Consequently, the marked increase in hippocampal activity that develops early in training is dependent only upon the paired CS-UCS conditioning procedure. Since it develops within a very few trials of training, it is likely to be the earliest, or certainly one of the earliest, neuronal indications that learning is occurring. In this sense, it might be considered an initial process in the formation of the "engram." This rapidly developing hippocampal activity is reminiscent of short-term or "primary" memory in human information processing theories (*8*), and is suggestive of various mnemonic functions hypothesized for the hippocampal formation (*9*).

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19 January 1976

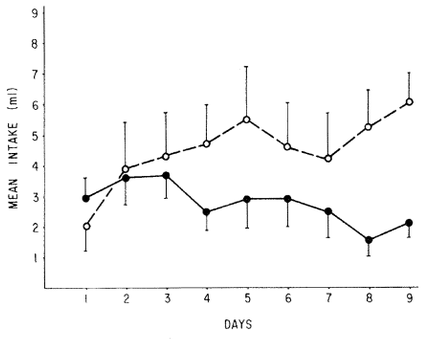
Rapid Discrimination of Rewarding Nutrient by the Upper Gastrointestinal Tract

Abstract. *When certain nutrients are injected into the stomachs of rats that are drinking one of two samples of nonnutrient, flavored water, the rats will (within a 10-minute session) choose the flavor paired with the nutrient. Such rewarding effects are obtained with predigested milk but not with similarly treated glucose or fresh milk. The results suggest the presence of rapidly acting, specialized, nutrient receptors in the upper gastrointestinal tract.*

It is not yet known how animals recognize some substances as food. Some investigators have suggested, by analogy with the work of Garcia *et al.* (*1*) on conditioned aversion, that the long-term beneficial aftereffects of a substance become conditioned to its taste (*2*). However, others, such as Gibbs *et al.* (*3*) and Snowdon (*4*), have postulated the existence of physiological mechanisms in the upper gastrointestinal tract, which signal the presence of food, presumably without an intervening process of learning. With the exception of an early experiment by Miller and Kessen (*5*), on the interpretation of which Holman (*6*) has cast doubt, support for the second view has been confined to work which shows reduction of intake after the injection of some nutrient into the upper gastrointestinal tract. However, we have shown that the injection of a palatable nutrient into the stomach of rats (1 ml of sesame oil) leads to a strong conditioned aversion to the fluid being drunk before the injection (*7*). Similarly, the injection of 1*M* glucose into the duodenum (0.6 ml/min, 3 ml total volume) also leads to a conditioned aversion to fluid drunk by rats just before the injection (*7*). The reduced intake after gastric injection may not be due to detection of nutrient by the gut as has been believed, but to some other cause. In Holman's own work (*6*) drinking of a flavored liquid was followed by an injection of nutrient into the stomach, and a preference for the flavored fluid developed. However, as trials were spaced 24 hours apart, a taste preference based on the long-term beneficial aftereffects of the nutrient may have accounted for the results; Holman did, in fact, interpret his findings in terms of such a hypothesis.

To provide a more stringent test of the hypothesis that the upper gastrointestinal tract immediately recognizes food, we gave rats a choice between two nonnutrient flavors. As the animal drank one of the flavored liquids, nutrient was pumped into its stomach through an implanted tube at the same rate as it drank. When the rat drank the other flavor, no nutrient was injected. Each daily session lasted 10 minutes. A successful choice of the liquid paired with the nutrient could then be made on only fairly immediate consequences of the arrival of nutrient in the upper gastrointestinal tract, especially because the rats almost invariably sample both flavors during the initial sessions. Such a choice does occur, but only when the injected nutrient has been digestively modified.

In the first experiment we implanted a Silastic tube in the stomachs of eight albino rats (300 to 350 g, male, Sprague-Dawley). They were given 2 weeks to recover and 1 week to become accustomed to a 22½-hour food and water deprivation schedule. They were then given a choice between two nozzles containing flavored water, one banana (0.5 percent Schilling banana flavoring) and the other almond (0.5 percent Schilling almond flavoring). When four of the rats drank the almond-flavored water, whole milk was injected through a long plastic connector into the stomach at the same rate and volume as they drank. When they drank banana-flavored water, nothing was injected. For the other four rats, the pairing between flavor and milk was reversed. There were nine daily 10-minute experimental sessions. In a second experiment, another eight rats had two Silastic tubes implanted into the stomach. The identical experiment was then



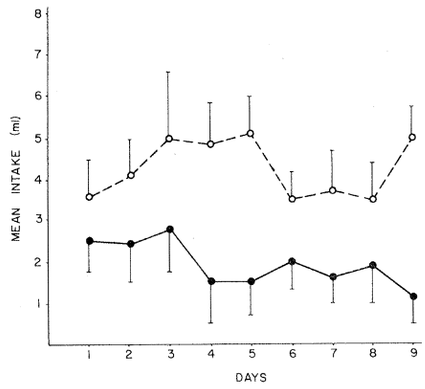


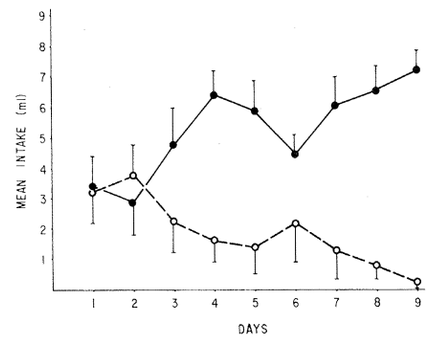
Fig. 1 (left). Mean amounts of flavored water drunk by rats on successive days of the first experiment ($N = 8$). Solid circles represent the mean amount of flavored water drunk paired with an equal simultaneous injection of whole milk. Open circles represent the mean amount drunk of differently flavored water that was unpaired with any injection. In all figures, the bars represent the standard error of the mean. Fig. 2 (right). Mean amounts of flavored water drunk by rats on successive days of the second experiment ($N = 8$). Solid circles represent the mean amount of milk-paired flavored water drunk. Open circles represent the mean amount of saline-paired flavored water that was drunk.

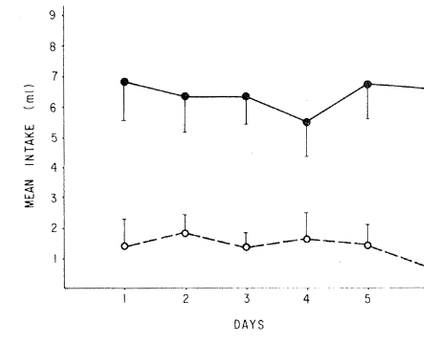
carried out except that one flavored liquid was paired with physiological saline (Tis-u-sol), while the other flavor was still paired with whole milk. The rates at which the milk and the saline were injected were the same as those at which the rats drank. The final volumes were also the same. The results of the two experiments were subjected to a three-way analysis of variance. There was no preference for the milk-paired flavor when the other flavor was either paired with nothing (Fig. 1) or with saline (Fig. 2) [$F(1, 14) = 3.94, P > .05$]. In fact, there is a nonsignificant overall tendency to prefer saline or nothing. Whether saline is injected or nothing is injected in the control condition makes no difference to the amount of milk-paired flavor chosen [$F(1, 14) = 0.08, P > .05$]. This indicates that stomach distention has little, if any, effect in producing reward.

The third experiment with eight new rats was in all respects the same as experiment 1, except that the whole milk injected had already been drunk by a donor rat. Just before the experimental session, the donor was allowed to drink milk to satiety. The milk was then extracted with a syringe from the donor's stomach through a Silastic tube implanted in the stomach and cooled to room temperature. The milk–digestive juice mixture was then injected in the same way as in the first experiment. An overwhelming preference for the nutrient-paired flavor became manifest [$F(1, 7) = 18.0, P < .01$] after 2 days of indifference [$F(8, 56) = 3.2, P < .01$] (Fig. 3). It could be objected that this preference was due to some rewarding consequence of greater stomach distention, although the results of experiments 1 and 2 make this unlikely. We hypothesized that the apparent indifference shown on the first 2 days was actually a partial avoidance that was due to the novelty of the stomach injection paired with one of the flavors.

Accordingly, in the fourth experiment, we paired an injection of liquid when the rat drank both the flavors. In order to produce no confounding of the two ingested liquids, we implanted two Silastic tubes into the stomach of the next eight rats. We continued to pair the digestive juice–milk mixture with one of the flavors. Paired with the other flavor was injected physiological saline (Tis-u-sol) matched in volume, rate of injection, and temperature. Our procedure was exactly the same as in the second experiment. The results are shown in Fig. 4. There was an overwhelming preference for the flavor paired with the nutrient solution [$F(1, 7) = 15.1, \alpha < .01$]. Even in the first 10-minute session, seven of the eight rats drank more nutrient-paired flavored water. The partially digested milk might be more rapidly absorbed than fresh milk, or its regurgitation and, therefore, taste might lead to the observed preference.

We performed an analogous experiment with glucose to determine if all food constituents had intragastric reinforcing properties. We injected 1M glucose mixed with digestive juice into the stomach of rats if they drank one of two flavored solutions. The design was similar to that of experiment 3, and eight new rats were used as recipients and four as donors. The differences were that the donor rats drank 1M glucose and that the recipients underwent a period of 6 days habituation to stomach injections, in which 1 ml of physiological saline was injected into the stomach when they drank water. During the experiment four of the





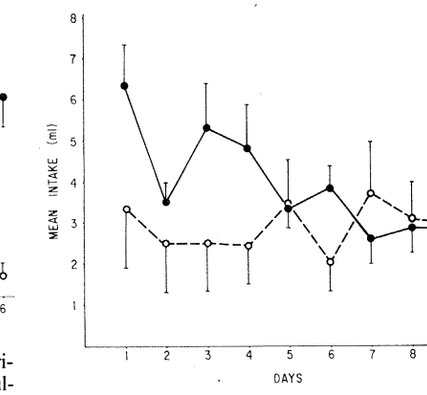


Fig. 3 (left). Mean amounts of flavored water drunk by rats on successive days of the third experiment ($N = 8$). Solid circles represent the total intake of flavored water paired with an equal simultaneous injection of nutrient (digestive juice–milk mixture). Open circles represent the total intake of water (flavored differently) that was unpaired with any injection. Fig. 4 (middle). Mean amounts of flavored water drunk by rats on successive days of the fourth experiment ($N = 8$). Solid circles represent the total intake of nutrient-paired flavored water (digestive juice–milk mixture). Open circles represent the total intake of the differentially flavored saline-paired water (Tis-u-sol). Fig. 5 (right). Mean amounts of flavored water drunk by rats ($N = 8$) on successive days of the fifth experiment. Solid circles represent the total intakes of flavored water paired with an equal simultaneous injection of digestive juice–glucose mixture. Open circles represent the total intake of water unpaired with any injection.

rats had almond flavor paired with the glucose–digestive juice mixture and the banana flavor with nothing. This pairing was reversed for the other four rats. There was no significant difference between the two groups [$F(1, 7) < 1.0$, not significant] (Fig. 5). There was also no significant interaction of glucose intake with days [$F(8, 56) = 1.65$, $P > .05$]. The mixture of glucose and digestive juice, which was injected intragastrically, seems to have no rewarding effect. Because the taste of glucose is highly palatable and glucose itself is rapidly absorbed, neither of these two factors seems responsible for the rewarding effect of the milk–digestive juice mixture. Further, the glucose mixture would also cause stomach distention; therefore, its lack of effect makes an explanation in terms of stomach distention even more implausible.

Our results show that some rewarding signals are rapidly generated by the upper gastrointestinal tract when nutrients arrive there. Why whole milk, unmixed with stomach digestive juices, had no such effect remains a problem for further research. However, it seems that the upper gastrointestinal tract can, at least under some conditions, recognize some components of food and signal their arrival rapidly to the central nervous system.

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4 December 1975; revised 17 February 1976

Lens Culinaris Lectin Immobilized on Sepharose: Binding and Sugar-Specific Release of Intact Tissue Culture Cells

Abstract. *Lens culinaris lectin (LCL) covalently linked to 2B Sepharose binds tissue culture cells to the matrix. This is prevented by hapten sugars specific for LCL. Unlike other immobilized lectins, lens culinaris lectin allows the removal of bound cells from the matrix on addition of the specific sugars in a concentration-dependent manner. Binding and release occur under physiological conditions. Released cells continue to grow.*

Lectins have proved to be an excellent tool for discovering cell surface changes that occur during neoplastic transformation (*1*) or during the cell cycle (*2*). Cell separation procedures utilizing these differences in the cell surface architecture would be of great interest. So far, immobilized lectins have been shown to induce specific, receptor-mediated binding of cells to solid supports. However, a hapten sugar–induced replacement of the cells under physiological conditions, even with mechanical aid, has been difficult if not impossible, as shown for concanavalin A (Con A) (*3*) and wheat germ agglutinin (*4*). Probably for the same reason, affinity chromatography with immobilized Con A yields only low amounts of glycoproteins upon elution with the specific sugar. This problem has been overcome to some degree by the use of lens culinaris lectin (LCL) (*5*).

We investigated the ability of immobilized LCL to induce cultured cells to bind to large agarose beads and to release them specifically on treatment with

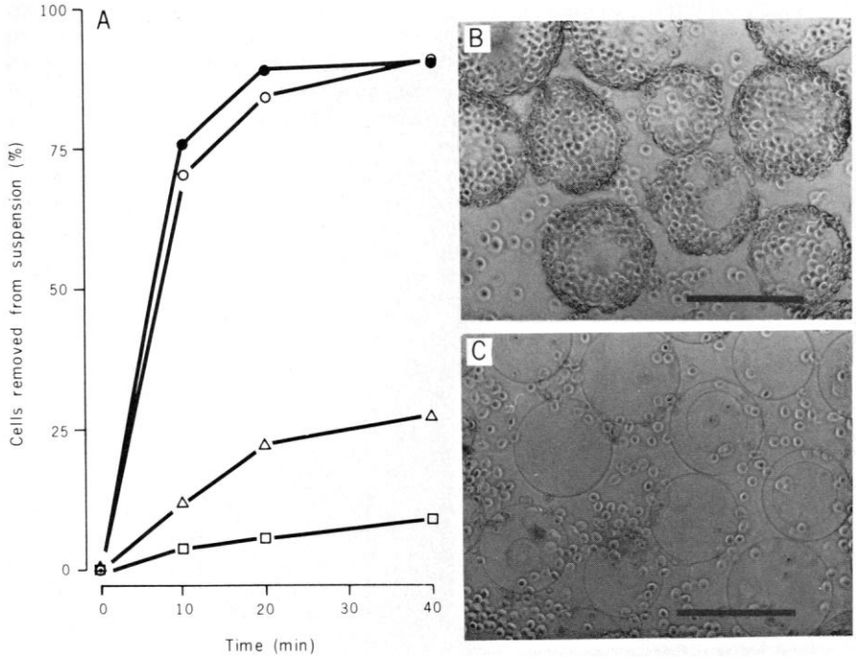

Fig. 1. Binding of HeLa cells to LCL-beads in the presence of different sugars (0.2*M*). (A) Time course of removal of cells from the supernatant in phosphate-buffered saline (•), D-galactose (○), methyl-α-D-glucopyranoside (△), and methyl-α-D-mannopyranoside (□). At the times indicated, two 100-μl portions were removed from the suspension and counted with a Coulter counter, model B. Maximum deviations do not exceed the symbols. (B and C) Phase contrast pictures taken from the D-galactose group (B) and the mannopyranoside group (C) at 20 minutes. Scale bar, 300 μm. HeLa cells were cultivated in Eagle's minimum essential medium supplemented with 10 percent calf serum. Suspensions were prepared from subconfluent cultures 1 to 3 days old. Dishes were washed two to four times with PBS-BSA without divalent cations but containing 2 m*M* EDTA until the cells came off. In the last wash as well as in all following steps a small amount of deoxyribonuclease I was included (about 20 to 30 units per milliliter). Before incubation the cells were washed with PBS-BSA. The cells (3×10^6 per group) were incubated with 0.5 ml of settled LCL-beads in a total volume of 5 ml in the presence of 0.2*M* sugars at room temperature. For the addition of the particular sugars, portions of 0.3*M* sugar solutions (physiological with regard to the osmotic pressure) containing 0.1 percent bovine serum albumin, 1 m*M* $CaCl_2$, 0.5 m*M* $MgCl_2$, and 0.1 m*M* $MnCl_2$ were mixed with PBS-BSA in ratios necessary to obtain the final concentrations. The LCL-beads were equilibrated with the particular solution for 30 minutes and then mixed with the cells, which had been suspended in the same solution 5 minutes before combining.