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.
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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.

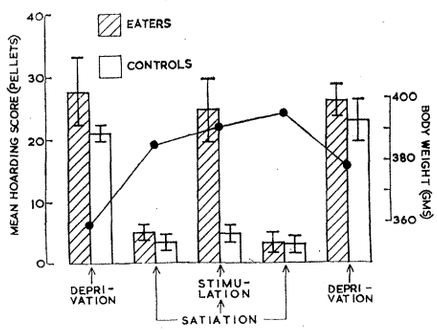

Fig. 1. Hoarding scores and body weights obtained from eating and noneating groups under five conditions. Results shown under each condition represent the combined means of six consecutive measures from each subject. The small bars represent standard errors of the means.

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

ratio of indigestible wooden blocks to pellets retrieved during stimulation was only 0.83 percent, a figure not significantly different from the proportion retrieved during deprivation (0.54 percent).

This experiment indicates that hoarding of food can be produced by electrical stimulation of those drive mechanisms in the hypothalamus which normally give rise to eating. Three theories of hoarding have been briefly referred to. The present result is evidence against a mechanism unrelated to physiological drive; it is also evidence against a nonspecific arousal effect manifesting as increased hoarding, since the control group showed exploratory behavior and other signs of response to stimulation, but no increase in hoarding. The result supports the supposition, implicit in the depletion hypothesis, that hoarding is brought about by a mechanism concerned in the regulation of body weight (*1, 2*). The experiment also throws light on how this mechanism, the lateral hypothalamic feeding area, monitors the nutritional requirements of the body. It has been suggested that the normal activity of the feeding area is spontaneous in origin and subject only to a braking action exerted by the hypothalamic ventromedial nucleus, the latter, in turn, being activated by the ingestion of food (*7*). However, since food ingestion inhibits further feeding but not hoarding (*1, 2*), it appears that the ventromedial nucleus acts only on certain efferent pathways from the lateral hypothalamus that subserve feeding, not on the lateral hypothalamus as a whole. This conclusion is consistent with duplex theories of hunger regulation which distinguish two separate regulatory mechanisms: a short-term control by the ventromedial nucleus, and an independently varying long-term regulation mediated by a proposed chemoreceptive mechanism in the lateral hypothalamus (*8*). The lateral hypothalamic mechanism, in responding to slow changes in metabolic demand, appears to be responsible both for the occurrence of eating in the nonsatiated animal, and regardless of activity in the ventromedial nucleus, for the motivation of hoarding.

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Mechanism of Delayed Reactions

Abstract. *Arsanilic acid conjugates of polymers of* L-*typrosine, glutamic acid, and alanine are immunogenic and can elicit hapten-specific, delayed-hypersensitivity reactions in sensitized guinea pigs. Conjugates of the* D-*amino acid polymers are neither immunogenic nor capable of eliciting delayed reactions. Mixtures of small amounts of conjugates capable of eliciting a delayed reaction with larger amounts of* D-*amino acid polymer conjugates produce only small delayed reactions. I suggest that the delayed reaction is an active response requiring the continued participation of immunogenic material in sensitized animals; it is not the reaction of preformed antibody-like material with the antigenic determinant.*

The discovery of hapten-specific delayed hypersensitivity produced by conjugates of arsanilic acid (*1*) has provided a useful model system for comparing various aspects of delayed sensitivity and antibody synthesis and reaction. In studies concerned with specificity it was found that, while guinea pigs could be sensitized with certain conjugates of arsanilic acid and poorly antigenic carriers, the delayed reactions could be elicited with virtually any conjugate (*2*).

These findings suggested that, as with antibody, the specificity of these reactions was directed toward the azobenzenearsonate group, which was effective on almost any unrelated carrier. In the course of these studies, several polymers of D-amino acids became available; their conjugates were therefore compared with those made from the corresponding L-amino acids regarding their ability to sensitize and elicit delayed reactions. The work I now report confirms other results that indicate that the conjugates of D-amino acid polymers are not antigenic (*3, 4*) when administered alone in Freund adjuvant.

Since such conjugates of D-amino acid polymers can elicit anaphylactic reactions with antibody (*3*), their failure to elicit delayed reactions in sensitized animals suggests that delayed hypersensitivity is not a passive reaction of the appropriate antigenic determinant with a preformed sensitizing moiety, akin to antibody reactions, but that it entails the active participation of a conjugate that is per se antigenic, much as in a secondary antibody response.

The conjugates used by me were prepared and purified, in a manner described (*1*), by coupling, at *p*H 8 to 9, overnight with diazotized arsanilic acid and by precipitation in acid. Conjugation was in the proportion of 10^{-5} mole of arsanilic acid per 10 mg of carrier. Samples of poly-L- and poly-D-glutamic-alanine-tyrosine (poly-L-GAT and poly-D-GAT) were donated by Paul Maurer, poly-D-tyrosine (poly-D-T) and poly-L- and poly-D-glutamic tyrosine (poly-L-GT and poly-D-GT) were donated by Michael Sela, and poly-L-tyrosine (poly-L-T) was purchased from New England Nuclear Corporation, Bedford, Massachusetts.

White, male, 400-g guinea pigs were injected in the four foot pads, each with a total of 0.1 ml of complete adjuvant containing 10^{-6} mole of the azobenzenearsonate conjugate of either D- or L-*N*-acetyltyrosine (ABA-tyr). Two weeks later they were shaved, depilated, and tested with the appropriate antigens injected intradermally in 0.1 ml of saline. Skin sites were examined after 3 hours for evidence of Arthus reaction and again after 24 hours for delayed reactions.

Previous study (*5*) had shown that guinea pigs immunized with either D- or L-ABA-tyr monomer uniformly developed delayed sensitivity to conjugates of guinea pig–serum albumin or poly-L-GAT, but not to poly-D-GAT. In order to ensure that the failure to produce skin reactivity with the D-polymer conjugate was not an artifact due to insufficient coupling or poor solubility, the experiment was repeated and enlarged to study the development of hapten-specific delayed sensitivity with several sets of D- and