# Appetitive conditioning with recovery from thiamine deficiency as the unconditioned stimulus

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*Pairing a taste with recovery from thiamine deficiency produced a preference for that flavor over tastes associated with deficiency and novel tastes in thiamine-deficient rats. The preference persisted after recovery from deficiency.*

When a rat is presented a distinctively flavored diet paired with X-irradiation, apomorphine injection, or the production of thiamine deficiency, aversion to that diet flavor quickly develops (Garcia, Kimeldorf, & Hunt, 1961; Garcia, Ervin, & Koelling, 1966; Rozin, 1967). Procedurally, these experiments follow a Pavlovian conditioning paradigm: A taste CS is followed by an aversive UCS that produces an unpleasant internal state (nausea). However, the conditions under which learning occurs with a taste CS and an internal aversive UCS seem different from those under which Pavlovian conditioning ordinarily occurs. Aversions have been produced with only a few pairings and with delays between CS and UCS of as long as 6 h or 12 h (Revusky, 1968; Smith & Roll, 1967).

The above findings have led Garcia & Ervin (1968) to conclude that learning which involves gustatory CSs and gastrointestinal UCSs proceeds through a different mechanism than does learning that involves exteroceptive CSs (e.g., a tone) and external UCSs (e.g., an electric body shock). Both Garcia & Ervin (1968) and Rozin (1967) have argued that high sensitivity to the association between gustatory CSs and gastrointestinal UCSs is of great survival value to the rat in its natural habitat. Thus, a specialized mechanism may have evolved through natural selection. If this argument is correct, it seems likely that the use of a gustatory CS and a positive gastrointestinal UCS should also lead to rapid appetitive conditioning. However, all of the above studies have employed an aversive UCS. Only one study has been interpreted as evidence for appetitive conditioning with a gustatory CS and a gastrointestinal UCS (Garcia, Ervin, Yorke, & Koelling, 1967). Garcia et al, using a one-bottle test, found that rats which had become thiamine deficient while drinking water and had then recovered from deficiency while drinking a saccharin solution, drank more of the saccharin solution than did rats that had become deficient while drinking saccharin and had recovered while drinking water. However, it is not entirely clear whether the rats developed a preference for the "recovery flavor," an aversion to the "deficiency flavor," or both, because only the recovery and deficiency flavors were tested. This distinction is important because it has been suggested (Rozin, 1967) that an aversion to the flavor associated with deficiency can account for all of the data on diet preferences in thiamine-deficient animals. In order to determine whether or not a preference for the recovery flavor has developed, a preference test involving a "neutral" flavor is needed.

In addition, Garcia et al found that the above difference occurred only when the rats were deficient in thiamine. When the rats were in the nondeficient state, the rats that had experienced saccharin in combination with thiamine injection drank no more saccharin than did the rats that had experienced saccharin in combination with thiamine deficiency. It is possible that a more sensitive preference test might reveal an intake difference when S is nondeficient.

## METHOD

Twenty male Sprague-Dawley rats, 6 months old at the beginning of the experiment, were fed a pelleted thiamine-deficient diet (Nutritional Biochemicals Corp.) ad lib. The Ss were given water for 30 min every 24 h. Each S's fluid consumption was measured to ±.5 ml during the daily drinking period. The Ss were randomly divided into four groups of five Ss each. Group 1 drank only water flavored with anise extract (.5 ml/100 ml), Group 2 drank only water flavored with banana extract (.3 ml/100 ml), Group 3 drank only water flavored with vanilla extract (.5 ml/100 ml), and Group 4 drank only tap water. The flavoring substances contained no thiamine.

After 20 days on this regimen, the mean fluid consumption had dropped to about 50% of consumption at the beginning of the experiment, indicating that the Ss were thiamine-deficient. On Day 21, each S was given a new flavor. As soon as S had consumed a few swallows of the new flavor, it was given an intramuscular injection of thiamine hydrochloride (200 μg/kg) and then allowed to complete its 30-min drinking period. The flavor paired with the injection for the various groups was: Group 1, vanilla; Group 2, anise; Group 3, banana; and Group 4, tap water. On Day 22, the animals were returned to the flavor originally paired with deficiency. The Ss were maintained on the "deficiency flavor" and the thiamine-deficient diet until their water intake again dropped to 50%, indicating deficiency. The injections were then administered a second time, paired with the "recovery flavor" for each group. The same procedure was followed for a third and a fourth injection.

When the Ss reached the criterion of deficiency after the fourth injection, all Ss were given the same three-flavor preference test. Bottles of anise-, banana-, and vanilla-flavored water were placed in random order on each cage, and the amount consumed from each bottle was measured after 30 min and again after 24 h. It may be seen that the design of the experiment was a simple Latin square, with three flavors and three conditions (paired with recovery, paired with deficiency, and novel flavor). The first three groups had each of the flavors paired with a different condition, with each combination of a flavor and a condition appearing in only one group (Table 1). The control animals had the same three-flavor test, but all three flavors were novel for the controls.

After being tested in the thiamine-deficient state, all Ss were given a diet of regular Purina chow and their "recovery flavor" of drinking water for the next 7 days, during which they recovered completely from vitamin deficiency. The same three-choice test was then administered to the recovered animals.

## RESULTS

Figure 1 shows the outcome of the preference tests. The left portion depicts the mean intake of the fluid paired with recovery, the fluid paired with deficiency, and the novel fluid, after 30 min and after

**Table 1**
**Experimental Design**

| | Group 1 | Group 2 | Group 3 | Group 4 |
|---|---|---|---|---|
| Paired with recovery | Vanilla | Anise | Banana | Tap water |
| Novel | Banana | Vanilla | Anise | Vanilla<br>Anise<br>Banana |
| Paired with deficiency | Anise | Banana | Vanilla | Tap water |

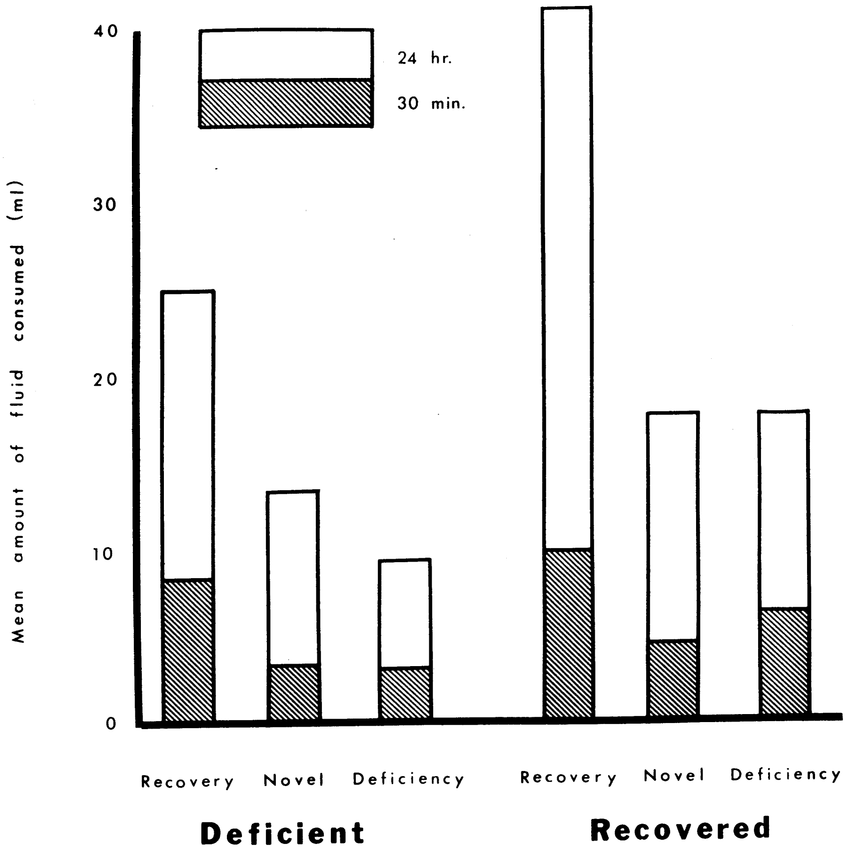


**Fig. 1. Mean amount of fluid consumed after 30 min and after 24 h of preference testing in the deficient and recovered states, for the taste paired with recovery, the novel taste, and the taste paired with deficiency.**

24 h of testing during the deficient state. The right portion of Fig. 1 shows the identical data for the test conducted in the recovered state.

A separate analysis of variance (Lindquist Design VII for replicated Latin square) was performed on the 30-min and 24-h intake data. Since the two analyses revealed identical findings, only the analysis of the 30-min data will be presented. The effect of flavors (anise, vanilla, and banana) was significant ($F = 22.62$, $df = 2/24$, $p < .001$), indicating that the Ss preferred some flavors to others. However, flavors did not interact significantly with any of the other variables. The effect of deficiency was also significant ($F = 5.53$, $df = 1/24$, $p < .025$), indicating that Ss drank more in the recovered than in the deficient state. The effect of conditions (paired with recovery, paired with deficiency, novel) was also significant ($F = 8.08$, $df = 2/24$, $p < .005$). Duncan's multiple-range test revealed that the Ss drank more of the recovery flavor than either the deficiency or novel flavors ($p < .05$). There was no significant intake difference between the novel and deficiency flavors. Finally, conditions did not interact significantly with deficiency, indicating that the preference for the recovery flavor was as great in the nondeficient as in the deficient state.

## DISCUSSION

This study demonstrates the development of a preference for a taste paired with recovery from thiamine deficiency. A large preference developed after only four pairings of the taste and injection of thiamine.

In contrast to the results of Garcia et al, the present experiment revealed a preference for the recovery flavor during both deficient and nondeficient states. This finding is important if one views the preference for the recovery flavor as a case of conditioned reinforcement. If the taste associated with recovery from thiamine deficiency acquires conditioned reinforcing properties, then S should show a preference for this taste in both the deficient and nondeficient states.

There were a number of differences between our procedure and that of Garcia et al that could account for the above discrepancy. We employed a preference test, while Garcia et al used an absolute intake measure. In addition, the test in the nondeficient state was here preceded by four recovery-flavor/thiamine-injection pairings *and* full recovery from deficiency in the presence of the recovery flavor. Garcia et al used only four pairings. Hence, our procedures provided S with a greater opportunity to associate taste and recovery than did that of Garcia et al.

It should be noted that although a preference for the taste paired with recovery from thiamine deficiency did develop, an aversion to the taste paired with deficiency did not develop. This is consistent with the findings of Rozin, Wells, & Mayer (1964). These investigators found that an aversion developed to the taste of a solid diet paired with thiamine deficiency, but that an aversion did not develop to the taste of a liquid paired with thiamine deficiency.

## REFERENCES


GARCIA, J., & ERVIN, F. R. Gustatory-visceral and telereceptor-cutaneous conditioning: Adaptation in internal and external milieus. Communications in Behavioral Biology, 1968, 1 (Part A), 389-415.

GARCIA, J., ERVIN, F. R., & KOELLING, R. A. Learning with prolonged delay of reinforcement. Psychonomic Science, 1966, 5, 121-122.

GARCIA, J., ERVIN, F. R., YORKE, C. H., & KOELLING, R. A. Conditioning with delayed vitamin injection. Science, 1967, 155, 716-718.

GARCIA, J., KIMELDORF, D. J., & HUNT, E. L. The use of ionizing radiation as a motivating stimulus. Psychological Review, 1961, 68, 383-395.

REVUSKY, S. H. Aversion to sucrose produced by contingent X-irradiation: Temporal and dosage parameters. Journal of Comparative & Physiological Psychology, 1968, 65, 17-22.

ROZIN, P. Thiamine specific hunger. In C. Code (Ed.), *Handbook of physiology*. Vol. 1. Washington: American Physiological Society, 1967.

ROZIN, P., WELLS, C., & MAYER, J. Specific hunger for thiamine: Vitamin in water versus vitamin in food. Journal of Comparative & Physiological Psychology, 1964, 57, 78-84.

SMITH, J. C., & ROLL, D. L. Trace conditioning with X-rays as an aversive stimulus. Psychonomic Science, 1967, 9, 11-12.