Dept Meeting, 13 Nov 2012, M. Umezaki

Essential Amino Acid Deficiency Enhances Long-Term Intake but Not Short-Term Licking of the Required Nutrient

Stacy Markison et al.

Journal of Nutrition, 129: 1604-1612, 1999

Abbrevation

EAA: Essential Amino Acid

LYS: Lysine

LYS-DEF: Lysine deficiency

THR: Threonine

THR-DEF: Threonine deficiency

GLY: Glysine

CON: Control

ABSTRACT. Rats can adjust their nutrient intake in response to nutritional deficiency. This phenomenon has been described extensively for sodium deficiency, whereas other nutrient deficiencies have not been explored thoroughly. Essential amino acid (EAA) deficiency represents a relevant model to describe adaptive changes in behavior resulting from deficiency. The purpose of these experiments was to examine more closely the behavioral responses that occur as a result of lysine (LYS) and threonine (THR) deficiency. Licking to LYS, THR, glycine and distilled water during 10-s trials was measured in control (CON) and EAA-deficient rats. Licking tests were conducted both before and after 23-h intake tests. Although EAA-deficient rats did not show increased licking to the deficient EAA in any of the brief-access tests, in all cases, they did initiate significantly more overall trials than did CON. The EAA-deficient rats also had elevated intake of the deficient EAA in long-duration tests. These findings suggest that LYS or THR deficiency does not emulate the behavioral properties of sodium deficiency in that it does not result in enhanced immediate licking responses to the limiting EAA in brief-access tests. Nevertheless, an appetite is expressed to the relevant EAA in a long-term intake test. J. Nutr. 129: 1604–1612, 1999.

Essential amino acid

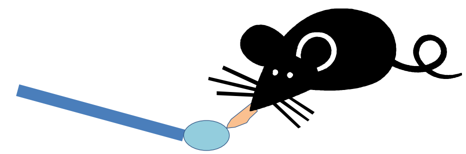
- an amino acid that cannot be synthesized *de novo* by the organism (usually referring to humans), and therefore must be supplied in the diet.

i.e., Histidine, Isoleucine, Leucine, Lysine, Methionine, Cysteine, Phenylalanine, Tyrosine, Threonine, Tryptophan, Valine

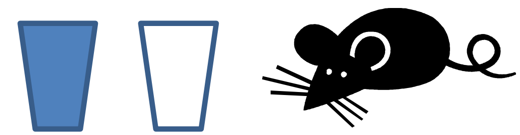
- Tissue protein synthesis is limited unless all required amino acids are available at the same time and in appropriate amounts at the site of tissue protein synthesis → Deficiency of an essential amino acid stop protein synthesis.

Deficiency-induced appetite: A mechanisms for Homeostasis

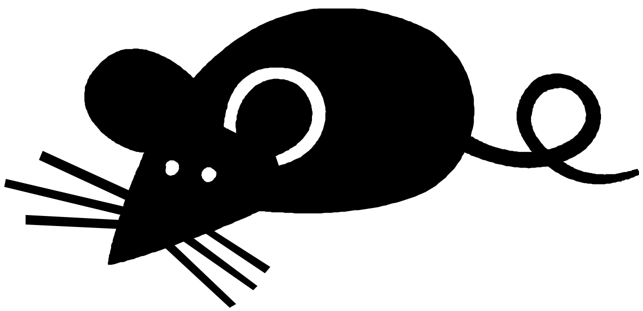
Licking test



Preference test



Deficiency in nutrient A



Appetite
/preference

Nutrient A

1. Immediate licking
(innate/instinct reaction under the control of oral sensors)

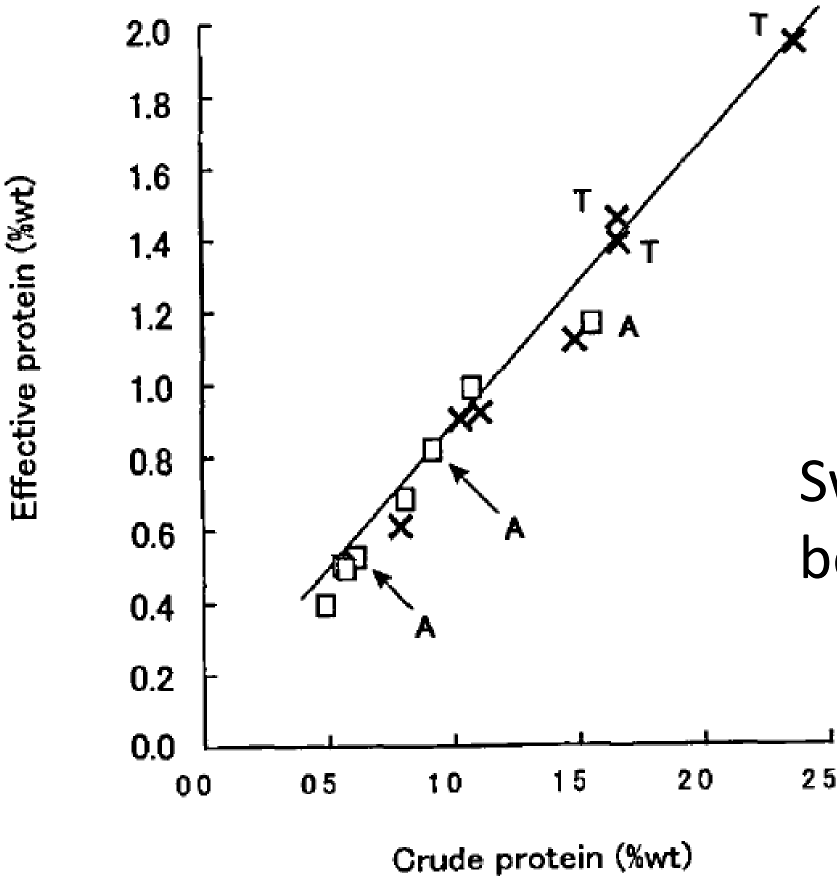
2. Preference during long-term intake tests
(innate/instinct reaction under the control of both oral-sensory and post-ingestive cues)

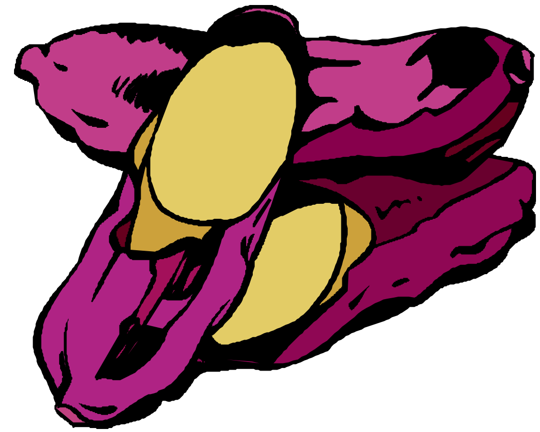
3. Licking after the preference test (a learned response under the control of oral sensors)

Deficiency-induced appetite: a case in essential amino acids?

Histidine (Rogers and Harper, 1970)
Lysine (Torii et al. 1986)
Tryptophan (Mori et al. 1991)

Selection of Cultivars of Sweet Potato in Papua New Guinea Highlands





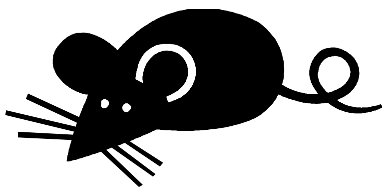
Sweet potato cultivars with better amino acid scores selected?

Umezaki et al. (2001)

Objectives

Lysin (LYS)
Threonine (THR)

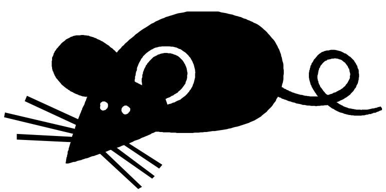
Deficiency in EAA



Appetite?

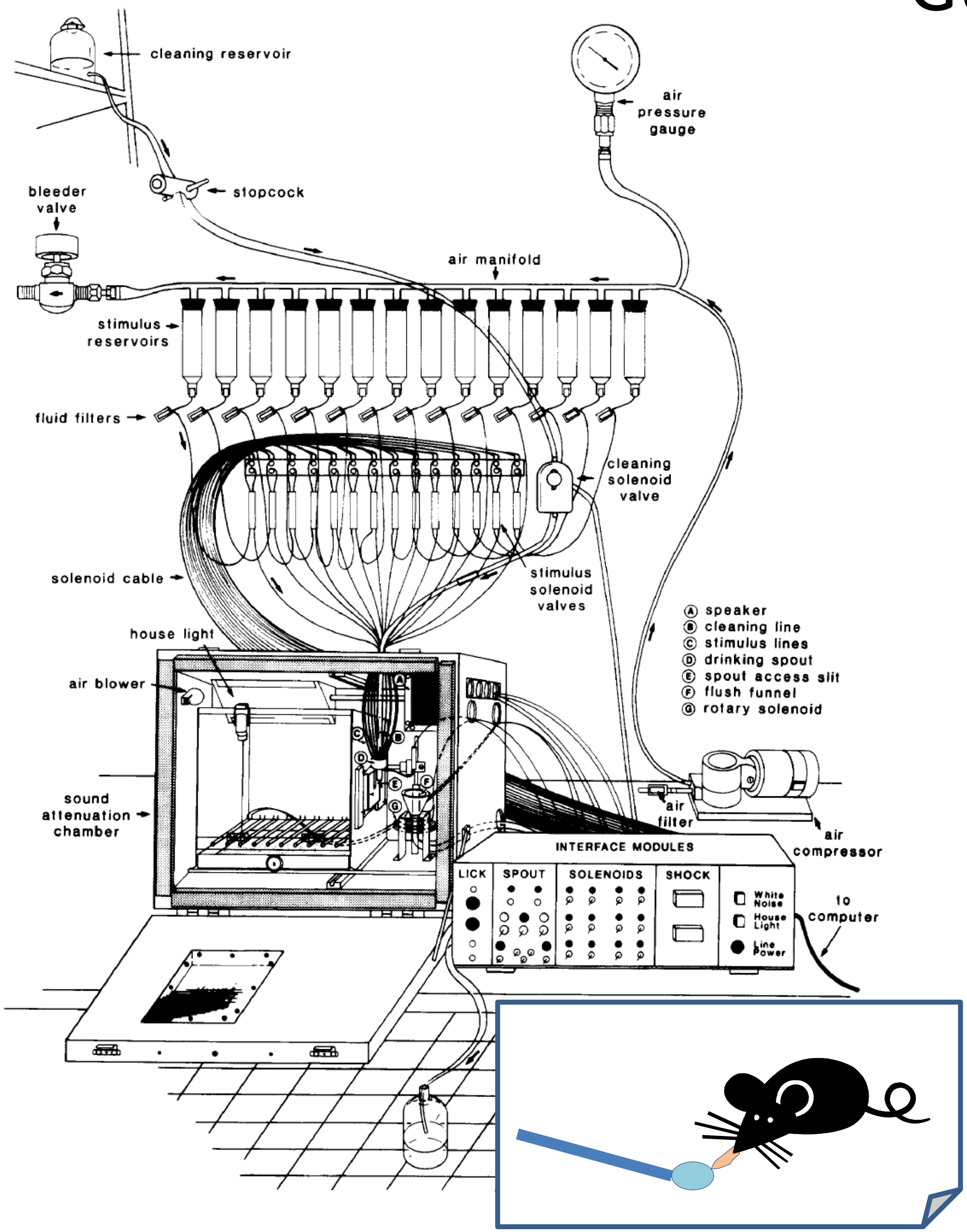
EAA

Control



1. Licking test (1^{st}, 2^{nd}, 3^{rd})
2. Preference test (1^{st}, 2^{nd})

Gustometer



Stimulus: (n=7)

- Distilled water
- LYS (0.2 mol/L)
- LYS (1.0 mol/L)
- GLY (0.1 mol/L)
- GLY (1.0 mol/L)
- THR (0.1 mol/L)
- THR (0.7 mol/L)

If two licks for either of stimulus, "trial" starts.

→ #trials
#licks/trial

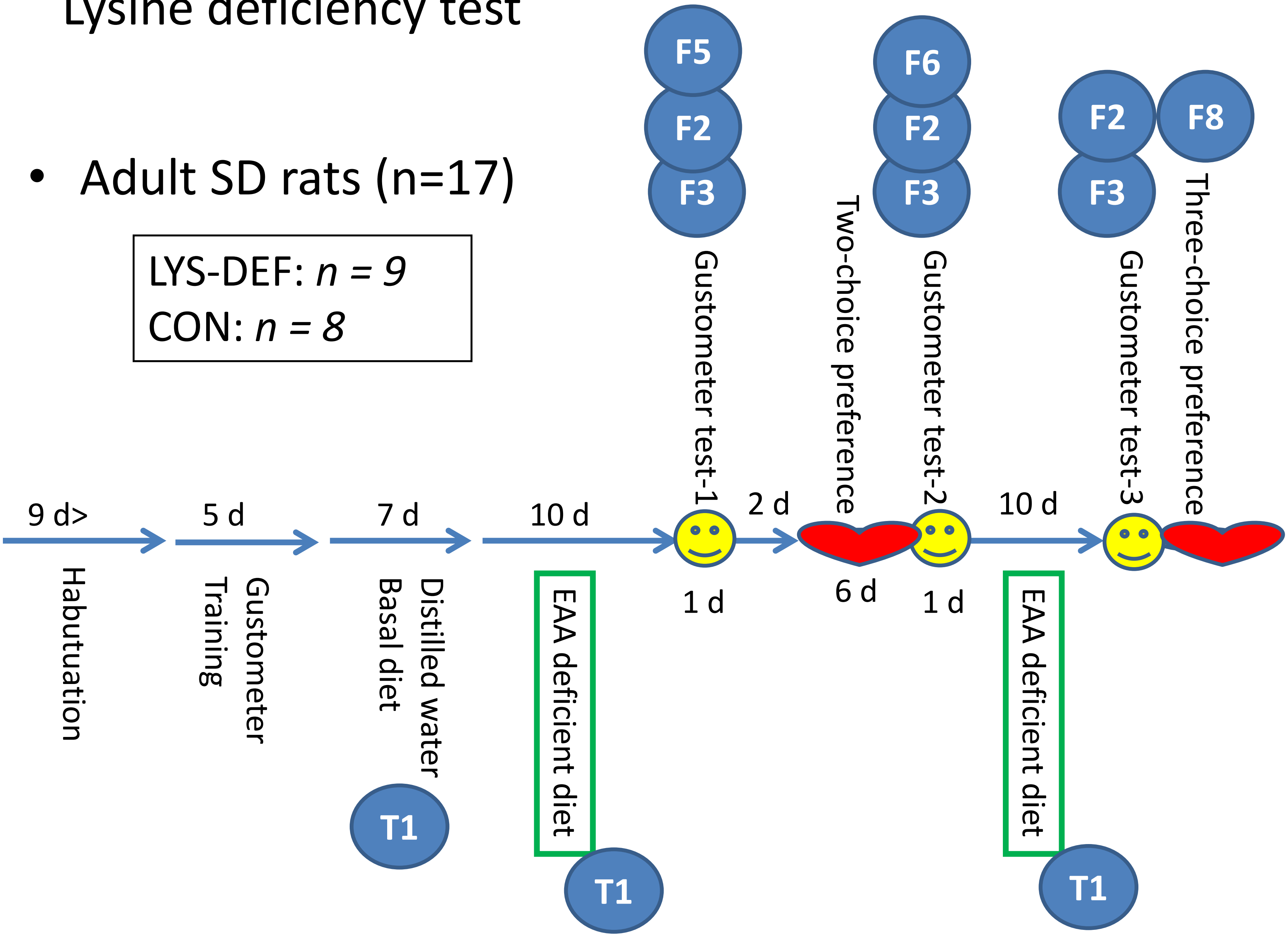
e.g., Stimulus= LYS (0.2), "trial" started, 8 licks/trial, rotation (6 seconds), stimulus =GLY (1.0), "trial" did not start, rotation (6 seconds), Stimulus=THR (0.1), "trial" started, 12 licks/trial, rotation…… ……………………….Totally 40 minutes

Spector et al. (1990)

Lysine deficiency test

- Adult SD rats (n=17)

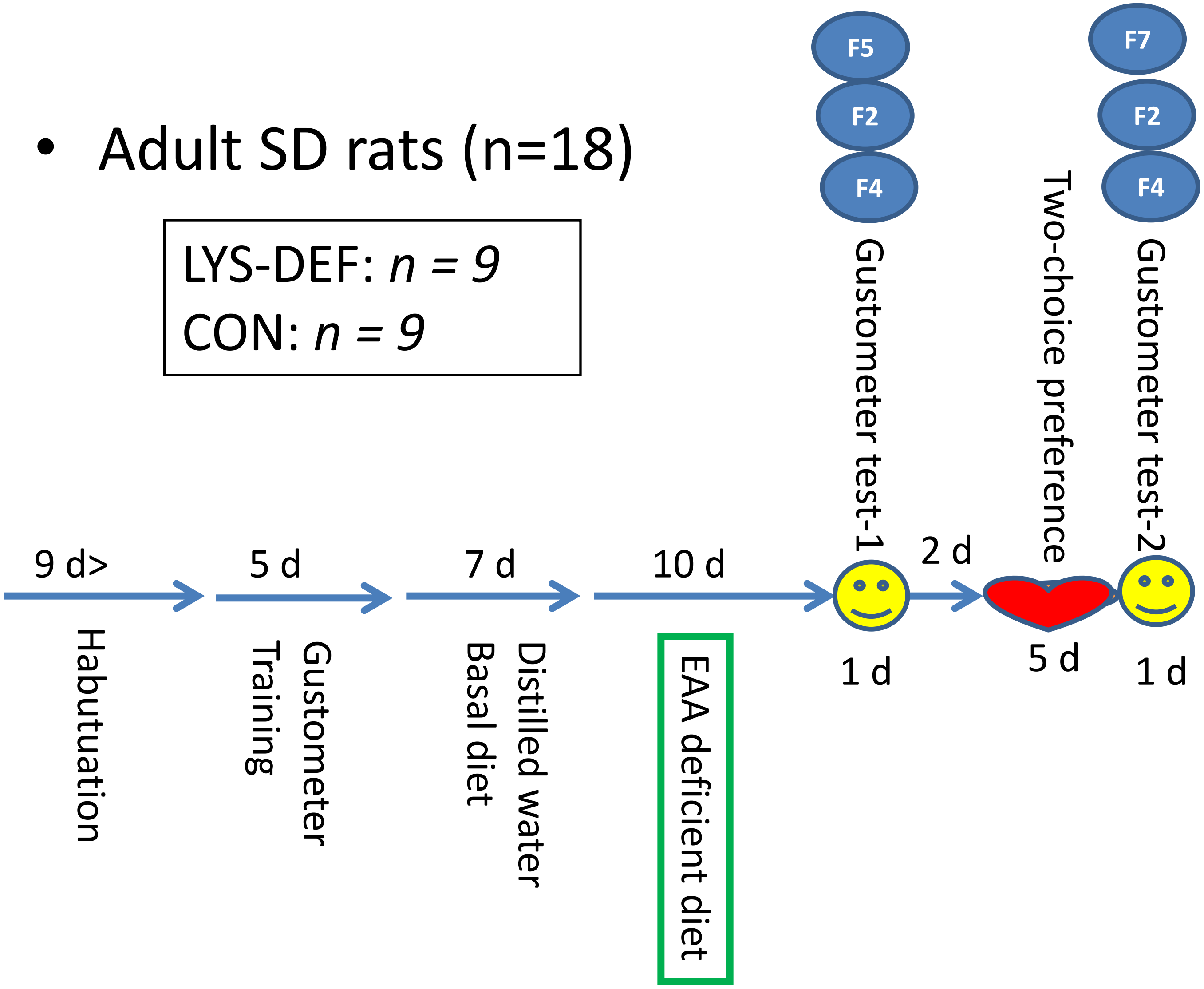
LYS-DEF: *n = 9*
CON: *n = 8*



Threonine deficiency test

- Adult SD rats (n=18)

LYS-DEF: *n = 9*
CON: *n = 9*



Message of each test

- 1. First gustometer test: Immediate licking
- (innate/instinct reaction under the control of oral sensors)

- 2. Choice tests: Preference during long-term intake tests (innate/instinct reaction under the control of both oral-sensory and post-ingestive cues)

- 3. Second/third gustometer tests: Licking after the preference test (a learned response under the control of oral sensors)

TABLE 1

Composition of Diets for Experiments 1 and 2[1]

Ingredient (g/kg diet)	LYS basal	LYS-CON	LYS-DEF	THR basal	THR-CON	THR-DEF
L-Arginine · HCl	10.0	10.0	10.0	10.0	10.0	10.0
L-Asparagine	10.0	10.0	10.0	10.0	10.0	10.0
L-Serine	3.5	3.5	3.5	3.5	3.5	3.5
L-Proline	10.0	10.0	10.0	10.0	10.0	13.74
Glycine	10.0	10.0	16.4	10.0	10.0	10.0
L-Glutamic acid	30.0	30.0	30.0	30.0	30.0	30.0
L-Alanine	3.5	3.5	3.5	3.5	3.5	3.5
L-Histidine · HCl · H_2O	3.0	11.0	11.0	3.0	11.0	11.0
L-Isoleucine	4.0	14.0	14.0	4.0	14.0	14.0
L-Leucine	4.0	18.0	18.0	6.0	20.0	20.0
L-Lysine HCl	4.0	20.0	1.0	8.0	24.0	24.0
L-Methionine	3.0	9.0	9.0	3.0	9.0	9.0
L-Cystine	2.0	6.0	6.0	2.0	6.0	6.0
L-Phenylalanine	4.5	14.5	14.5	4.5	14.5	14.5
L-Tyrosine	2.5	8.5	8.5	2.5	8.5	8.5
L-Threonine	4.0	8.0	8.0	2.0	6.0	2.0
L-Tryptophan	1.0	3.0	3.0	1.0	3.0	3.0
L-Valine	5.0	15.0	15.0	5.0	15.0	15.0
Sucrose	256.4	223.0	230.03	254.2	220.73	220.82
Cornstarch	512.79	445.89	460.06	508.29	441.46	441.63
Corn oil	50.0	50.0	50.0	50.0	50.0	50.0
Mineral mix, AIN-76[2]	50.0	50.0	50.0	50.0	50.0	50.0
Sodium acetate	6.8	17.1	8.5	8.5	18.8	18.8
Vitamin mixture[3]	10.0	10.0	10.0	10.0	10.0	10.0
Ethoxyquin	0.01	0.01	0.01	0.01	0.01	0.01
Choline chloride	—	—	—	1.0	1.0	1.0

[1] Abbreviations: LYS, lysine; CON, control; DEF, deficient; THR, threonine.

[2] Mineral mixture 170915; Teklad Test Diets, Madison, WI (AIN 1977).

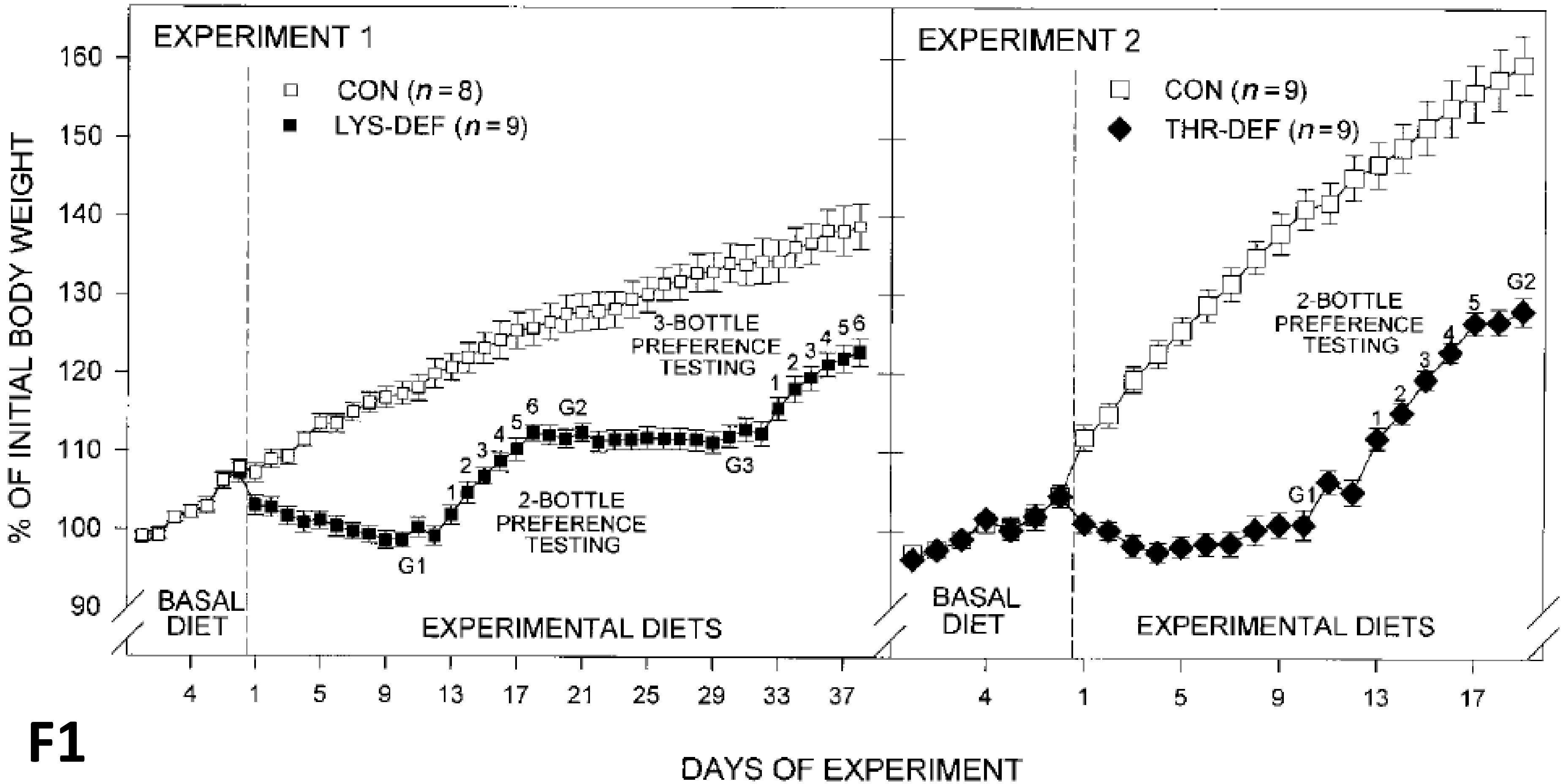
[3] Vitamin mixture 40060; Teklad Test Diets, Madison, WI (mg/kg diet): *p*-aminobenzoic acid, 110; ascorbic acid, 1017; biotin, 0.4; vitamin B-12, 30; calcium pantothenate, 66; choline dihydrogen citrate, 3497; folic acid, 2; inositol, 110; menadione, 50; niacin, 99; pyridoxine · HCl, 22; riboflavin, 22; thiamin · HCl, 22; retinyl palmitate, 40; vitamin D-3, 4; vitamin E acetate, 242; cornstarch, 4667.

TABLE 2

Outline of general procedures

	Experiment 1: LYS-DEF	Experiment 2: THR-DEF	Purpose
Sample size	LYS-DEF: $n = 9$ CON: $n = 8$	THR-DEF: $n = 9$ CON: $n = 9$	
Initial body weight[2]	387 ± 16.5 g	223 ± 16.3 g	
Gustometer training	5 d: water and sucrose	5 d: water and sucrose	Train rats to lick in gustometer.
Dietary manipulation	7 d: basal diet 10 d: exp diets	7 d: basal diet 10 d: exp diets	Deplete rats of a specific EAA.
Gustometer Test 1	10-s licking to water, lysine, glycine, threonine	10-s licking to water, lysine, glycine, threonine	Is there an innate, specific, taste-guided EAA appetite?
Two-choice preference	6 d: lysine vs. water	5 d: threonine vs. water	Is EAA appetite expressed in a long-term test?
Gustometer Test 2	(same as Gust. Test 1) 10-s licking to water, lysine, glycine, threonine	(same as Gust. Test 1) 10-s licking to water, lysine, glycine, threonine	Is an EAA appetite observed in a short-term paradigm after long-term experience with the EAA and repletion?
Dietary manipulation	10 d: deficient diet		Ensure that rats are EAA-DEF during gustometer testing.
Gustometer Test 3	(same as Gust. Test 1 & 2) 10-s licking to water, lysine, glycine, threonine		Is an EAA appetite observed in a short-term paradigm when rats are depleted during testing?
Three choice preference	lysine, threonine, water		Will LYS-DEF rats prefer lysine in the presence of another EAA?

[1] Abbreviations: LYS, lysine; DEF, deficient; THR, threonine; CON, control; EAA, essential amino acid.
[2] Mean ± SD.



F1

Percentage change from initial body weight of control and essential amino acid– deficient rats plotted over the course of the experiments. Values are means 6 SEM. Control rats in both experiments (CON) had steady growth rates. Lysine-deficient rats (LYS-DEF) gained weight only when they were given lysine solution on the home cage (during two- and three-bottle preference testing). Threonine-deficient rats (THR-DEF) grew only when they were given threonine solution on the home cage (during two bottle intake testing). Rats in Experiment 1 underwent additional testing resulting in more days in the experiment as depicted by the horizontal axes.

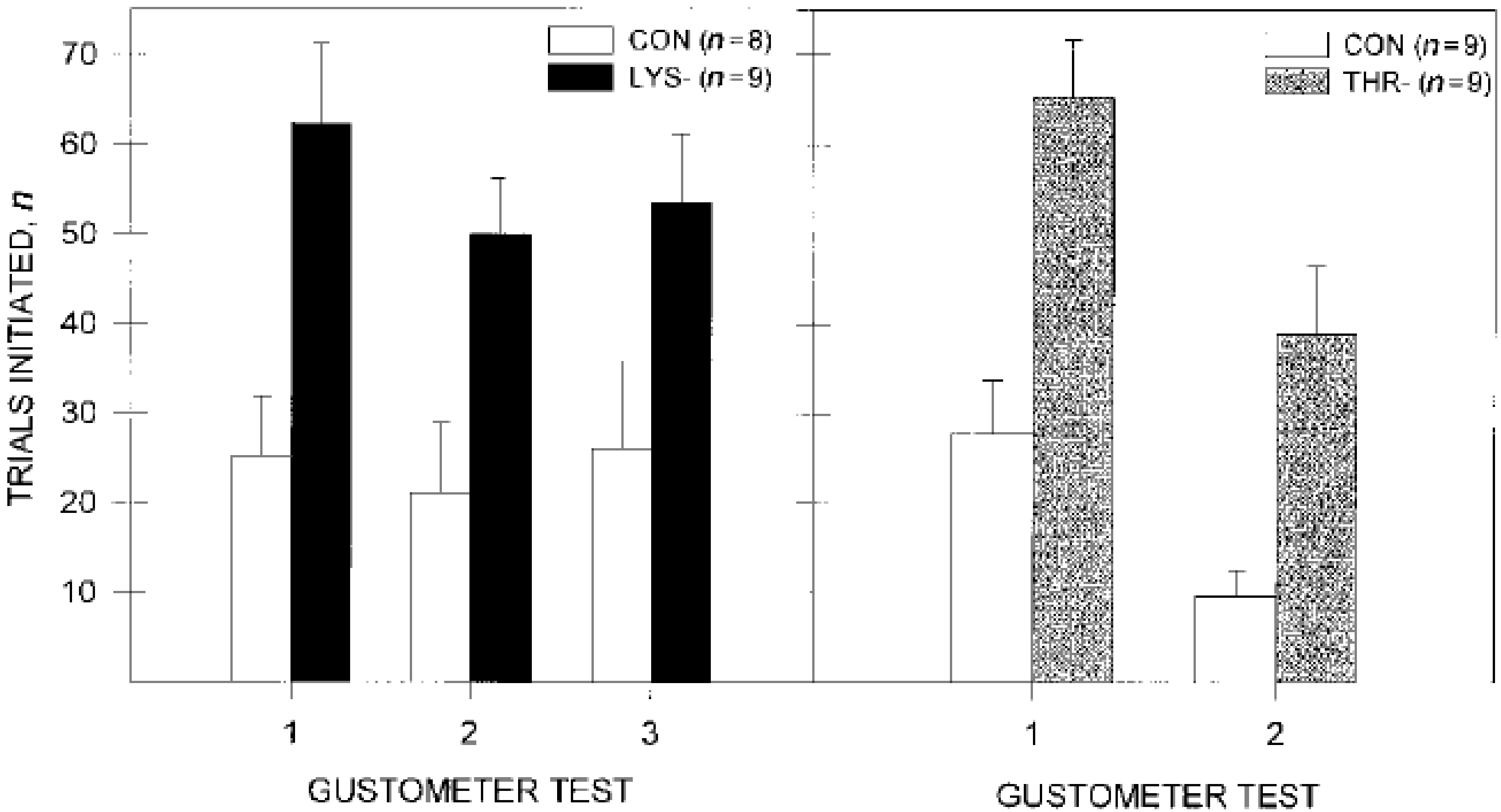


FIGURE 2 Number of trials initiated for the control and essential amino acid–deficient rats plotted for each gustometer test. Values are means + SEM. Control rats in both experiments (CON) initiated fewer trials than both the lysine-deficient rats (LYS-DEF) in Experiment 1 and the threonine-deficient rats (THR-DEF) in Experiment 2.

Finding:
LYS-DEF rats did not specifically licked lysine.

LYS-DEF RATS

1. innate/instinct reaction under the control of oral sensors → NO

2. a learned response under the control of oral sensors) → NO

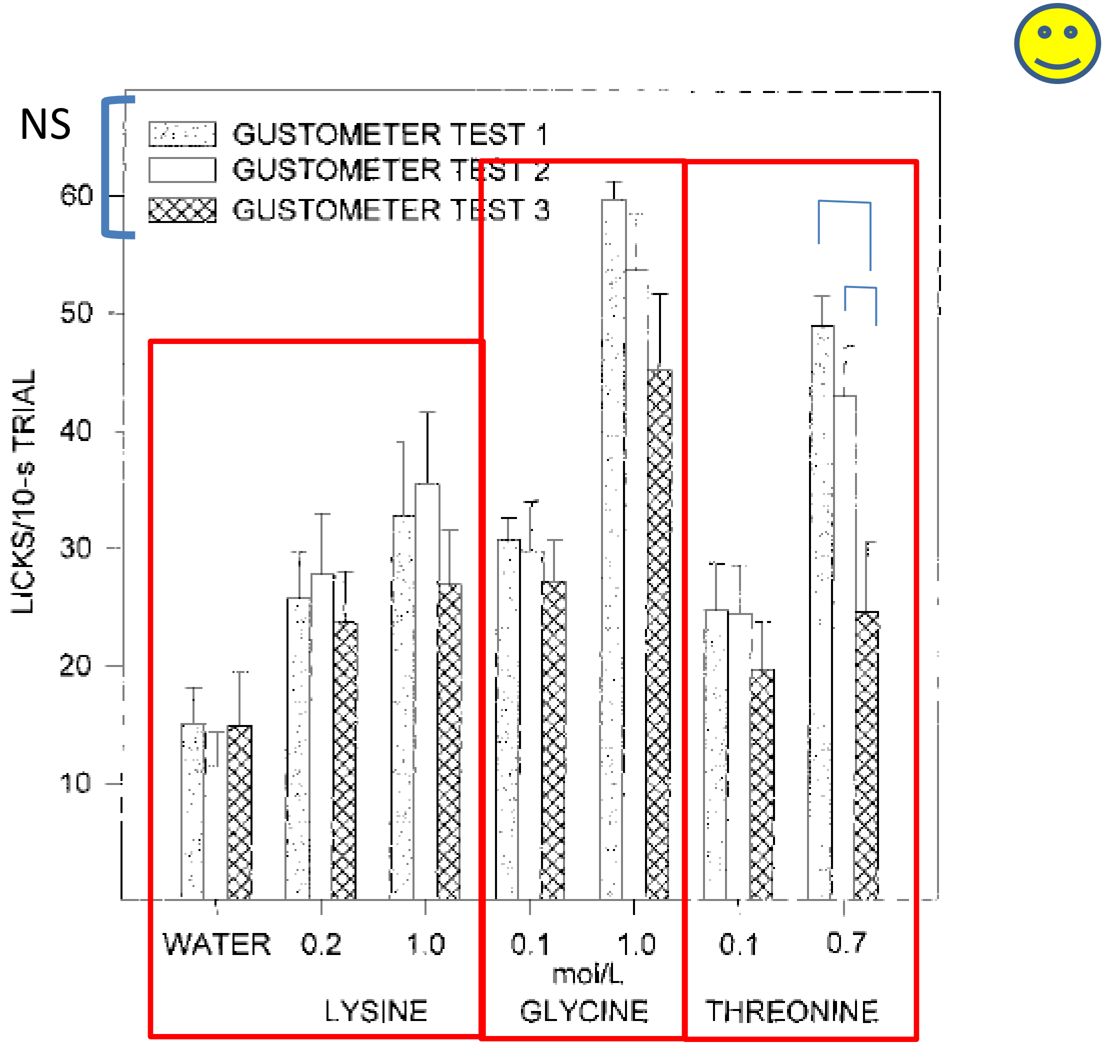


FIGURE 3 Number of licks lysine-deficient (LYS-DEF) rats took in response to each stimulus during each of three gustometer tests. Values are means + SEM, $n = 8$. Lysine-deficient rats did not show an initial heightened responsiveness to lysine nor did they increase licking to lysine on the second or third tests.

Finding:
THR-DEF rats did not specifically licked THR.

THR-DEF RATS

1. innate/instinct reaction under the control of oral sensors → NO

2. a learned response under the control of oral sensors) → NO

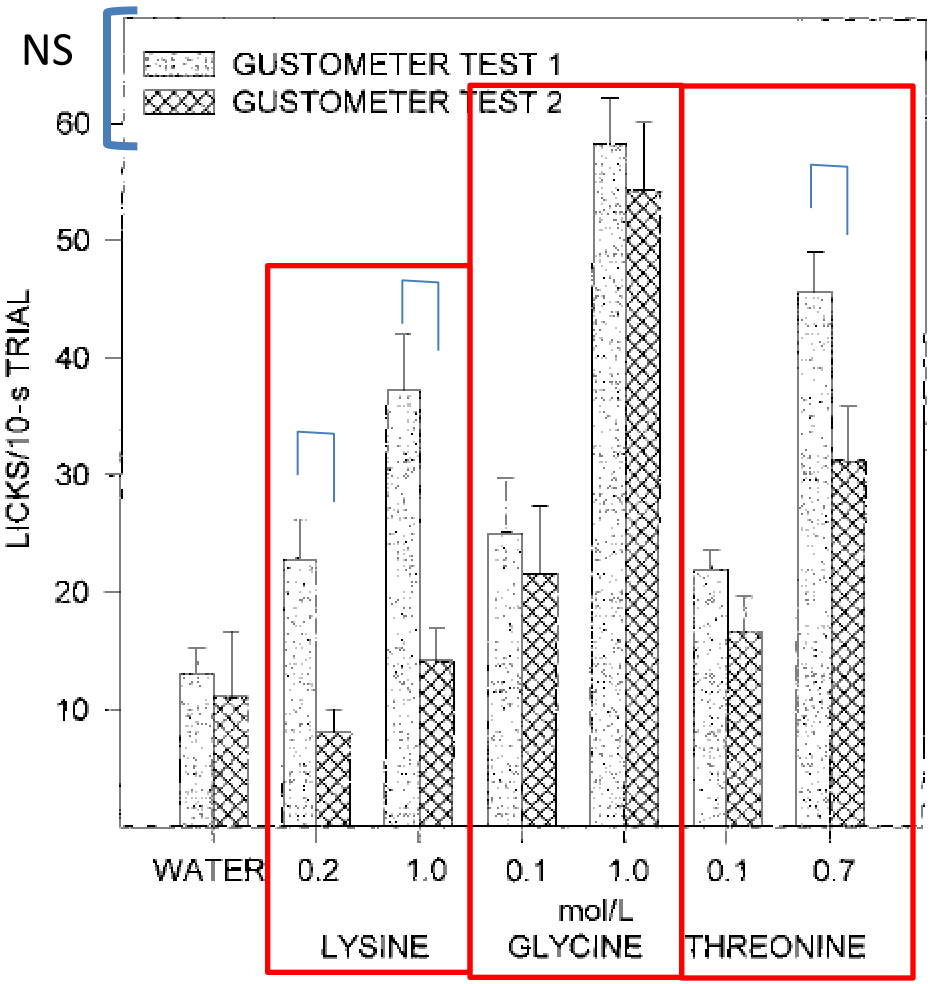


FIGURE 4 Number of licks threonine-deficient (THR-DEF) rats took in response to each stimulus during each of three gustometer tests. Values are means + SEM, $n = 7$. Threonine-deficient rats did not reveal an innate, specific appetite during the first licking test nor did they increase threonine responding during the second.

Finding: LYS-DEF rats and THR-DEF rats showed identical licking profiles

EAA-DEF: innate/instinct reaction under the control of oral sensors → NO

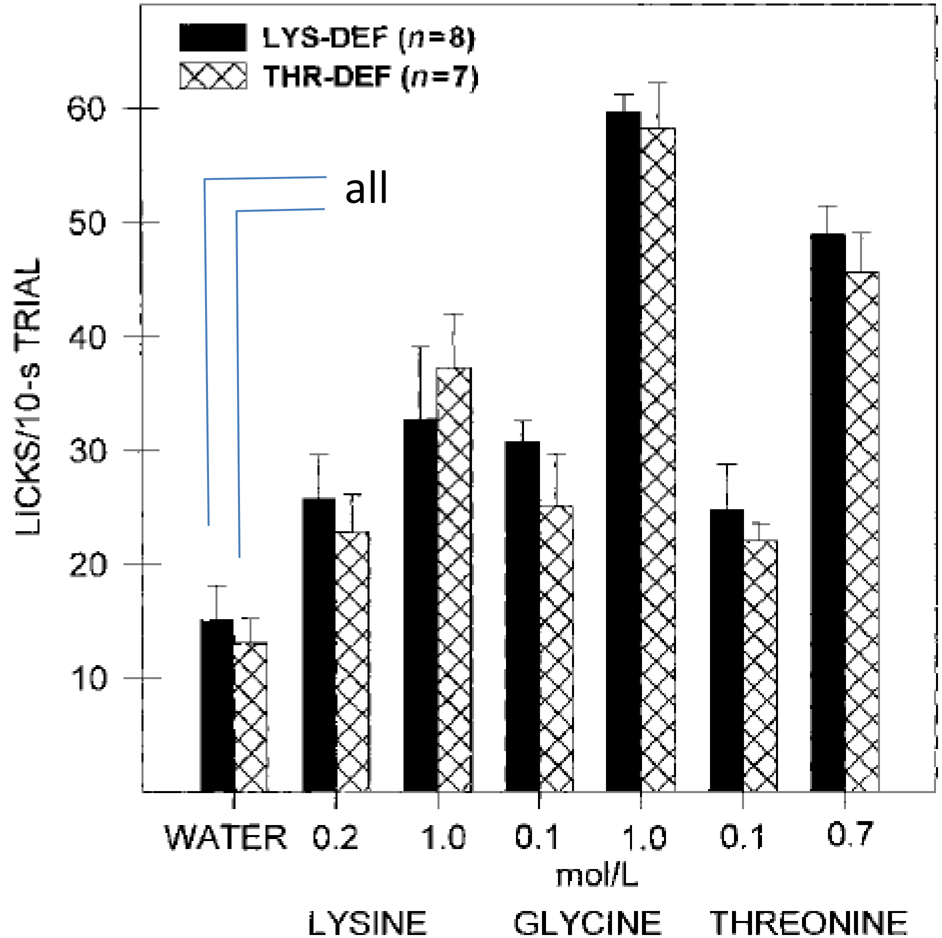


FIGURE 5 Number of licks lysine-deficient (LYS-DEF) and threonine-deficient (THR-DEF) rats took in response to each stimulus during the initial gustometer test. Values are means + SEM. These data are replotted from Figures 3 and 4 for purpose of comparison. The two essential amino acid–deficient groups showed almost identical licking profiles regardless of whether they were deficient in lysine or threonine.

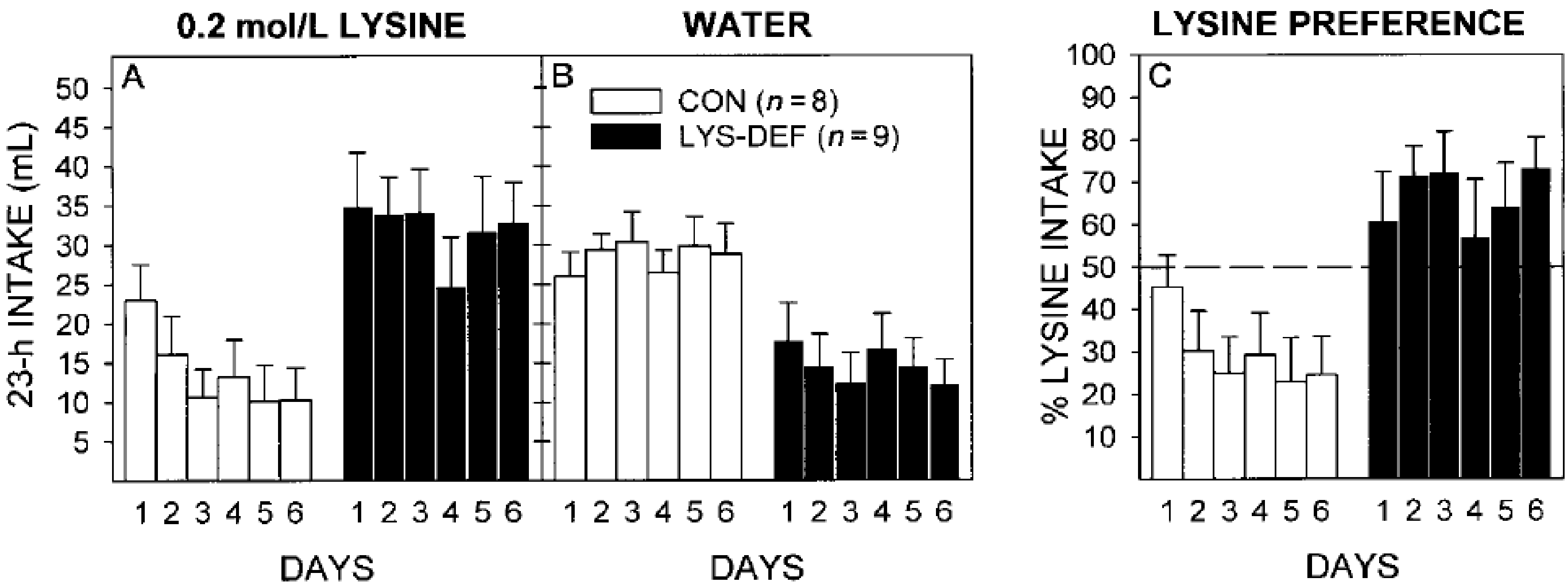


FIGURE 6 Twenty-three–hour intake in milliliters of 2.0 mol/L lysine (*panel A) and water (panel B) and the* mean percentage of total intake that was lysine (*panel C) plotted by days of* testing for control (CON) and lysine deficient (LYS-DEF) rats. Values are means and SEM.

Finding: Lysine-deficient rats showed significantly higher lysine intake and preference relative to controls.

LYS-DEF rats: innate/instinct reaction under the control of both oral-sensory and post-ingestive cues → YES

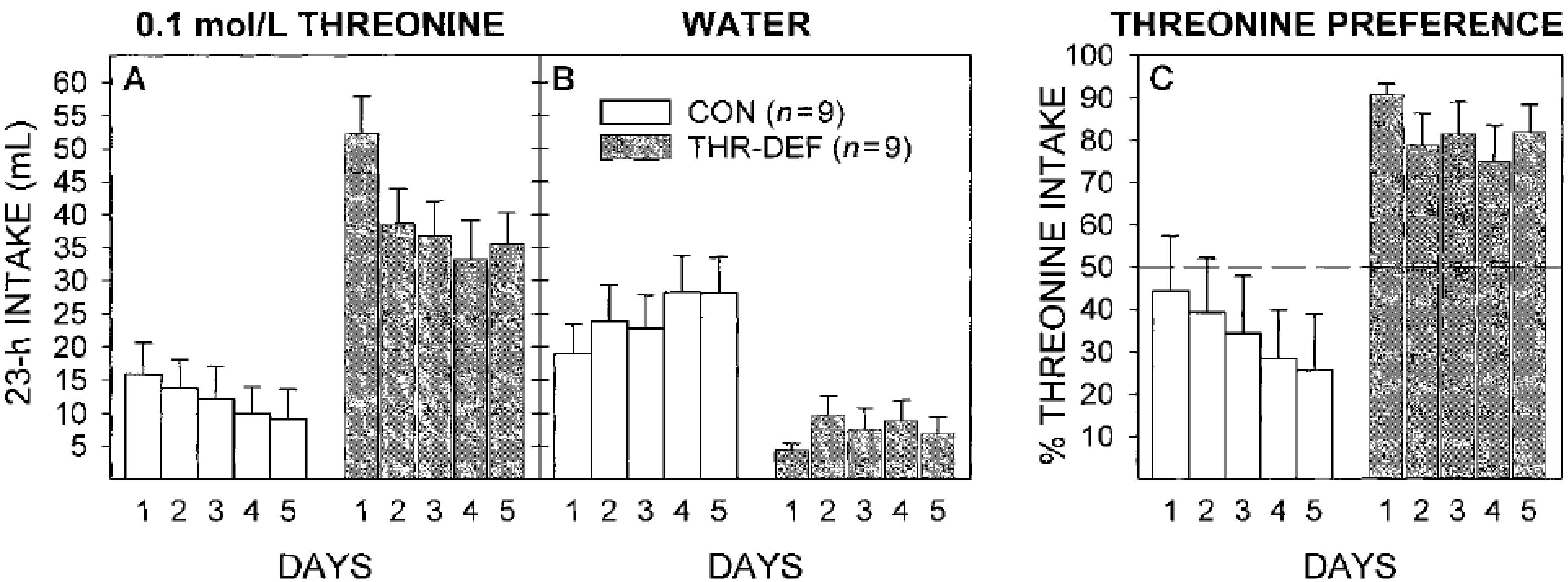


FIGURE 7 Twenty-three–hour intake in milliliters of 0.1 mol/L threonine (*panel A) and water (panel B) and the* mean percentage of total intake that was threonine (*panel C) plotted by* days of testing for control (CON) and threonine-deficient (THR-DEF) rats. Values are means + SEM.

Finding: THR-DEF rats showed significantly higher THR intake and preference relative to controls.

THR-DEF rats: innate/instinct reaction under the control of both oral-sensory and post-ingestive cues → YES

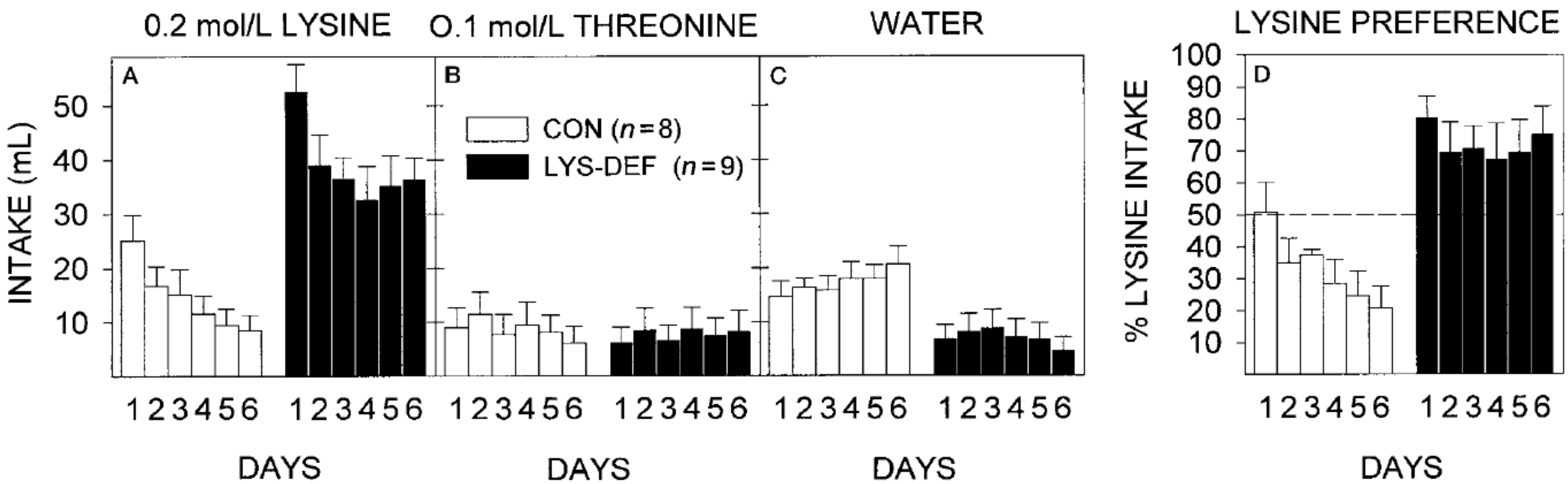


FIGURE 8 Twenty-three–hour intake in milliliters of 2.0 mol/L lysine (panel A), 0.1 mol/L threonine (panel B) and water (panel C) and the mean percentage of total intake that was lysine (panel D) plotted by days of testing for control (CON) and lysine-deficient (LYS-DEF) rats. Values are means 1 SEM.

Finding: Lysine-deficient rats showed significantly higher lysine intake and preference relative to controls. LYS-DEF rats did not show higher intake of THR.

Findings

- LYS-DEF/THR-DEF rats did not specifically licked deficient amino acid.
- LYS-DEF/THR-DEF rats showed higher intake of deficient amino acid.

Not innate and taste-guided appetite.

Appetite controlled by both oral sensory and post-ingestive cues?

Discussion

Comparison with Sodium deficiency induced appetite:

1. Innate or unconditioned appetite. No leaning required;
2. Appetite is specific to sodium.
3. Sodium-specific appetite has been demonstrate in short-duration and long-duration tests.
4. Sodium-specific appetite is taste-guided; experiments that minimize post-ingestive signals.

EAA deficiency cause:

1. ~~Innate or unconditioned appetite~~. ←No difference btw LYS-DEF and THR-DEF; comparison with CON not possible.

2. ~~Appetite is specific to sodium.~~ ←EAA deficient rats appear to be "motivated"; increase appetitive behavioral responding.
3. Sodium-specific appetite has been demonstrate in ~~short-duration and~~ long-duration tests. ←EAA appetite is under the control of both oral-sensory and post-ingestive cues.

   How? Information from oral sensors, sensors in portal vein and other sensors are integrated in frontal association cortex, which stimulate specific appetite? (Torii, 2010)

4. ~~Sodium-specific appetite is taste-guided; experiments that minimize post-ingestive signals~~. ← EAA appetite is under the control of both oral-sensory and post-ingestive cues.

Why rats could not specify RYS/THR after preference test?
Why rats could not learn?

1. Amino Acid concentration was not sufficient for taste system?

- Rats responded more to amino acid solutions than to water
- Electrophysiologic measures demonstrate that the gustatory system responds to the concentrations of amino acids used in these experiments.
- Pritchard and Scott (1982) found the lowest concentration that evoked a response in the whole nerve to be 0.8 mmol/L for LYS, 20 mmol/L for THR and 3.5 mmol/L for GLY.

2. Methodological flaw?

-Not prefect procedure to produce ”Conditioned preference”, because no flavor was added to nonnutritive substance (i.e., water) which is required by Pavlovian discipline. ?

Question Remained:

Do rats learn an association between the taste of the amino acid and the beneficial results of ingesting it when they are deficient?

Does this association lead to a conditioned preference for the EAA that guides future behavior?

Do EAA-deficient rats increase intake of the EAA based solely on need at the time of ingestion, responding to more immediate post-ingestive feedback?