fungi (*7*), and as a model of the latter condition, one can visualize the loop that forms when two points on a length of hose or rope are grasped and twisted in opposite directions (*8*). It is evident from the timing of bud formation by *A. dactyloides* and from the alignment and union of buds with growing tips of the curled hyphae to form traps, that the causes and levels of control responsible for morphogenesis are numerous and complex.

Ring closure is induced by touch and by increased temperature. It is extremely fast, requiring less than 0.1 second, and, on inflation, the cell volume more than triples (Fig. 1H). Under natural conditions, rings constrict when nematodes move into their openings. In the laboratory, micromanipulation with a fine needle can be substituted for prey, but application of water at 50°C, as described by Muller (*9*), is the simplest means of inducing ring constriction. We were successful in triggering closure of rings electrically, but activity was localized to the area immediately surrounding the cathodic microelectrode. The mechanism of cell inflation has not been adequately explained. Touch and temperature somehow initiate an irreversible change in wall structure, decreasing wall pressure and increasing permeability of the cell to water. Plasmolytic estimates of the osmotic potential of ring cells before and after inflation indicate that the concentration of solute in stimulated cells must triple (*9*), but the timing and rate of this process and the nature and origin of the osmotically active material are not known as yet.

In order to determine whether inflation is metabolically linked and requires an expenditure of energy on the part of the cell, *A. dactyloides* was grown on the surface of cornmeal extract agar in petri dishes and treated at *p*H 6.0 with solutions of iodoacetate, mercuric chloride, sodium azide, and sodium cyanide at concentrations ranging from 10^{-1} to $10^{-4}M$. After being exposed to the inhibitors for 15 minutes, ring closure was induced electrically. Cells that had been treated with iodoacetate, mercuric chloride, and sodium cyanide at 10^{-1} and $10^{-2}M$ failed to inflate. However, more dilute solutions of these compounds were inactive, and the system was not influenced adversely by $10^{-2}M$ or less of sodium azide. The fact that inflation was resistant to catalytic quantities of various metabolic inhibitors indicates that it is a passive rather than an active process and supports the suggestion that physicochemical phenomena, including changes in osmotic potential, and not energy requiring biochemical reactions, are operative in ring closure.

M. L. HIGGINS
DAVID PRAMER
Department of Biochemistry and Microbiology, College of Agriculture and Environmental Science, Rutgers–The State University, New Brunswick, New Jersey

References and Notes

1. W. J. Nickerson, Ed., *Biochemistry of Morphogenesis* (Pergamon, New York, 1959); E. C. Cantino, in *Microbial Reaction to Environment*, G. G. Meynell and H. Gooder, Eds. (Cambridge, New York, 1961), p. 243; S. Bartnicki-Garcia, *Bacteriol. Rev.* **27**, 293 (1963); M. Sussman, *Ann. Rev. Microbiol.* **19**, 59 (1965); B. E. Wright, *Science* **153**, 830 (1966).
2. D. Pramer and S. Kuyama, *Bacteriol. Rev.* **27**, 282 (1963); D. Pramer, *Science* **144**, 382 (1964).
3. Our film "Carnivorous fungi" is available on loan from R. W. Sarber, Executive Secretary, American Society for Microbiology, 115 Huron View Blvd., Ann Arbor, Mich.
4. M. L. Higgins and J. K. G. Silvey, *Trans. Amer. Microscop. Soc.* **85**, 390 (1966).
5. Sage Instruments, Inc., White Plains, N.Y.
6. C. Drechsler, *Mycologia* **29**, 447 (1937).
7. W. Shropshire, *Physiol. Rev.* **43**, 38 (1963); K. M. Hartmann, H. Menzel, H. Mohr, *Planta* **64**, 363 (1965).
8. We are indebted to Dr. S. Bartnicki-Garcia for first suggesting this analogy.
9. H. G. Muller, *Trans. Brit. Mycol. Soc.* **41**, 341 (1958).
10. Paper of the Journal Series, New Jersey Agricultural Experiment Station, Rutgers–The State University, New Brunswick. Supported in part by NSF grants G19211 and G15950.

10 October 1966

Opposite Synaptic Actions Mediated by Different Branches of an Identifiable Interneuron in Aplysia

Abstract. *Among the identifiable cells in the abdominal ganglion of* Aplysia californica *are five that generate bursting rhythms endogenous to the cells. In the four bursting cells of the left upper quadrant the rhythm is modulated by a unitary inhibitory postsynaptic potential; in the bursting cell of the right lower quadrant the rhythm is modulated by a unitary excitatory postsynaptic potential. Both the excitatory and inhibitory postsynaptic potentials are mediated by separate branches of a single interneuron. The pharmacological properties of the double action interneuron as well as those of the follower cells suggest that a single transmitter (acetylcholine) is involved in both the excitatory and the inhibitory action of the interneuron.*

Certain chemical transmitter substances can produce different actions at different synapses. For example, acetylcholine (ACh) mediates excitation at the vertebrate neuromuscular junction and inhibition at the sinoauricular node of the vertebrate heart (*1*). In the marine mollusc *Aplysia* ACh appears to be, in the same ganglion, the excitatory synaptic transmitter on certain cells (D cells) and the inhibitory synaptic transmitter on other (H cells) (*2*). These findings indicate that the nature of synaptic transmission is determined not only by the chemical structure of the transmitter substance but also by the properties of the postsynaptic cell (*2, 3*). It is therefore theoretically possible for a single interneuron to produce opposite synaptic actions with the same transmitter via different branches on different follower cells. One cell would then serve as an inhibitory interneuron for some cells and as an excitatory interneuron for others. Although such interneurons have been postulated (*2*) and their presence inferred from indirect data (*4*), no such interneuron has yet been described.

In the course of studying direct and common connections among identifiable cells in the abdominal ganglion of *Aplysia californica*, we have encountered a cell that mediates inhibition to some cells in the ipsilateral hemiganglion and excitation to some cells in the contralateral hemiganglion. We now report some properties of this interneuron and of some of the cells to which it is synaptically connected (*5*).

Within the abdominal ganglion of *Aplysia californica* there are 30 cells that can be identified on the basis of a number of physiological and morphological criteria (*6*). Figure 1A illustrates the position of the 19 identifiable cells on the dorsal surface. The five cells whose connections we will describe are stippled on the drawing. These cells appear to form a distinct functional group since they are the only identifiable cells to show a regular bursting rhythm and since they all send their efferent axon into the pericardial branch of the genital nerve (*6*).

There is good evidence that in all five neurons the bursting rhythm is

endogenous to the cell and is not dependent on afferent input (*7*). However, these cells do receive intermittent synaptic bombardment from interneurons, and this input can modulate the natural pacemaker rhythm (*6, 8*). In the isolated and unstimulated ganglion, the main synaptic modulating effect on the four bursting cells in the left upper quadrant is a unitary inhibitory postsynaptic potential (IPSP). By recording (with intracellular microelectrodes) two at a time from these four cells we could show that the IPSP's were synchronous; this suggests that each cell received an inhibitory branch from a common interneuron (Fig. 1B). One of the several modulating synaptic inputs on the bursting cell in the right lower quadrant was a unitary excitatory postsynaptic potential (EPSP). Simultaneous recordings from this cell (R_{15}) and one of the cells in the left upper quadrant (L_3) showed that the EPSP's in R_{15} and the IPSP's in the cells of the left upper quadrant were produced synchronously, suggesting that they are mediated by different branches of the same interneuron (Fig. 1C). Since the synchronous postsynaptic potentials in L_3 and R_{15} are of opposite sign, there is a tendency for them to shift the phase of the bursting rhythm of the two follower cells by 180 degrees.

Synchronous postsynaptic potentials of opposite signs can be seen among other cells in the ganglion (*8*). One such situation was previously encountered by Strumwasser (*4*) who suggested that these opposite synaptic actions were mediated by different branches of a single interneuron. A direct test of the hypothesis requires that the interneuron mediating these synaptic actions be identified and that its direct connections with the follower cells be established by both anatomical and electrophysiological methods.

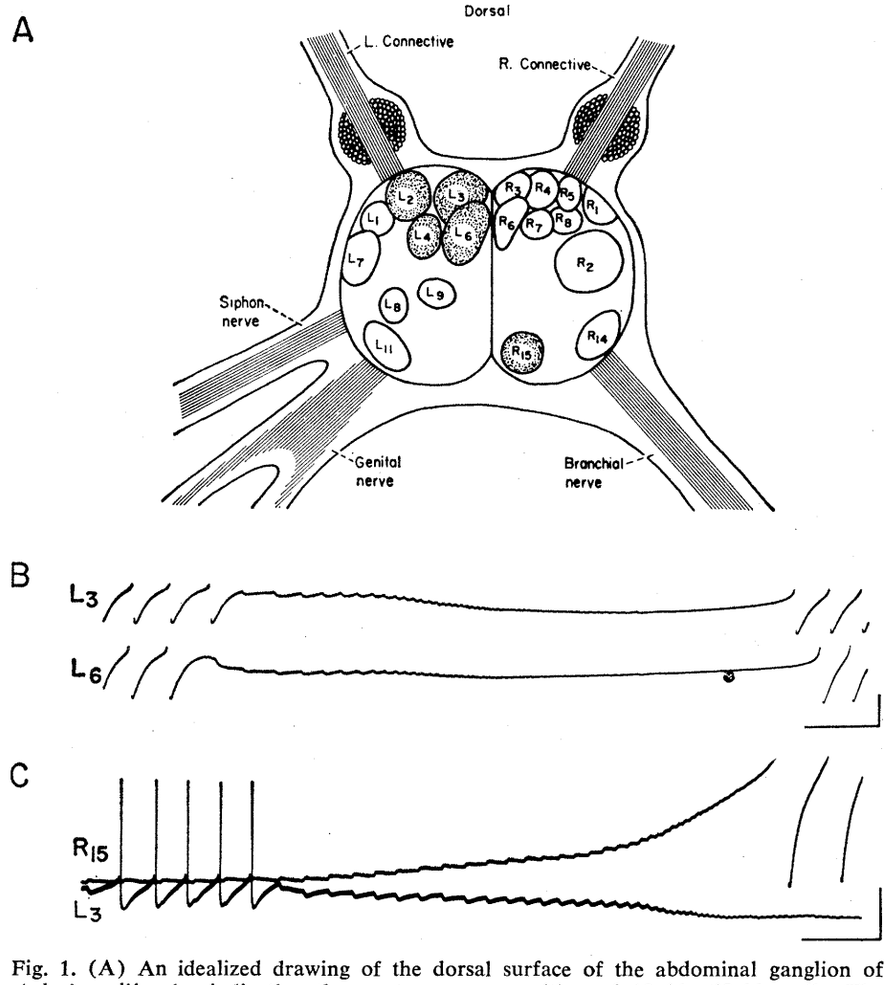

Fig. 1. (A) An idealized drawing of the dorsal surface of the abdominal ganglion of *Aplysia californica* indicating the most common position of 19 identifiable cells. The five bursting cells of the ganglion L_2, L_3, L_4, L_6, and R_{15} are stippled. (B) Simultaneous intracellular recordings from L_3 and L_6, two bursting cells of the left upper quadrant, illustrating synchronous IPSP's in both cells. The IPSP's tend to bring the follower cells into a synchronous beat. Voltage calibration is 15 mv; time calibration is 2.5 seconds. (C) Simultaneous intracellular recordings from a bursting cell of the left upper quadrant (L_3) in the lower trace and of one from the right lower quadrant (R_{15}) in the upper trace. The EPSP in R_{15} and the IPSP on L_3 are synchronous and tend to pull the bursting rhythm of the follower cells out of phase. Voltage calibration is 7.5 mv for the upper trace and 30 mv in the lower trace. The time calibration is 2.5 sec.

By searching among the cells of the ganglion, we have found the interneuron that produces the IPSP's in each of the four bursting cells of the left upper quadrant and the EPSP's in the bursting cell of the right lower quadrant. Figure 2 is a simultaneous recording from the interneuron and two follower cells. Action potentials initiated by intracellular stimulation of the interneuron invariably produced EPSP's in one follower cell (L_3) and IPSP's in the other (R_{15}). In Fig. 2A, the membrane potentials of follower cells are at the resting level, and the IPSP is hyperpolarizing. In Fig. 2B follower cells are hyperpolarized about 10 to 15 mv. The EPSP remained essentially unchanged, but the IPSP, as expected, inverted to a depolarizing postsynaptic potential.

In both parts of Fig. 2 a number of oscilloscopic traces have been superimposed to illustrate that the postsynaptic potentials in the follower cells will follow the action potential of the interneuron with constant and short latency and in an all-or-none manner. These data are consistent with there being unitary monosynaptic connections between the interneuron and the follower cells. We have obtained anatomical confirmation of monosynaptic connections by tracing processes of the interneuron, in serial sections examined with the light microscope, to a point where they make direct contact with each of the five follower cells.

The question naturally arising at this point is whether the EPSP's and IPSP's recorded from the follower cells are mediated by different transmitters released by the interneuron at the different terminals, or whether, in agreement with Dale's principle (*9*), the same transmitter is released at all terminals but has opposite actions on the different postsynaptic cells.

Available evidence from the work of Tauc and Gerschenfeld (*2*) favored the notion that it was the same transmitter, namely acetylcholine. To test this possibility, we injected ACh iontophoretically on each of the follower cells by means of an external microelectrode placed on the surface of the cell body (Fig. 3, A and B). Acetylcholine caused a depolarization of R_{15},

thus simulating the excitatory effect of the interneuron on this cell (Fig. 3A); it caused hyperpolarization and inhibition of L_3, again simulating the effect of the interneuron on this cell (Fig. 3B).

To test further the hypothesis that the same transmitter, ACh, mediated these opposite effects, we bathed the ganglion in curare to see whether we could block the natural transmitter released by both branches. Figure 3C illustrates simultaneous recordings from the interneuron and two follower cells (L_3 and R_{15}), which receive inhibitory and excitatory postsynaptic potentials respectively. Both the EPSP and the IPSP were blocked by *d*-tubocurarine.

Our results, based upon the identification of an interneuron and its follower cells, provide evidence for the existence of an interneuron that can produce opposite synaptic actions in different follower cells. Morphological evidence shows that the interneuron makes direct contact with the follower cells, electrophysiological evidence demonstrates opposite synaptic actions mediated by the same interneuron on different identified follower cells, and pharmacological evidence suggests that each branch of the interneuron releases the same transmitter, acetylcholine, which produces opposite synaptic effects on the appropriate follower cells.

An obvious functional consequence of such an arrangement is economy of number, which may be particularly important in the central nervous system of *Aplysia* where the total number of neurons is small (about 10^4).

A comparison of these results in *Aplysia* with those from the two types of vertebrate cells (the spinal motoneuron and the cerebellar Purkinje cell), in which the synaptic actions of different branches have been examined, is interesting. In each of these cells only a single action has been demonstrated (*9*). For example, in the motoneuron, a cholinergic cell, both the peripheral (neuromuscular) and central (collateral-Renshaw cell) terminals release ACh, but the transmitter produces only excitation at both junctions. Recurrent inhibition is mediated by a specialized, noncholinergic inhibitory interneuron (*9*). As a result of these findings, Eccles has proposed that vertebrate neurons invariably produce only a single action and that cells are specialized for either inhibition or excitation (*9*). Although it is possible that double action neurons are limited to invertebrates (*5*), the number of instances in which a vertebrate neuron has been shown to mediate only a single action is small. Consequently, the alternative and more economical mode of operation provided by interneurons with a double action may yet be found in the vertebrate brain.

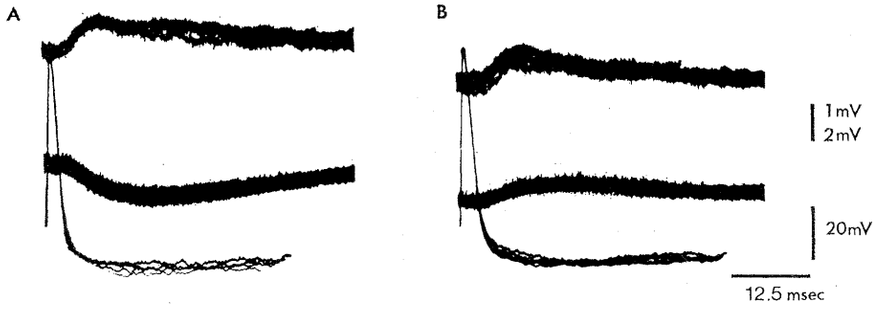

Fig. 2. Intracellular recordings from the interneuron (lower trace) and from two bursting cells, R_{15} (upper trace) and L_3 (middle trace). The interneuron was stimulated intracellularly, and its action potential was used to trigger the oscilloscope time base. Several sweeps were superimposed. The action potential in the interneuron produces an EPSP in R_{15} and IPSP's in L_3. Both postsynaptic potentials are all-or-none and of constant latency. In A the membrane potential of the follower cells is at the resting level. In B the membrane potential of the two follower cells has been hyperpolarized causing the hyperpolarizing postsynaptic potential of L_3 to be inverted to a depolarizing postsynaptic potential.

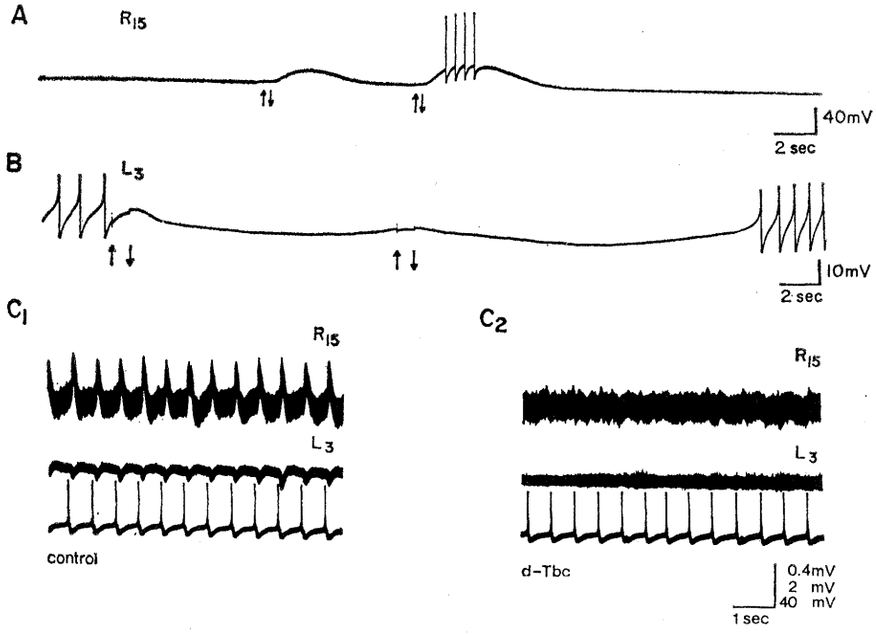

Fig. 3. (A and B) Responses of R_{15} and L_3 to iontophoretic application of ACh to the synapse-free cell body by means of an external micropipette. The duration of the Ach pulse is indicated by the arrows. The second pulse in A is stronger than the first. Cell R_{15} depolarizes, and L_3 hyperpolarizes in response to Ach simulating, in each case, the sign of the synaptic potential produced in these cells by the interneuron. (C) Simultaneous recordings from interneuron (bottom trace) and from R_{15} and L_3. C_1, control; C_2, 5 minutes after application of *d*-tubocurarine (d-Tbc, 10^{-4} g/ml), which blocked both postsynaptic potentials. Capacitatively coupled recordings in upper two traces (time constant, 0.1 second).

E. R. KANDEL
W. T. FRAZIER*
Departments of Physiology and *Psychiatry, New York University Medical School, New York*
R. E. COGGESHALL
Department of Anatomy, Harvard Medical School, Cambridge, Massachusetts

References and Notes

1. J. del Castillo and B. Katz, *J. Physiol.* **125**, 546 (1954); *Nature* **175**, 1035 (1955).
2. L. Tauc and H. M. Gerschenfeld, *Nature* **192**, 366 (1961).
3. H. Grundfest, *Physiol. Rev.* **37**, 337 (1957).
4. F. Strumwasser, *Proc. Intern. Union Physiol. Sci. Intern. Congr.* 22nd, Leiden, 1962, vol. 2, No. 801 (1962).
5. We have recently learned of the discovery of a double-action neuron in the last abdominal ganglion of the lobster (M. Otsuka, E. A. Kravitz, D. D. Potter, personal communication). In this ganglion the inhibitory neurons to muscle are electrically coupled

with the symmetrical inhibitory neuron on the other side. These cells thus mediate chemical inhibition to muscle (by the release of γ-aminobutyric acid) and electrical excitation to the contralateral inhibitory interneuron.
6. W. T. Frazier, E. R. Kandel, I. Kupfermann, R. Waziri, R. E. Coggeshall, in preparation.
7. F. Strumwasser, in *Circadian Clocks*, J. Aschoff, Ed. (North-Holland, Amsterdam, 1965), pp. 442–62; R. Waziri, W. T. Frazier, E. R. Kandel, *Physiologist* **8**, 190 (1965); B. Alving, in preparation.
8. E. R. Kandel, W. T. Frazier, R. Waziri, R. E. Coggeshall, in preparation.
9. J. C. Eccles *The Physiology of Synapses* (Springer-Verlag, Berlin, 1964); ———, R. Llinas, K. Sasaki, *J. Physiol.* **182**, 316 (1966).
10. This research was supported by NIH grants NB-04550-03 and NB-05980-01, and by NSF grant NB-3595. We thank Susan Smith for technical assistance.
* Present address: Yerkes Regional Primate Research Center, Emory University, Atlanta, Georgia.

14 November 1966

Lateral Hypothalamus: Hoarding Behavior Elicited by Electrical Stimulation

Abstract. *Electrical stimulation of those points in the lateral hypothalamic area of the brain that promote feeding, but not of other points, elicited intense hoarding activity in satiated rats, similar to that produced by long-term food deprivation. This result suggests that hoarding of food is organized by a hypothalamic drive mechanism sensitive to the effects of long-term nutritional depletion.*

Laboratory rats will not hoard food which is continuously available, but if they are placed on intermittent deprivation schedules for some days they will then begin to hoard at least as much while satiated as when they are hungry (*1, 2*). Since short-term hunger is thus neither a sufficient nor a necessary condition for hoarding to occur, the hoarding of food was originally ascribed to the cumulative effects of "bodily depletion" as opposed to short-term deprivation (*1*). This explanation anticipates the distinction made in recent years between the long-term and short-term regulation of food intake (*3*), the former process presumably being based on nutritional status; the latter, on the temporarily satiating effect of the act of food ingestion. Other explanations of hoarding invoke instinctive processes not directly related to physiological drive (*4*), or ascribe it to nonspecific arousal associated with drive states in general (*5*). These explanations were tested in an experiment in which electrical stimulation was administered to a hypothalamic area of the brain controlling the hunger drive (*6*).

The cages used for the observation of hoarding each contained a partially enclosed home area with nesting materials, and a water bottle. For 10 minutes daily, 100 pellets (1.8 g each) and an equal number of similar wooden blocks were presented in the unenclosed part of the cage, and hoarding scores were obtained by counting the number of food pellets carried into the home area during this time. Daily records were made of body weights, and of the time spent eating during trials. Electrode leads were carried by the rats during all trials, regardless of whether or not electrical stimulation was to be administered.

The subjects used were ten adult male rats with 0.01-inch (0.0254-cm) nichrome electrodes, insulated to within 0.5 mm of the tips, permanently implanted in the lateral hypothalamus (de Groot coordinates A5-A6, 1.5, 2.5). All subjects showed high response rates in postoperative tests for self-stimulation. One animal died during the experiment and two others were rejected because they failed to hoard. The seven remaining animals included four experimental subjects which showed immediate eating in response to continuous hypothalamic stimulation, and three subjects which failed to eat and served as controls.

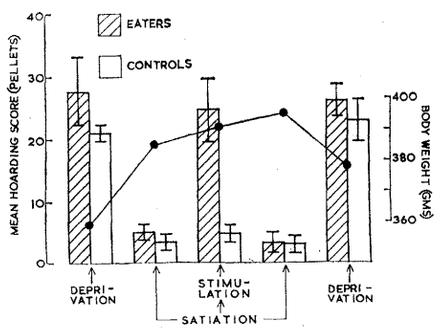

Fig. 1. Hoarding scores and body weights obtained from eating and noneating groups under five conditions. Results shown under each condition represent the combined means of six consecutive measures from each subject. The small bars represent standard errors of the means.

All subjects were given a single hoarding trial once a day for five 6-day periods; experimental conditions were changed for each period to give the sequence deprivation, satiation, satiation plus hypothalamic stimulation, satiation, deprivation. Up to 3 unscored days were allowed after changes in dietary schedule to allow body weights and hoarding scores to stabilize.

Under the deprivation conditions, all pellets were removed from the cages 16 hours before each trial, and at the end of each trial the accumulated hoard was adjusted to 20 pellets and left in the cage for 8 hours. In satiation trials the same procedure was followed, but a wire-mesh hopper was continuously present in the unenclosed part of the cage, so that pellets could be nibbled at any time but not removed by the rat. During stimulation trials, a continuous 50-cy/sec hypothalamic stimulus was administered at an intensity approximately two-thirds of the minimum current that had been found to maintain self-stimulation. This intensity produced stimulus-bound eating in the experimental group, but only behavioral arousal and exploration in the control group.

The mean hoarding scores and the changes in body weight recorded in the two groups during the five experimental periods are summarized in Fig. 1. Hoarding scores in both groups averaged less than six pellets per trial during satiation, but showed a fivefold increase after 2 to 3 days' partial deprivation had produced a 5 to 7 percent loss of body weight.

Hypothalamic stimulation had no significant effect on hoarding by the control group: the stimulation scores were not significantly different from the scores recorded during satiation. But the corresponding scores for the experimental group stood in sharp contrast: stimulation during satiation led to immediate and sustained hoarding at a level quite as high as during the deprivation conditions at the beginning and end of the series. This effect was highly significant for each subject (Mann-Whitney $U \leq 1.0$, $p < .001$), and it was not simply a matter of the stimulated animals fetching pellets for immediate consumption: the experimental subjects spent nearly half of each stimulation trial transporting the pellets, and in the remaining time they were able to eat no more than two pellets (3.6 g), far less than they collected. Nevertheless, the hoarding was clearly food-oriented, since the