Time Course and Pattern of Compensatory Ingestive Behavioral Adjustments to Lysine Deficiency in Rats\textsuperscript{1,2}

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ABSTRACT We and others have demonstrated that rats deficient in an essential amino acid (EAA) will consume sufficient quantities of the lacking nutrient to produce repletion when it is made available in solution. In the current series of experiments, we made rats deficient in lysine (LYS) by limiting the level of this EAA in the diet. We then examined licking behavior during ~23-h two-bottle intake tests over 4 consecutive days. In three separate experiments, rats were presented with the following: 1) 0.1 mol/L LYS and water, 2) 0.2 mol/L threonine (THR) and water and 3) 0.1 mol/L LYS and 0.2 mol/L THR. Lysine-deficient (LYS-DEF) rats drink significantly more LYS than did nondepleted controls (CON) when this amino acid was available. Meal pattern analysis revealed that the enhanced intake of LYS occurred as a function of a greater number of ingestive bouts, not changes in bout size. A cumulative analysis of LYS intake between CON and LYS-DEF rats revealed that a potentiation of intake developed within 30 min of sampling the solution when LYS and water were available and within 90 min when LYS and THR were the contrasting choices. In conclusion, increased LYS intake in the deficient rats occurs relatively rapidly and appears to be at least somewhat specific. Moreover, LYS deficiency does not seem to enhance the palatability of the limiting amino acid as judged by behaviors such as lick rate and bout size. Instead, LYS-DEF rats relieve the deficiency by increasing the number of drinking episodes initiated. J. Nutr. 130: 1320–1328, 2000.

KEY WORDS: • meal pattern analysis • threonine • rats • specific appetite • two-bottle test

Essential amino acids (EAA),\textsuperscript{1} the building blocks of protein, must be obtained from the diet because the body cannot produce them. Severe decreases in levels of an EAA impair physiologic function and reduce feeding in rats (for reviews see Harper et al. (1970) and Gietzen (1993)), making this particular dietary component an especially interesting model for studying recovery from nutrient deficiency. Rats can recover from such deficiencies when given selections of food items or solutions replete in the specific EAA they are lacking. EAA appetite has been demonstrated in choice tests for deficiency of threonine (Gietzen et al. 1992, Leung et al. 1986), tryptophan (Mori et al. 1991), isoleucine (Naito-Hoppe et al. 1993), methionine (Feng et al. 1975), histidine (Rogers and Harper 1970, Sanahuja and Harper 1962), lysine (LYS; Markison et al. 1999, Torii et al. 1986) and valine (Murphy and King 1989). Consistent with previous work, we have recently shown that lysine-deficient (LYS-DEF) rats preferred LYS solution when it was presented (with water or another amino acid) on the home cage during long-term (i.e., 23-h) intake tests (Markison et al. 1999). Interestingly, however, there were no observable differences between dietary groups in their licking responses to LYS during short-duration tests (10 s). This finding suggests that “LYS appetite” is not immediate. Rather, enhanced LYS consumption, as a result of deficiency, appears to develop over time. The fact that LYS-DEF rats preferred LYS in long-duration tests, but not short-duration tests, raises critical questions concerning when and how EAA appetite is expressed over 23-h periods. We used a meal pattern analysis to address these issues.

The amount consumed (of either a solid or liquid nutritive stimulus) over the course of a day is a result of the combination of bout size and number. Meal pattern analysis quantifies these and other components of ingestive behavior. In doing so, it is possible to determine how various gustatory, physiologic, pharmacologic or surgical manipulations influence intake variables (Davis 1989). Meal pattern analysis is an ideal tool with which to explore the deficient rat’s adaptive increased intake of LYS. This type of detailed analysis can set the boundaries for the search of the physiologic mechanisms underlying recovery from LYS deficiency. For example, the development of an appetite can be tracked temporally; at what point in time do LYS-DEF rats begin drinking more LYS relative to their non-

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\textsuperscript{5} Abbreviations used: CON, control; EAA, essential amino acid(s); LYS, lysine; LYS-DEF, lysine-deficient; THR, threonine.

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deficient counterparts? Given that LYS-DEF rats do not show an amplified response to LYS immediately (Markison et al. 1999), the characterization of this time course is important and may reveal the minimum amount of time that is required for the signal(s) of repletion to be interpreted and associated with taste cues, thereby setting the functional boundaries for any search for the underlying physiologic mechanism(s).

Meal pattern analysis represents a model of homeostasis-based behavior that can be compared with other depletion-induced compensatory ingestive responses, both learned and unlearned. There are several ways in which LYS-DEF rats can enhance their intake of LYS. It is possible that LYS-DEF rats will increase bout size (number of licks/bout); this could be interpreted as reflecting a shift in the palatability of LYS. That LYS deficiency enhances the palatability of LYS would be further supported by increases in rate (licks/s) of ingestion during a bout. An analysis of the meal patterns of LYS-DEF rats ingesting LYS would yield information pertaining to the rat's hedonic evaluation of this stimulus. The LYS-DEF rat may also increase LYS intake by increasing the number of bouts initiated. This outcome would suggest that deficiency may not render LYS more "palatable," but may increase ingestion by virtue of relieving the deficiency. In other words, the LYS-DEF rat might treat LYS as a sick human would treat a bitter-tasting medicine. Ultimately, the question that remains is how does the animal make these adjustments in the service of correcting the deficiency? Without such knowledge, a comprehensive understanding of the physiologic/neural mechanisms subserving such adaptive ingestive behavior will be difficult to attain.

Additionally, we examined the chemospecificity of elevated intake in LYS-DEF rats to explore the nature of the appetite stimulated by specific EAA deficiency. In particular, does amino acid deficiency promote an appetite that is distinct for the needed amino acid or does it generalize to other amino acids? This type of "general appetite" has been shown to occur in calcium-deficient rats. Calcium deficiency induced up to an eightfold elevation in daily intake of 0.3 mol/L NaCl relative to control rats (Tordoff et al. 1990).

Each of the following questions was addressed in this study: 1) What adjustments in meal pattern (e.g., bout number, bout size), if any, accompany the deficiency-induced enhanced LYS responsiveness? 2) Does enhanced intake occur in response to another nonlimiting amino acid, THR? 3) What is the developmental time course of the enhanced LYS responsiveness (determined by cumulative licking over 23 h) during both one- and two-amino acid choice tests? Potentially, the results generated from the LYS-DEF rat may be generalizable to behavioral recovery from other specific nutrient deficiencies.

**MATERIALS AND METHODS**

**Animals.** Adult male Sprague-Dawley rats (Charles River, Wilmington, MA) were studied. In Experiment 1, 14 rats weighing an average of 364 ± 22.6 g at the start of the experiment were used. Two groups (n = 16/group), weighing 415 ± 20.4 and 283 ± 8 g at the start of the experiments, were used in Experiments 2 and 3, respectively. Rats were housed singly in modified hanging wire mesh cages (described in detail below) in a room with a r 12-h light-dark cycle (lights on 0800–2000 h). Rats had free access to a nonpurified powdered diet (Purina Chow 5001; Ralston-Purina, St. Louis, MO) and distilled water before the beginning of the experiments and during the spout-licking habituation phase. When the experiments began, rats were given the experimental diets and distilled water. During testing, rats' solutions were present on the cage (described below). The protocols described here were approved by the Institutional Animal Care and Use Committee of the University of Florida.

**TABLE 1**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>LYS basal</th>
<th>LYS-CON</th>
<th>LYS-DEF</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>g/kg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L-Arginine HCl</td>
<td>10.0</td>
<td>10.0</td>
<td>10.0</td>
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<tr>
<td>L-Asparagine</td>
<td>10.0</td>
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<tr>
<td>L-Serine</td>
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<td>3.5</td>
<td>3.5</td>
</tr>
<tr>
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<td>10.0</td>
</tr>
<tr>
<td>Glycine</td>
<td>10.0</td>
<td>10.0</td>
<td>16.4</td>
</tr>
<tr>
<td>L-Glutamic acid</td>
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<td>30.0</td>
<td>30.0</td>
</tr>
<tr>
<td>L-Alanine</td>
<td>3.5</td>
<td>3.5</td>
<td>3.5</td>
</tr>
<tr>
<td>L-Histidine HCl·H2O</td>
<td>3.0</td>
<td>11.0</td>
<td>11.0</td>
</tr>
<tr>
<td>L-Isoleucine</td>
<td>4.0</td>
<td>14.0</td>
<td>14.0</td>
</tr>
<tr>
<td>L-Leucine</td>
<td>4.0</td>
<td>18.0</td>
<td>1.0</td>
</tr>
<tr>
<td>L-Lysine HCl</td>
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<td>20.0</td>
<td>1.0</td>
</tr>
<tr>
<td>L-Methionine</td>
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<td>9.0</td>
</tr>
<tr>
<td>L-Cystine</td>
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<td>6.0</td>
</tr>
<tr>
<td>L-Phenylalanine</td>
<td>4.5</td>
<td>14.5</td>
<td>14.5</td>
</tr>
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<td>L-Tyrosine</td>
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<td>8.5</td>
</tr>
<tr>
<td>L-Threonine</td>
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<td>8.0</td>
</tr>
<tr>
<td>L-Tryptophan</td>
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<td>3.0</td>
</tr>
<tr>
<td>L-Valine</td>
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<td>15.0</td>
</tr>
<tr>
<td>Sucrose</td>
<td>256.4</td>
<td>222.0</td>
<td>230.03</td>
</tr>
<tr>
<td>Cornstarch</td>
<td>512.70</td>
<td>446.89</td>
<td>460.06</td>
</tr>
<tr>
<td>Corn oil</td>
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</tr>
<tr>
<td>Mineral mix, AIN-76²</td>
<td>50.0</td>
<td>50.0</td>
<td>50.0</td>
</tr>
<tr>
<td>Sodium acetate</td>
<td>6.8</td>
<td>17.1</td>
<td>8.5</td>
</tr>
<tr>
<td>Vitamin mixture³</td>
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</tr>
<tr>
<td>Ethoxyquin</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
</tr>
</tbody>
</table>

1 Abbreviations: LYS, lysine; CON, control; DEF, deficient.
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, 3.45; folic acid, 2; inositol, 110; menadione, 50; niacin, 99; pyridoxine HCl, 22; riboflavin, 22; thiamin·HCl, 22; retinyl palmitate, 40; cholecalciferol, 1.0; tocopherol acetate, 324; cornstarch, 4657.

**Experimental diets.** Specific amino acid deficiency was achieved by providing rats with a diet that contained a low proportion of LYS (see Table 1). First, rats were given free access to a basal diet for 7 d. The basal diet consisted of 11% protein and all the EAA, but was slightly limiting in the EAA. LYS. Rats were fed the basal diet before the diet to reduce stores of circulating, unmetabolized free amino acids and proteins so that deficiency could be induced rapidly upon feeding the EAA-deficient diet [see Gietzen and Beverly (1992)]. After 7 d of consuming the basal diet, rats were divided into two groups, matched as closely as possible for body weight and basal diet intake. The control (CON) group was fed a complete, amino acid–balanced diet consisting of 20.4% protein and a total of 2% LYS. The LYS-DEF diet consisted of 19.4% protein and contained a total of only 0.1% LYS. Nitrogen levels were equated between the two diets by adding the nonessential amino acid glycine to the LYS-DEF diet. Diets were obtained from Harlan Teklad (Madison, WI) in powdered form and stored at 4°C.

**Chemical stimuli.** Amino acids (i.e., L-lysine and L-threonine) f.r solutions were obtained from Sigma Chemical (St. Louis, MO). Stimuli were remade daily with room-temperature distilled water. The concentrations of LYS and THR for these experiments were determined on the basis of previous research; thus we did not simply use concentrations of equal molarity. The selected concentrations were above the threshold for the chorda tympani nerve as determined by electrophysiologic recordings (Pritchard and Scott 1982). Furthermore, in short-duration tests (which are believed to be guided by oral sensory input), rats licked these concentrations of LYS and THR significantly more than water, indicating the behavioral relevance of these amino acid stimuli (Markison et al. 1999).
**TABLE 2**

**General procedures for Experiments 1, 2 and 3**

<table>
<thead>
<tr>
<th>Experiment 1 LYS vs. WAT</th>
<th>Experiment 2 THR vs. WAT</th>
<th>Experiment 3 LYS vs. THR</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sample size</strong></td>
<td><strong>Sample size</strong></td>
<td><strong>Sample size</strong></td>
</tr>
<tr>
<td>LYS-DEF, n = 7; CON, n = 7</td>
<td>LYS-DEF, n = 8; CON, n = 8</td>
<td>LYS-DEF, n = 8; CON, n = 8</td>
</tr>
<tr>
<td>364 g ± 22.6</td>
<td>415 g ± 20.4</td>
<td>283 g ± 8.0</td>
</tr>
<tr>
<td>Spout-licking habituation</td>
<td>Spout-licking habituation</td>
<td>Spout-licking habituation</td>
</tr>
<tr>
<td>Sipper tube lengths varied over 3 d.</td>
<td>Sipper tube lengths varied over 3 d.</td>
<td>Sipper tube lengths varied over 3 d.</td>
</tr>
<tr>
<td>7 d—basal diet</td>
<td>7 d—basal diet</td>
<td>7 d—basal diet</td>
</tr>
<tr>
<td>10 d—exp. diets</td>
<td>10 d—exp. diets</td>
<td>10 d—exp. diets</td>
</tr>
<tr>
<td>Choice test 1 (4 d)</td>
<td>Choice test 1 (4 d)</td>
<td>Choice test 1 (4 d)</td>
</tr>
<tr>
<td>0.2 mol/L LYS vs. water</td>
<td>0.1 mol/L THR vs. water</td>
<td>0.2 mol/L LYS vs. 0.1 mol/L THR</td>
</tr>
</tbody>
</table>

1 Abbreviations: BW, body weight; CON, control; LYS, lysine; LYS-DEF, lysine-deficient; THR, threonine.

**Apparatus.** The apparatus used to measure licking over the 23-h period was a revised version of that described previously by Spector and Smith (1984). Briefly, 16 Hoelte hanging wire mesh cages were modified to include three ports for measurement of liquid and food intake. There were two slits in the back of the cage allowing access to two stainless steel drinking spouts located just outside the cage. Thus, the rat’s tongue had to protrude just beyond the slit opening to contact the spout. The rat was required to extend its head inside a feeding compartment on the front of the cage for access to food. Phototransistors and infrared light-emitting diodes were positioned so that kicks on either of the two spouts and entries into the feeder interrupted a beam. The beam interruptions were transmitted to a computer and recorded in 6-s bins. Intake patterns were measured for 23 h, allowing a 1-h period each day for refilling solution bottles and food cups, and measuring body weight.

**General procedure.** The procedures for Experiments 1, 2, and 3 are summarized briefly in Table 2. The rats in all three experiments were habituated to the Hoelte cages described above for at least 3 d before presentation of the basal diet. The length of the sipper tubes was varied over the 3 d such that on d 1, the tubes were long enough to protrude through the slit and into the cage. On d 2, the tubes were slightly shorter, and on d 3, the rats were presented with the tube length that was used throughout the rest of the experiment. These tubes did not protrude into the cage; the rat was required to extend its tongue through the slit, outside of the cage, to make contact with the spout. This was done to prevent accidental beam breaks. During the spout-licking habituation and the dietary manipulation phases of the experiments, rats had access to two bottles filled with distilled water. After habituation, all rats were fed the basal diet for 7 d. For the next 10 d, the rats in the top two rows of cages were fed the LYS-DEF diet, whereas the bottom two rows of rats were fed the CON diet. Body weight was measured daily and used to monitor the deficiency; other investigators have shown that when rats are fed a diet that is devoid of or low in a single EAA, food intake is decreased and weight loss occurs (Elvehjem and Krehl 1955, Harper 1958).

After the depletion phase of the experiment, LYS-DEF and CON rats were presented with a choice of solutions. In Experiments 1 and 2, rats were presented with distilled water and one amino acid solution, 0.2 mol/L LYS or 0.1 mol/L THR, respectively. In Experiment 3, rats were presented with a choice between two amino acids, 0.2 mol/L LYS and 0.1 mol/L THR. Body weight, and diet and solution intakes were measured daily between 1400 and 1500 h.

**Statistics.** Body weights were converted to percentage of initial body weight by dividing each rat’s body weight on a given day by its body weight on the day before the basal diet was presented. These values were then compared using two group × day ANOVA for the two diet presentation phases of the experiment (7 d of basal diet feeding and 10 d of experimental diet feeding). Subsequent Bonferroni-adjusted independent (unpaired) Student’s t tests were performed between groups for each day of the experiment.

When rats were given two-bottle intake tests that included LYS, in all experiments, LYS-DEF rats (as a group) significantly increased their daily LYS intake relative to CON rats (see Table 3). There was variability among individuals, however. A few rats showed only modest increases or did not increase LYS intake. It was not meaningful to include such rats in the analysis of the meal patterns because the goal of this analysis was to describe changes in meal pattern that correspond to the adaptive preference for LYS. Therefore, rats were excluded from statistical analyses if they did not fall above the 95% confidence interval of the CON group with respect to both 4-d LYS intake and 4-d LYS preference. Because this was the case, one rat in Experiment 1, one rat in Experiment 2 and two rats in Experiment 3 were not included in the analysis. It should be emphasized that the main effect of dietary treatment group on LYS intake was still significant when these rats were included in the analysis (Table 3).

Intake measured in milliliters and LYS preference (LYS intake divided by total intake, then multiplied by 100) were examined with group × day ANOVA. Significant main effects of day were examined using Bonferroni-adjusted paired t tests. Significant group × day interactions were examined using Bonferroni-adjusted unpaired t tests comparing groups on each of the four test days.

In addition to intake measured in milliliters, the number of licks (in 6-s bins) that each rat took at each drinking spout (i.e., left bottle, right bottle) during 23-h periods was determined. For the cumulative licking analysis, these data were collapsed into 15-min intervals, providing the number of licks taken to each of the stimuli during 92 consecutive 15-min bins. The number of licks per bin was counted beginning after a given rat’s first lick. This was done to track the time course of intake relative to the rat’s first exposure to the stimulus. Unpaired t tests at each of the 92 time intervals on d 1 were used to compare cumulative licking between dietary groups.

For meal pattern analysis, the lick data were summarized into bouts. Typically, rats eat and drink in bouts of behavior, which can be operationally defined. A bout was defined using the following three criteria: 1) a bout started with 3 licks within a 6-s bin; 2) a bout ended when there were no licks for 5 consecutive minutes; and 3) bouts <30 licks were excluded from the bout analysis. These criteria allow for a comprehensive characterization of the pattern of test stimulus ingestion. Typically, >95% of the total number of licks are included in bouts. Thus, these criteria truly capture the feeding behavior and describe it accurately. When the total lick data were divided into meal patterns, three variables were examined, i.e., bout number, bout

**TABLE 3**

Four-day lysine intake with all rats included in the analysis

<table>
<thead>
<tr>
<th>Experiment 1 LYS vs. WAT</th>
<th>Experiment 2 THR vs. WAT</th>
<th>Experiment 3 LYS vs. THR</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>LYS mg/d</strong></td>
<td><strong>LYS mg/d</strong></td>
<td><strong>LYS mg/d</strong></td>
</tr>
<tr>
<td>43.4 ± 20.1</td>
<td>46.8 ± 15.4</td>
<td>53.8 ± 8.4</td>
</tr>
</tbody>
</table>

1 Abbreviations: CON, control; LYS, lysine; LYS-DEF, lysine-deficient; THR, threonine; WAT, water.

2 Values are means ± sd; *significantly different from water or threonine intake, P < 0.01.
size (licks/bout) and ingestion rate (lick/s). Increases in overall intake can occur as a function of increases exclusively in either bout number or bout size (licks/bout), or a combination of changes in both variables.

Meal pattern variables were examined with two-way (group x day) ANOVA. Significant day main effects were examined using Bonferroni-adjusted paired t-tests. Significant group x day interactions were examined using Bonferroni-adjusted unpaired t-tests comparing groups on each of the four test days.

**RESULTS**

**Body weight.** The body weight results from all three experiments were similar (Fig. 1). There were no differences between the body weights of the dietary groups during the 7-d basal diet phase in any of the three experiments. The basal diet contained sufficient protein and was appropriately balanced with regard to amino acid content as demonstrated by the fact that all rats gained weight over the 7-d basal diet feeding period. 

[Experiment 1: F (1,6) = 132.0, P < 0.001; Experiment 2: F (1,6) = 113.0, P < 0.001; Experiment 3: F (1,6) = 205.0, P < 0.001]. Groups did differ as a function of what diet they were fed during the first 10 d when they were switched to the experimental diets [Experiment 1: F (1,12) = 61.97, P < 0.001; Experiment 2: F (1,14) = 29.57, P < 0.001; Experiment 3: F (1,14) = 129.5, P < 0.001]. Subsequent Bonferroni-adjusted t-tests revealed that the rats fed the LYS-DEF diet had significantly lower body weights relative to their CON group by d 1 after the diet was presented in Experiment 3 (all P < 0.05) and by d 2 in Experiments 1 and 2 (all P < 0.05). These results indicate that feeding rats a diet with a reduced level of LYS produces a substantial decline in body weight indicative of the deficiency.

**Experiment 1: lysine vs. water.** Figure 2 (upper panel) shows that, overall, LYS-DEF rats consumed significantly more LYS [F (1,11) = 20.22, P < 0.001] and less water [F (1,11) = 46.49, P < 0.001] than did CON rats. This pattern of two-bottle intake resulted in a significantly greater LYS preference [F (1,11) = 67.44, P < 0.001]. There were also significant main effects of day for LYS preference [F (3,33) = 3.43, P < 0.05], but no significant interactions in any of the analyses.

Meal pattern analysis revealed that LYS-DEF rats increased LYS intake by initiating significantly more LYS bouts relative to CON [Fig. 2, middle panel; F (1,11) = 25.32, P < 0.001]. Furthermore, LYS-DEF rats took significantly fewer water bouts [F (1,11) = 17.82, P < 0.001], thus resulting in a greater percentage of total bouts as LYS [F (1,11) = 56.24, P < 0.001]. A direct comparison of lysine intake and bout number reveals the close parallel between the two measures.

The other measures from the meal pattern analysis (i.e., bout size and ingestion rate) did not appear to be substantially influenced by deficiency. LYS bout size did not vary between groups. For water, however, CON rats took significantly larger bouts compared with LYS-DEF rats [F (1,10) = 6.16, P < 0.05]. Drinking rate was unaffected by LYS deficiency for both stimuli. However, there was a significant effect of day for LYS [F (3,24) = 4.63, P < 0.01]. Post-hoc tests (Bonferroni-adjusted paired t-tests, collapsed over groups because there were no interactions) revealed that LYS drinking rate was significantly slower on d 1 relative to d 3 and 4 (all P < 0.05).

**Experiment 2: threonine vs. water.** Not surprisingly, LYS-DEF rats did not increase intake of THR, a nonlimiting EAA, when it was offered on the home cage for ~23 h (see Fig. 4). There were no significant main effects of group for THR intake, water intake or THR preference. There were there any significant main effects of day or interaction. Both number and bout size also did not differ between groups.

Although there were no group differences for intake, LYS-DEF rats might have preferred THR at some time interval during the 23-h measurement period. This possibility, however, was not supported by the cumulative lick analysis. Figure 5 shows the number of cumulative licks that rats took during d 1 of THR and water presentation. At no time interval did the dietary groups differ in responsiveness to THR or water.

**Experiment 2: lysine vs. water.** As expected there were no differences between the dietary groups in response to THR (see Figs. 4 and 5). Thus, LYS intake was measured to ensure...
FIGURE 2 Twenty-three hour intake in milliliters of 0.2 mol/L lysine and water, and the percentage of total intake that was lysine all plotted by days of testing for control (CON) and lysine-deficient (LYS-DEF) rats (upper panel), and the number of bouts for 0.2 mol/L lysine and water and the percentage of the total bouts that were lysine all plotted by days of testing for control (CON) and lysine-deficient (LYS-DEF) rats (lower panel). Values are means ± SEM. Lysine-deficient rats drank more lysine than controls by increasing the number of ingestive bouts that they took.

that the typical increase in LYS responsiveness would be observed. As predicted (Fig. 6), LYS-DEF rats drank more LYS [F(1,13) = 41.92, P < 0.001] and less water [F(1,13) = 30.49, P < 0.001] relative to CON, resulting in a significantly greater preference for LYS [F(1,13) = 46.05, P < 0.001]. Consistent with the results from Experiment 1, this group of LYS-DEF rats also took significantly more LYS bouts [F(1,13) = 10.11, P < 0.01] and fewer water bouts [F(1,13) = 17.35, P < 0.01], resulting in a greater LYS preference [F(1,13) = 35.42, P < 0.001]. The results for bout size were also congruent with those from Experiment 1; there were no differences between dietary groups for LYS [F(1,10) = 0.40, P = 0.54] or water [F(1,8) = 0.11, P = 0.75]. Although the rats in this experiment were given experience with THR before they were ever presented with LYS solution, their intake and meal pattern results replicate those of Experiment 1. Although not depicted on the body weight graph, LYS-DEF rats in Experiment 2 did significantly gain weight over the 4 d they had access to LYS and water.

Experiment 3: lysine vs. threonine. When LYS and THR were presented together, LYS-DEF rats showed a pattern of behavior very similar to that described above for Experiment 1 (LYS vs. water). In response to LYS deficiency, rats increased intake of LYS even in the two-amino acid choice situation (Fig. 7, upper panel). LYS-DEF rats drank significantly more LYS than CON [F(1,11) = 36.28, P < 0.001] and less THR [F(1,11) = 2.86, P < 0.001], resulting in a significantly greater LYS preference [F(1,11) = 36.23, P < 0.001]. Furthermore, there was a significant main effect of day for LYS intake [F(3,33) = 3.92, P < 0.01] and LYS preference [F(3,33) = 3.42, P < 0.05], but no interactions. Post-hoc tests (collapsed over groups) revealed that both LYS intake and LYS preference decreased on d 2 and 4 relative to d 1 (all P < 0.05).

Bout number accounted for the increased intake (Fig. 7, lower panel); LYS-DEF rats took significantly more bouts of LYS [F(1,12) = 14.45, P < 0.01] and fewer of THR [F(1,12) = 19.58, P < 0.01] relative to CON, resulting in a significantly greater percentage of LYS bouts [F(1,12) = 45.62, P

FIGURE 3 Number of cumulative licks for lysine (upper panel) and water (lower panel) plotted in 15-min intervals during the first 23-h amino acid presentation period. Values are means; solid and dashed lines represent SEM for the control (CON) and lysine-deficient (LYS-DEF) groups, respectively. Time is plotted on a logarithmic scale to resolve the onset of the apparatus more clearly. Notice that the increase in the slope of cumulative intake is attributable primarily to the onset of the dark phase (at −min 300). Controls took fewer cumulative licks to lysine and more to water relative to lysine-deficient rats. As represented by the asterisk, this difference was significant by 30 min after sampling the LYS.
< 0.01]. There was a significant group × day interaction for both LYS bout number \( F(3,36) = 4.99, P < 0.01 \) and the percentage of total bouts that were LYS \( F(3,36) = 3.10, P < 0.01 \). Bonferroni-adjusted paired t tests revealed that LYS-DEF rats took significantly more bouts of LYS only on days 2 and 4, but LYS-DEF took a greater percentage of LYS bouts relative to CON on all 4 days (all \( P < 0.05 \)).

Neither bout size nor ingestion rate differed between the two dietary groups for LYS or THR. Collectively, these findings demonstrate that the enhanced LYS intake by LYS-DEF rats was effected entirely through adjustments in bout number rather than bout size regardless of whether the rats were presented with a choice between LYS and water or between LYS and THR.

During 1 of 4 LYS and THR presentation, the LYS-DEF group took significantly more cumulative licks to LYS relative to CON by 90 min after sampling the LYS (all \( P < 0.05 \)); the groups differed for all later intervals, except minute 225 and 300 (Fig. 8). There was considerable overlap in THR cumulative licking; the CON took significantly more cumulative licks to THR beginning at minute 855 (14 h, 15 min) and each interval thereafter. Interestingly, the addition of another chemical cue (i.e., THR) to the choice appeared to delay the expression of the LYS appetite.

**DISCUSSION**

Animals must select balanced diets in their natural environments for optimal growth and reproduction. By examination of total intake, we and others have shown that rats do indeed successfully obtain needed nutrients such as EAA (Giertz et al. 1992, Leung et al. 1968, Markison et al. 1999, Mori et al. 1991, Murphy and King 1989, Naito-Hoopes et al. 1993, Peng et al. 1975, Rogers and Harper 1970, Sanahuja and Harper 1962, Torii et al. 1986). Specifically, rats fed an imbalanced diet ingested adequate quantities of LYS solution over days such that their rate of growth became equal to that of CON rats fed nutritionally complete amino acid diets (Markison et al. 1999). In these experiments, licking behavior was surveyed to give a better description of the behavioral basis of this deficiency-induced potentiation of LYS intake.

Enhanced intake of LYS in LYS-DEF rats occurred as early as 30 min after sampling when LYS was tested against water, and ~90 min after sampling when LYS was tested against another EAA, THR. Although this is a fairly rapid onset of LYS appetite, it is likely that rats formed an association between the taste of LYS and its repetitively postgestive consequences within this time frame. In the case of sodium deficiency, an appetite believed to be “innate,” rats given sodium salts showed immediate enhanced licking (Handal 1965, Markison et al. 1995, Nachman 1962). In the case of LYS deficiency, immediate responsiveness was not observed (Markison et al. 1999).

Interestingly, the presence of a second amino acid (THR) did not eliminate the development of LYS preference. However, its expression was delayed (i.e., 90 min after stimulus sampling). This delay in preference can likely be attributed to the complexity of the discrimination. In Experiment 1, rats merely had to choose between a solution of water (a familiar
stimulus) or LYS (a "novel" stimulus), whereas in Experiment 3, rats were required to choose between two novel solutions, LYS and THR.

The latency of LYS-DEF rats to ingest LYS, determined in these experiments, generally parallels findings by other investigators in terms of detection of and behavioral reaction to dietary manipulations. Of course, measurement was made of the deficient rat's latency to prefer the needed amino acid when it was presented in solution. Another approach has been to examine the rat's latency to show an anorectic response to the presence of an insufficient diet (EAA imbalanced or deficient; Elvehjem and Krehl 1955, Harper 1958). Interestingly, the emergence of the anorectic response in EAA-deficient rats occurs within time frames very similar to what has been shown here for enhanced solution intake. For example, Leung and Rogers (1986) presented rats with a choice of sufficient and THR-deficient diets. Two to three hours after presentation of the diets, rats expressed a preference for the THR-balanced diet. This latency is slightly longer than in other experiments; however, in this paradigm, rats were required not only to reject the imbalanced diet but then demonstrate a preference for the sufficient diet. Furthermore, this paradigm required rats to make a complex discrimination. The discrimination was between two diets that presumably had similar sensory properties.

FIGURE 6 Twenty-three hour intake in milliliters of 0.2 mol/L lysine and water, and the percentage of total intake that was lysine all plotted by days of testing for control (CON) and lysine-deficient (LYS-DEF) rats (upper panel); and the number of bouts for 0.2 mol/L lysine and water and the percentage of the total bouts that were lysine all plotted by days of testing for control (CON) and lysine-deficient (LYS-DEF) rats (lower panel). Values are means ± SEM. These data were collected after intake tests with threonine and water. Although lysine-deficient rats did not increase threonine intake, they did increase lysine intake consistently (by increasing bout number). These findings are almost identical to those of Experiment 1 and support the reliability of this effect.

FIGURE 7 Twenty-three hour intake in milliliters of 0.2 mol/L lysine and 0.1 mol/L threonine, and the percentage of total intake that was lysine all plotted by days of testing for control (CON) and lysine-deficient (LYS-DEF) rats (upper panel), and the number of bouts for 0.2 mol/L lysine and 0.1 mol/L threonine and the percentage of the total bouts that were lysine all plotted by days of testing for control (CON) and lysine-deficient (LYS-DEF) rats (lower panel). Values are means ± SEM. Even when faced with a more difficult choice test (i.e., two amino acids), lysine-deficient rats still expressed a lysine preference by increasing bout number.
concluded that deficient rats may choose novel diets as a strategy to overcome nutritional deficiency. This raises the question whether EAA deficiency produces a chemospecific appetite or a general increase in consumption of any novel available substance. It is possible that the inception of a learned preference for Lys is merely a preference for anything novel. Only after the association of Lys taste and repletion consequences does a lasting Lys preference develop. This possibility was investigated in Experiment 2 by a close examination of responsiveness to THR, a novel stimulus that does not lead to recovery in the LYS-DEF rat. If EAA deficiency merely stimulates an appetite for "novelty," then one would have expected LYS-DEF rats to (at least) initially show an increase in responsiveness to any chemical and continue to ingest only those that were associated with positive postingestive benefits. Although ingestion in only 15-min intervals was analyzed, there was no evidence of increased consumption of THR, the nonlimiting amino acid, at any time during testing. These results demonstrate at least some degree of chemospecificity in behavioral responsiveness to Lys after deficiency and do not support the notion that LYS-deficient animals simply increase consumption of any novel stimulus.

Another important and consistent finding in this series of experiments was that enhanced intake of Lys in LYS-DEF rats can be accounted for by an increase in bout number and not an increase in bout size. In other words, LYS-DEF rats initiate more ingestive bouts of lysine relative to controls, but once a drinking episode is begun, they do not increase the amount they consume. This may mean that LYS deficiency does not serve to elevate the "palatability" of LYS. The definition and utility of the term palatability have been the center of some debate over the years (Berridge 1996, Kissileff 1990, Pfaffmann 1960, Ramirez 1990, Rogers 1990, Young 1959). For our purposes, palatability can be considered a hypothetical construct that cannot be observed directly, but can be inferred from or defined operationally by certain behaviors including those described by meal pattern variables (i.e., bout size, bout number) and within-bout ingestion rate [see Smith (2000)].

Sucrose, for example, can be considered palatable because it serves as an effective reward in operant paradigms (Conover and Shizgal 1994, Reilly 1999). Furthermore, a so-called "palatable" stimulus such as sucrose elicits distinct adjustments in meal pattern variables. By way of comparison, it is useful to review briefly meal pattern analysis findings by other investigators that have used sucrose. Spector and Smith (1984) showed that as the concentration of sucrose was increased up to midrange concentrations, nondeprived rats responded by increasing both bout size and bout number. Smith et al. (1987) showed that when rats were given access to 32% sucrose, it was ingested in prolonged bouts. These experiments support the notion that in response to "palatable stimuli" such as sucrose, rats increase bout number as well as bout size.

In contrast to behavioral responding to sucrose, the LYS-DEF rat consuming Lys increased only bout number and not bout size. It is tempting to speculate that under conditions of deficiency the hedonic value of Lys would increase; bout size and bout number would be elevated relative to water intake (in LYS-DEF rats) and relative to LYS intake in nondepleted control rats. But this was not the case. Whether LYS-DEF rats were presented with a choice between Lys and water or between LYS and THR, they increased LYS intake consistently as a function of bout number, not size. That LYS deficiency does not appear to enhance the palatability of LYS is further supported by the analysis of lick rate. Lick rate has been used as an index of palatability (Davis & Smith 1988, Davis 1989, Smith 2000). For example, when rats consumed

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**FIGURE 8** Number of cumulative licks for 0.2 mol/L lysine (upper panel) and 0.1 mol/L threonine (lower panel) plotted in 15-min intervals during the first 23-h amino acid presentation period. Values are means; solid and dashed lines represent SEM for the control (CON) and lysine-deficient (LYS-DEF) groups, respectively. Time is plotted on a logarithmic scale to resolve the onset of the appetite more clearly. Notice that the increase in the slope of cumulative intake is attributable primarily to the onset of the dark phase, which occurred at 8 min. Controls took fewer cumulative licks to lysine and more to threonine relative to lysine-deficient rats. Interestingly, the addition of another chemical cue (i.e., threonine) to the choice test appeared to delay the expression of the lysine appetite from 30 to 90 min. The asterisk represents the first time interval in which the lysine-deficient group took significantly more licks to lysine relative to controls.
sucrose, within-bout drinking rate increased with concentration (Spector and Smith 1984). However, the LYS-DEF rat showed no differences in lick rate to LYS relative to water or nondeficient controls.

Collectively, the pattern analysis of ingestive behavior in LYS-DEF rats revealed the following: 1) LYS deficiency produces a specific appetite that is expressed relatively quickly but not immediately. In two-bottle tests, the appetite emerged as early as 30 min after sampling when LYS was presented with water, and as late as 90 min when LYS was tested with THR. 2) LYS deficiency does not seem to enhance the palatability of the limiting amino acid as judged by behaviors such as lick rate and bout size. Instead, it appears that LYS-DEF rats relieve the deficiency by increasing the number of drinking episodes initiated. These findings highlight the advantages of analyzing ingestion as a series of moment-by-moment behavioral events, instead of examining merely total output (e.g., intake).

LITERATURE CITED