

Conditioned taste aversion in rats for a threonine-deficient diet: demonstration by the taste reactivity test

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Abstract

Rats avoid a diet that is deficient in one or more essential amino acids (EAAs). This phenomenon is thought to involve the development of a “learned aversion” for the sensory properties or spatial placement associated with the deficient diet. The dietary self-selection technique has been widely used to show this avoidance of the deficient diet. Because avoidance does not necessarily imply taste aversion, we used the Taste Reactivity Test initially created by Grill and Norgren (1978) to analyze the affective reactivity pattern of rats that ingested a threonine-deficient diet. The results showed that there was an increase in the aversive responses when ingesting the threonine-deficient (Thr-Dev) diet, compared to a control diet, without changes in the hedonic responses. The aversive reactions were mainly gaping, and to a lesser extent chin rubbing and head shaking. This asymmetrical shift in the Thr-Dev diet palatability is consistent with a two-dimensional hypothesis of palatability, indicating that the aversive palatability of the deficient diet was increased while the positive palatability did not change. Further evidence indicates that rats do not develop a normal behavioral satiety sequence after ingesting the threonine-deficient diet. These results indicate that a true aversion is formed to the taste of a diet that is deficient in an essential amino acid. © 2000 Elsevier Science Inc. All rights reserved.

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1. Introduction

The essential amino acid (EAA) composition of a diet is known to influence food intake. Most animals, including rats, reduce their food intake after ingesting diets that are imbalanced in essential amino acid content (i.e., devoid of one or more EAAs or deficient in a particular EAA relative to the other EAAs; for review, see [1]). This phenomenon occurs quickly, sometimes within hours of the first ingestion (e.g., [2–4]), except for a lysine deficiency [5]. The suppression of intake depends on several factors, including (a) the limiting EAA [6], (b) the prefeeding diet condition [7], and (c) the feeding schedule [8].

The anorectic response to an EAA-deficient diet is thought to involve two distinctive phases: the initial recognition of the deficiency [9,10], and the subsequent reduction in food intake together with development of a “learned aversion” for the sensory qualities of the deficient diet [11–13] or for its spatial placement [14]. Different areas of the

brain have been implicated in these two phases of response to an EAA-deficient diet [15]. The anterior piriform cortex (APC) has been demonstrated to be important in the initial recognition phase (e.g., [16–19]). Lesions of the central nucleus of the amygdala [18] or of the parabrachial nucleus [20], by comparison, appear to disrupt the second phase, or “learned aversion” for a threonine-deficient diet.

Previous studies have used preference tests and intake measures to infer the existence of a conditioned aversion for an EAA-deficient diet (e.g., [5,12,13,21,22]). For example, rats that have been gastrically infused with an EAA-deficient solution later avoid food presented with the odor associated with the infusion [11,23]. Such studies have elegantly demonstrated conditioned avoidance of flavors associated with EAA-deficient diets. They have inferred indirectly that a conditioned aversion exists based on the avoidance. In other words, such studies infer that flavors associated with EAA-deficient diets are “not liked” after observing that they were “not wanted.” The assumption that “unwanted” foods are also “disliked” is usually valid, but not always [24,25]. Berridge distinguished this two notions of food reward as follows: “Liking corresponds closely to the concept of pal-

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atability; wanting, by comparison, corresponds more closely to appetite or craving” and assumed that “. . .wanting and liking have separable underlying brain substrates” [24].

Studies using the “taste reactivity test” developed by Grill and Norgren [26], which measures affective facial and somatic reactions to tastes, have shown that strong food avoidance sometimes can be conditioned without food aversion. For example, Parker and colleagues have demonstrated that conditioned food avoidance produced by pairing a flavor with rewarding drugs such as amphetamine, nicotine, or morphine fails to be accompanied by aversive taste reactivity components (chin rubs, gapes, etc.), whereas conditioned avoidance produced by pairing with lithium chloride causes subsequent conditioned aversive reactions to the taste [9,10,27–29]. Even unconditioned aversive stimuli may differ in their ability to produce conditioned aversion to a taste: both electric footshock and lithium chloride are able to serve as unconditioned stimuli to produce conditioned avoidance of a taste, but that only lithium chloride produced aversive reactions to the taste [30]. Pairing of a taste with electric foot shock produced taste avoidance but not taste aversion. Similarly, suppression of intake without aversion can be produced by physiological states such as caloric satiety [31], or by pharmacological states such as dopamine antagonist administration [32], or by brain lesions such as amygdala lesions or 6-hydroxydopamine lesions [25,33]. For example, electrolytic lesion of the amygdala in rats disrupt natriophilia (preference for salty solution after physiological sodium depletion), but not the positive hedonic reaction to the taste of salt [33]. 6-Hydroxydopamine lesions of midbrain dopamine projections produce aphagia for all foods, but similarly fail to produce aversion or to reduce hedonic reactions to palatable foods [25,33].

The aim of the present work was to test whether rats that learn to avoid a diet devoid of threonine will respond with active aversive affective reactions to its taste. The taste reactivity test was used to study affective reactions emitted by rats during voluntary intake of a threonine-deficient diet or a control diet. The results suggest that, unlike the manipulations discussed above, avoidance of EAA-deficient diets reflects a true taste aversion.

2. Materials and methods

2.1. Subjects and procedure

The subjects were 18 male Wistar rats (Iffa Credo, L'Arbresle, France) weighing 393 ± 11 g at the start of the experiment. They were housed in individual cylindrical cages, controlled for temperature ($21 \pm 1^\circ\text{C}$) and lighting (0600–1800 h light; 12:12-h cycle). The experimental chamber consisted of a circular Plexiglas tank (height: 320 mm; diameter: 300 mm), with a ring inside the chamber, attached to the wall, for (receiving) the food cup and a water bottle at the opposite side. The rats were habituated to the test chambers for 3 days prior to the testing.

In the first group, nine rats received a moistened diet (powder: tap water; 2:1 so that it was semiliquid in texture) devoid of threonine (Thr-Dev) for 3 days (test on Day 3), and then 3 more days in the test cage on a control diet corrected for the threonine deficiency (Cor). Between the Thr-Dev and Cor sessions, the rats were replaced in their home cages and received during the next 3 days a regular stock diet to replete the threonine deficiency (Extralabo M25C from Pietremont, l'Arbresle, France). The threonine-devoid diet (Thr-Dev; metabolizable energy content of 15.4 kJ/g) and control diet (Cor; energy content of 15.6 kJ/g), which was identical except that an equivalent load of glucose and starch (1:1) was substituted for threonine, were obtained from INRA (Unite de Preparation des Aliments Experimentaux, Jouy en Josas, France) (Table 1; [13]).

Tap water was provided ad lib throughout the experimental procedure, but food was available only from 1800–1000 h.

On the third day of each diet session, affective reactions emitted by the rats were videorecorded while they ate dur-

Table 1
Composition of the experimental diets (g/kg)

Diet	Thr-Dev	Cor
Essential amino acids ^a	124	124
Nonessential amino acids ^b	77	77
L-Threonine	—	6
Salt mixture ^c	45	45
Vitamin mixture ^d	10	10
African peanut oil ^e	20	20
Rapeseed oil ^e	30	30
Cellulose ^f	20	20
Maize starch ^g	337	334
Glucose ^h	337	334
Total ⁱ	1000	1000

Thr-Dev, threonine-devoid diet.; Cor, corrected diet.

^aProvided (g/kg diet): L-methionine 10, L-cystine 6, L-histidine 12, L-lysine 15, L-isoleucine 15, L-leucine 21, L-phenylalanine 15.5, L-tryptophan 4, L-valine 16, L-tyrosine 9.5.

^bProvided (g/kg diet): glutamic acid 30, L-glycine 10, 10, L-arginine 10, L-alanine 3.5, L-asparagine 10, L-proline 10, L-serine 3.5.

^cProvided (g/kg diet): calcium phosphate 17.1, potassium phosphate 10.8, calcium carbonate 8.1, magnesium sulfate 4.0, sodium chloride 3.16, sodium fluoride 0.037, potassium chromate 0.002, potassium iodide 0.002, ammonium molybdate 0.001, cobalt carbonate 0.001, sodium selenite 0.001.

^dProvided (per kg of diet): retinyl acetate 5 mg, cholecalciferol 62.5 μg , D, L- α -tocopherol acetate 5 mg, menadione 1 mg, thiamin 10 mg, riboflavin 10 mg, nicotinic acid 45 mg, D-calcium panthotenate 30 mg, pyridoxine HCl 10 mg, inositol 50 mg, s-biotin 0.2 mg, pteroylmonoglutamic acid 2 mg, cyanocobalamin 13.5 μg , ascorbic acid 100 mg, *p*-amino benzoic acid 50 mg, choline chloride 750 mg.

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^hRoquette Frères, 59022 Lille, France.

ⁱThese ingredients were purchased and prepared at the Institut National de la Recherche Agronomique, Unite de Preparation des Aliments Experimentaux, Centre de recherche de Jouy en Josas, Domaine de Vilvert, 78352 Jouy-en-Josas Cedex, France.

ing the first hour of food presentation (between 1800–1900 h). The video camera (Sony-Handycam Vision, DCR-PC7E Pal) was placed outside the chamber at approximately 300 mm in front of the ring for the food cup. The video signal was recorded on conventional VHS tape (SONY, Premium V, E120) at 50 frames per second using a recorder (Philips VR 9489/39N VHS). For a second group of nine rats, the same diets were presented, but in the opposite sequence to counterbalance any order effect (the two sessions were also separated by 3 days on the regular stock diet).

2.2 Videoanalysis of taste reactivity

Videotapes were analyzed by a slow-motion playback (frame by frame to 1/6th of the normal playing speed) to count taste reactivity components. Taste reactivity components were classified as either hedonic, aversive, or neutral, as previously described [26,34]. Hedonic components were: rhythmic tongue protrusion (TP), lateral tongue protrusion (LTP), and paw licking (PL). Aversive reactions were: gape (G), head shaking (HS), paw treading (PT), forelimb flailing (FF), and chin rubbing (CR). Rhythmic mouth movement (MM) was classified as a neutral expression. Each occurrence of the behavior was scored for TP, LTP, G, HS, PT, FF, and CR. To avoid biasing of taste reactivity category scores, the more frequent PL and MM components were scored in 5-s time bins [34]. General locomotor activity of the rat was also scored each time it occurred: this included locomotion (walking and running), rearing (defined as the rat standing up on its two hind limbs), and jumping (in a manner directed at escape from the experimental chamber). From the 1-h of videorecording, only selected minutes (meals) were used for video analysis. These were the first 2.5 min and the final 2.5 min of a meal, during active ingestion or interaction with the food. All rats interacted with the food (tasting, sniffing, handling the food cup) during at least 5 min per trial, allowing us to approximately balance the two groups in amount of exposure to the sensory properties of the food. The first 2.5 min were scored separately from the final 2.5 min to detect within-meal changes in taste reactivity. Only one meal (for the Thr-Dev group) was less than 5 min in duration, and it was excluded from the analysis. An intermeal interval of more than 10 min was set to define two distinct meals.

2.3 Statistical analysis

Results were expressed as means with standard errors (SEM). Hedonic, aversive and neutral components were compared by a two-way analysis of variance (ANOVA), with diet (Cor versus Thr-Dev) and trial session (1 versus 2) as factors, using version 5.1 of Statistica™ (Statsoft® Tulsa, OK). A within-group comparison was made (ANOVA with repeated measures), when data for the two trials were collapsed, using the same program.

3. Results

3.1. Food intake

Ingestion of the Thr-Dev diet was reduced by at least 40% compared to intake of the control diet in each trial. However, the order of presentation of the diets influenced cumulative food intake of both the diets, $F(5, 70) = 2.96$, $p < 0.05$. Rats ingested 25% more of the Thr-Dev diet, when it was presented after the Cor diet, than when they received Thr-Dev diet first, $F(1, 14) = 10.28$, $p < 0.01$. This effect was less visible for the Cor diet. There was a reduction of 8% in intake of the Cor diet when it was presented after the Thr-Dev diet (relative to Cor presentation first), but this effect was not significant ($p = 0.09$).

When the analysis was restricted to food intake during the first hour of each test, there was a highly significant interaction between diet \times order of presentation in the cumulative food intake, $F(5, 70) = 9.02$, $p < 10^{-4}$; Fig. 1).

Rats that had initially received the Cor diet did not immediately reduce their intake when given the Thr-Dev diet, but did reduce their first-hour intake by the second day ($p < 0.01$). The opposite was not observed for rats that were switched from Thr-Dev to Cor diets. The first-hour intake of Cor diet was never elevated over the prior intake of Thr-Dev diet.

The temporal profiles of the three major categories of behavior (eating, grooming, and resting) implicated in the development of satiation [35,36] are plotted in Fig. 2.

For the Cor group, the three behaviors appeared in a well-defined sequence: eating behavior, followed by grooming behavior, followed by resting (Fig. 2).

Most eating behavior occurred during the first 15 min following Cor diet presentation. For the Thr-Dev group (Fig. 2), the well-defined sequence of the three behaviors was lost, and they were mostly distributed uniformly.

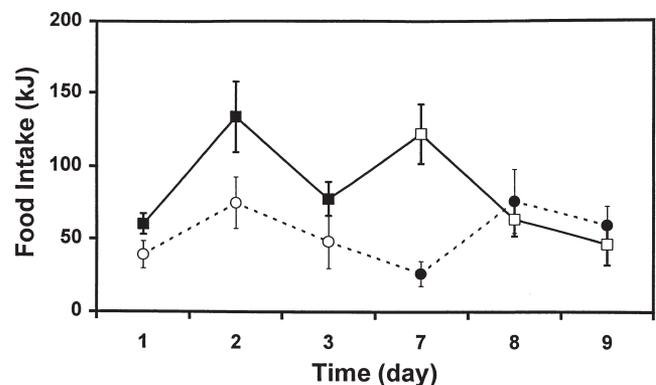


Fig. 1. Means (\pm SEM) of cumulative food intake during the first hour of presentation of each diet. The first group ($n = 9$) received the Thr-Dev diet from Days 1 to 3 (open circles) and COR diet from Days 7 to 9 (black circles). The second group ($n = 9$) received the diets in the opposite order (Cor: black squares; Thr-Dev open squares).

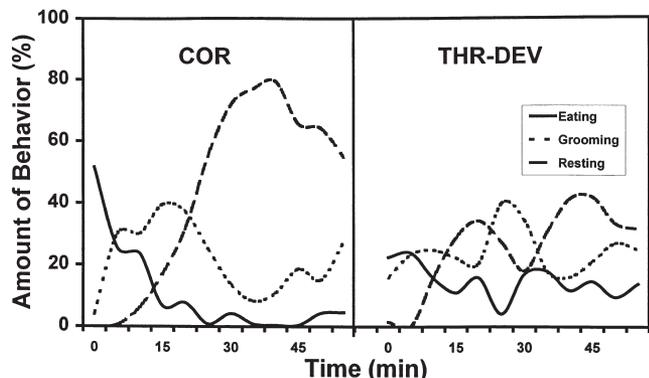


Fig. 2. Temporal profile of the Behavioral Satiety Sequence for the Cor and Thr-Dev diets ($n = 18$). Each behavior (eating, grooming, and resting) is expressed as the percentage of time occurring within a 5-min bin.

3.2. Taste reactivity

The total number of hedonic (TP+ LTP+ PL) and aversive (G+ HS+ FF+ PT+ CR) reactions during the recording hour (Fig. 3) were collapsed across trial sessions because there was no session effect on the number of hedonic reactions, $F(1, 32) = 0.2, p = 0.7$, aversive reactions, $F(1, 32) = 1.2, p = 0.28$, or neutral reactions, $F(1, 32) = 0.98, p = 0.33$.

On the third day with each diet, rats emitted three times the number of aversive reactions while consuming the threonine-devoid diet (Thr-Dev) compared to the control diet (Cor), $F(1, 17) = 11.6, p < 0.004$. By contrast, there was no difference between diets in the number of hedonic reactions or of neutral reactions. The number of hedonic, aversive and neutral reactions per meal are shown in Fig. 4. Ingestion of the Thr-Dev diet was associated with a twofold increase in the number of aversive reactions per meal compared to the Cor group, $F(1, 17) = 9.27, p < 0.01$. The apparent trend

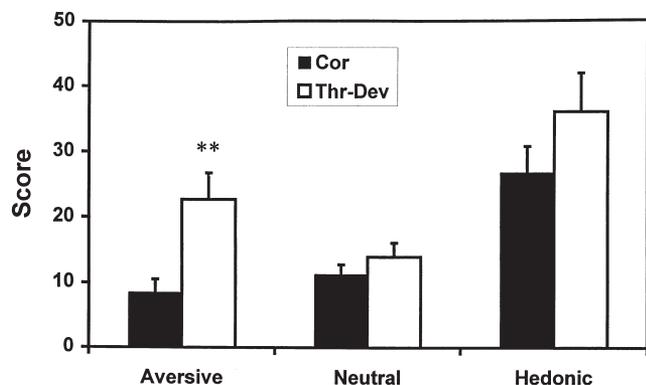


Fig. 3. Means (\pm SEM) of the overall number of hedonic, aversive, and neutral reactions after ingestion of a diet devoid of threonine (Thr-Dev) or corrected from the deficiency (Cor). ** $p < 0.01$ between the two diets using an ANOVA with repeated measures (within-group comparison; $n = 18$).

towards reduction in the number of hedonic and neutral reactions for the Thr-Dev group was only marginally significant ($p = 0.09$ and $p = 0.06$, respectively).

When separate aversive taste reactivity components were analyzed in detail (Table 2), it was found that the number of gapes was elevated fourfold during ingestion of the Thr-Dev diet compared to the Cor diet, $F(1, 17) = 10.04, p < 0.01$. The number of head shakes, $F(1, 17) = 4.88, p < 0.05$ and chin rubs, $F(1, 17) = 6.54, p < 0.05$, was also elevated during ingestion of the Thr-Dev diet compared to the Cor diet, although to a lesser degree. Finally, there was a tendency for paw treading to increase during ingestion of the Thr-Dev diet, but this elevation reached only marginal statistical significance ($p = 0.056$). There also appeared to be a slight trend to increase paw licking, but the effect was also not significant ($p = 0.09$).

3.3. General locomotion

General locomotion was increased by 67% during ingestion of the Thr-Dev diet compared to the Cor diet, $F(1, 17) = 22.56, p < 0.001$. The general increase in activity arose principally from a near twofold increase of the walking, $F(1, 17) = 21.18, p < 0.001$, and rearing measures, $F(1, 17) = 13.41, p < 0.005$. Running was not increased by ingestion of the Thr-Dev diet, but there was a tendency for an increase in jumping (escape) that came close to statistical significance ($p = 0.053$).

4. Discussion

The present study aimed to ascertain whether a true aversion becomes established to a threonine-deficient diet (Thr-Dev), using the taste reactivity test [26]. Our results indicate that rats emit more aversive reactions (gapes, chin rubs, head shakes) to a threonine-deficient diet than to a control diet. This increase in aversive reaction occurred even though the rats ingested the diet voluntarily. Taste reactivity measures have been applied to voluntary intake in several

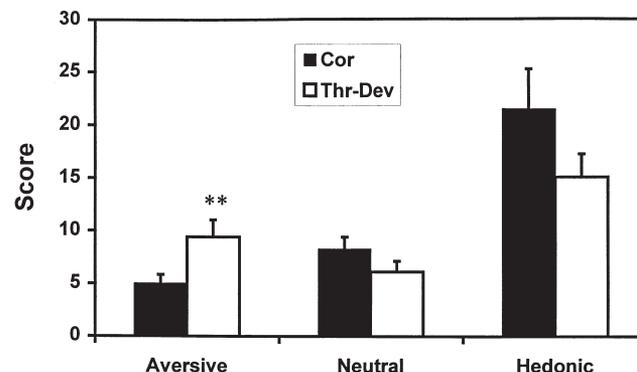


Fig. 4. Mean (\pm SEM) number of hedonic, aversive, and neutral reactions, per meal, during ingestion of a diet devoid of threonine (Thr-Dev) or of the nutritionally corrected diet (Cor). ** $p < 0.01$ between the two diets using an ANOVA with repeated measure (within group comparison; $n = 18$).

Table 2
Affective reactivity pattern during ingestion of the diet devoid of threonine (Thr-Dev) and corrected from the threonine deficiency (Cor)($n = 18$)

Affective reaction ^a	Cor	Thr-Dev
Hedonic		
TP	10 ± 2	9 ± 2
LTP	9 ± 2	12 ± 4
PL	8 ± 3	15 ± 4
Neutral		
MM	11 ± 2	14 ± 2
Aversive		
FF	3 ± 1	4 ± 2
G	3 ± 1	12 ± 3**
HS	1 ± 1	3 ± 1*
PT	1 ± 1	3 ± 1
CR	0	0.3 ± 0.1*

Mean ± SEM of the affective reactivity responses. Asterisks mean a statistical difference between the two diets using an ANOVA with repeated measure: * $p < 0.05$; ** $p < 0.01$.

^a(L)TP: (lateral) tongue protrusion; PL: paw licking; MM mouth movement; FF: forelimb flailing; G: gape; HS: head shaking; PT: paw treading; CR: chin rubbing.

earlier studies [30,37–39], and two of those studies similarly used taste reactivity measures in a voluntary intake paradigm to demonstrate conditioned aversions established by pairing a food with lithium chloride [30,40]. Our results show the method also detects an aversion conditioned as a nutritional consequence of a dietary imbalance in essential amino acids.

4.1 Food intake

The two diets were highly similar in sensory properties. When rats were switched from the Cor diet to the Thr-Dev diet (or vice versa), their food intake changed gradually across the first 2 days with the new diet. From the first day of ingestion of the Thr-Dev diet, food intake diminished by almost 40% compared to the Cor diet. The sensory similarity of the two diets may also be responsible for the presentation order effect on food intake [41]. The rats appeared to generalize from the sensory properties of one diet to the other. However, by the second day with each diet they had clearly learned about its distinct nutritional consequences, and they modulated their intake accordingly. By the third day, when the taste reactivity was measured, the rats also had altered their affective perception of the diet's palatability.

4.2 Affective reactivity pattern

Rats emitted many more facial and somatic aversive responses when they ingested the diet devoid of threonine, both overall and per meal. The increase in overall aversive reactions was mainly due to a fourfold increase in gaping, and to less of an increase in head shaking and chin rubbing. These components are sensitive measures of conditioned aversion, and are also enhanced by associative pairing of a food with lithium chloride [30,39,42–44]. In the present case, aversive reactions increased to the Thr-Dev diet, but hedonic reactions did not change. Thus, the negative or

aversive palatability of the diet appeared to have been selectively elevated without a change in its positive hedonic palatability. This asymmetrical shift in the Thr-Dev diet palatability is consistent with a two-dimensional hypothesis of palatability, which posits that aversive palatability is reacted to separately from positive hedonic palatability [45,46]. That suggests that the postingestive consequences of an amino acid-deficient diet may act primarily upon the negative aversive perception of food palatability. A similar unidimensional increase in aversive reactions, without change in hedonic reactions, was reported [44], after conditioning a taste aversion by pairing of a taste and LiCl administration in a temporally overlapping fashion. Thus, while reciprocal aversive increases and hedonic decreases are common results in taste aversion conditioning experiments, unidimensional shifts limited to aversion appear to be conditioned in some naturalistic situations.

Analysis of the affective reactivity pattern during ingestion of the Thr-Dev diet show that there was no increase of aversive responses during the meal. Thus, ending of the meal does not result from a rise in aversion at the end of the Thr-Dev meal. Because we measured taste reactivity only on the third day of each diet, we cannot say whether aversive reactions also differed on earlier days. One remaining question is the time course of relative development for anorexia and aversion. Observation of affective reactivity during the first phase of anorexia on initial days would lead to further information about whether anorexia and aversion develop in parallel, or whether one precedes the other.

4.3 Loss of behavioral satiety sequence

The normal satiety sequence of eating, grooming, and resting behavior [47–49] was totally lost after ingestion of the Thr-Dev diet. This may indicate that the Thr-Dev diet fails to produce normal physiological anorexia (satiety); it, instead, produces a pathological process similar to LiCl poisoning [50,51]. Loss of the satiety sequence was associated with the general increase in locomotor activity in rats that ate the Thr-Dev diet. Increases in general activity have been related to some "need states" like food and water deprivation, or deprivation of a specific nutrient (reviewed in [52]). Some authors have defined this increase in activity as a frustration state [53]. In our case, by the third day of ingesting the threonine-deficient diet, the rats had incurred both an energy-deficit, due to the 50% reduction in cumulative energy intake, and a specific threonine deficiency. It remains an open question as to whether locomotion was due to these need states, was due to a response to the aversive properties of the food, or was due to a response to frustration, or a combination of these factors.

5. Conclusion

This study showed that an aversive palatability shift is produced in the perception of a threonine-deficient diet after

3 days of ingestion. The palatability shift is illustrated by an increase in the number of active aversive reactions made to the Thr-Dev diet. We, therefore, conclude that anorexia to an EAA-deficient diet is mediated, at least in part, by an aversion that develops to the taste of the diet. Future experiments may show whether this aversion appears concomitantly with the early anorexia, or whether the anorexia appears first.

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