 1984, Measurement and control of dopamine and serotonin release from *Limax* ganglia *in vitro, Soc. Neurosci. Abstr.* **10:**690.

Zs-Nagy, I., and Sakharov, D. A., 1970, The fine structure of the procerebrum of pulmonate molluscs, *Helix* and *Limax, Tissue Cell* **2:**399–411.