

# **Nutritional deficiency of an essential amino acid L-Lysine (physiological recognition and responses)**

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## Abstract

A risk of deficiency in the essential amino acid, L-Lysine (Lys) exists in developing regions where cereals supply the major proportion of energy. Dietary Lys inadequacies have also been reported among the elderly in developed countries. However, little is known about the physiological consequences of such a deficiency. Experimental animals respond to a Lys-deficient diet with anorexia and an increase in taste preferences for a previously aversive (bitter) Lys solution. These responses are triggered by metabolic and blood-borne factors, including a rapid decline of plasma Lys, suppression of plasma activin A and up-regulation of the vagal Lys sensors. All those factors inform the brain about a dietary Lys deficiency. Magnetic resonance imaging (MRI), single neuron activity electrophysiological recording and circadian microdialysis have been used to identify the lateral and the ventromedial areas of the hypothalamus as the main regulators of the physiological responses to Lys deficiency. Applied to awake, Lys-deficient rats, these techniques have also shown that the neural plasticity of specific neurons and a rapid decline of norepinephrine release in the ventromedial area contribute to taste preference changes that accompany recovery from Lys deficiency.

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## ***Introduction***

Amino acids (AA) are the building blocks for proteins, and their nutritional homeostasis must remain well preserved. The cephalic and visceral stimuli that are detected during a protein meal yield nutritional information about its AA content and aid in its digestion (1). In normal conditions, protein-based foods having a pleasant taste [salty, *umami* (savory or broth-like taste)] are preferred, while those that signal toxicity or spoilage (bitter, sour) are avoided. However, both animals and humans must have a mechanism for preference changes, based on nutritional balance of the essential AA. If the balance is disturbed and AA is deficient, animals start searching for AA and the previous taste preference shifts (2). The anorectic response to an AA-deficient diet is brain-mediated (3). Among other essential AA deficiencies, L-Lysine (Lys) has been detected as a limiting amino acid (AA) in the diet of the elderly in developed countries. Low Lys availability occurs in several developing countries, where a significant portion of the population is dependent predominantly on cereals for their protein supply. Traditionally, many populations supplement their wheat-based diets with Lys-rich legumes and beans, but recent deteriorating economic conditions have worsened this dietary balance. In fact, double-blind trials in rural areas of China and Pakistan showed that Lys supplementation of wheat flour significantly improved the growth of children, along with a number of immunological indicators (5). This short review will discuss the brain mechanisms for the recognition of Lys deficiency in rats.

## ***Fluid-Intake and Operant-Type Behavior in Lys-Deficient Rats***

Lys-deficient rats prefer a solution containing Lys during long-term intake tests, showing that the animals are able to compensate for a deficient AA diet by consuming the missing AA in solution (6). Although a selection of 15 AA solutions was presented to the deficient rats, they preferred the Lys solution, although it had an initially unpleasant, bitter taste. Several days after selecting the Lys solution, their growth recovered and a normally observed preference for monosodium L-glutamate (MSG), which produces an *umami* taste, was observed.

An operant behavior test was developed to analyze the behavior of rats fed a Lys-deficient diet. As in the simple choice, behavioral test described above, operant box-housed rats specifically selected a diet containing Lys. A bilateral microinjection of a Lys solution into the hypothalamic area of the brain (hypothalamus) suppressed the Lys-seeking behavior in the Lys-deficient animals, pointing to the hypothalamus as a fundamental regulator of physiological responses to Lys deficiency. There are several humoral and afferent neural factors that might have contributed to the hypothalamic ability to recognize Lys deficiency.

## ***Humoral and Afferent Neural Factors in Lys-Deficiency***

Ingesting a Lys-deficient diet causes a rapid decline of plasma Lys, while the brain Lys levels and daily patterns of other AA remain unchanged (6). This finding led to a hypothesis that a peripheral, not a central, sensor detects Lys deficiency in the circulating blood and alimentary organs. Neural information that is sent from such a hepatoportal sensor via vagal afferent inputs may be one way the brain is informed about the level of peripherally circulating Lys. To evaluate such a possibility, an *in situ* preparation was used, by cannulating the portal vein. With a dissection microscope, the hepatic vagus branch was isolated and divided. An isolated nerve filament from the peripheral cut end was placed on a pair of silver wire recording electrodes to record afferent activity. Afferent nerve activity was amplified in a condenser-coupled amplifier. All nerve activity was analyzed by a spike counter after conversion of raw data to standard pulses (7). While a putative vagal sensor was activated by Lys in rats fed both a normal and a Lys-deficient diet, the lowest effective concentration in the deficient rats was one hundred fold lower than that found in their normally-fed counterparts. Neither essential L-AA nor D-Lys activated the Lys sensor in the Lys-deficient animals. Thus, the recognition of Lys deficiency appears functional only after Lys is absorbed from the small intestine into the portal vein. Generally, this absorption mechanism may play an important role in maintaining homeostasis of each AA, and the portal vein would seem to be an appropriate area for recognizing an AA deficiency. However, the physiological importance of Lys suggests that another afferent mechanism also exists for maintaining a nutritional balance of Lys.

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A very sensitive assay for many humoral factors is a feeding assay of the *Hydra Japonica* (2). Using this assay, an additional humoral factor effective during Lys deficiency was identified. An increase in serum inhibin and activin A was observed in normal rats, while serum activin A activity was significantly suppressed under Lys deficiency. In the brain, the lateral and the ventromedial hypothalamus (LH and VMH respectively) were also positively stained for the activin A during the Lys deficiency. In addition, activin infused into the LH significantly decreased the Lys-seeking behavior of operant-housed rats fed a Lys-deficient diet (2). Since both the LH and the VMH are known as “feeding centers”, a possibility of interactions between the blood-bind and the hypothalamic activin A in adaptive responses to Lys deficiency is apparent.

The above-mentioned findings indicated the fundamental importance of the brain during the Lys deficiency. For the non-invasive recording in the brain of the Lys-deficient animals, we have developed three original *in vivo* methods. Because feeding/drinking behavior is a very complex process, all three methods were based on recording in awake rats.

## ***Brain Studies: Magnetic-Resonance Imaging***

A magnetic-resonance imaging (MRI) system, which included a stable 4.7 Tesla magnet with a small bore, was developed for discrete imaging of neural activity in awake rats (for technical details, see Ref. 8). A polystyrol block with a head-shaped hole was used for painless alignment of the rat's head inside the magnet. In this way, head motion was effectively minimized. Male Sprague-Dawley rats, with the above-described polystyrol block attached to their heads, were fed a normal or a Lys-deficient diet for seven days. Each rat was fitted with a silicon tube for bolus injections, of either a Lys solution or saline, into the abdominal cavity. Following the injection of Lys solution to Lys-deficient rats, increased oxygenation was observed at 30 minutes in the hypothalamus, including the LH and the VMH (2). Recovery was observed from 100 minutes after the injection. There were no significant changes in other brain areas. No changes were observed in control rats injected with Lys solution. Consequently, the important role of the hypothalamus in the initial recognition of Lys deficiency was further supported.

## Brain Studies: Electrophysiological Recording

This original electrophysiological technique (9) enabled recording of single neuron activity in the hypothalamic area of awake, 24-hour water-deprived, rats during fluid licking. The rat's head was painlessly fixed in a manner similar to that described for the MRI technique. Rats were offered a wide array of solutions and trained

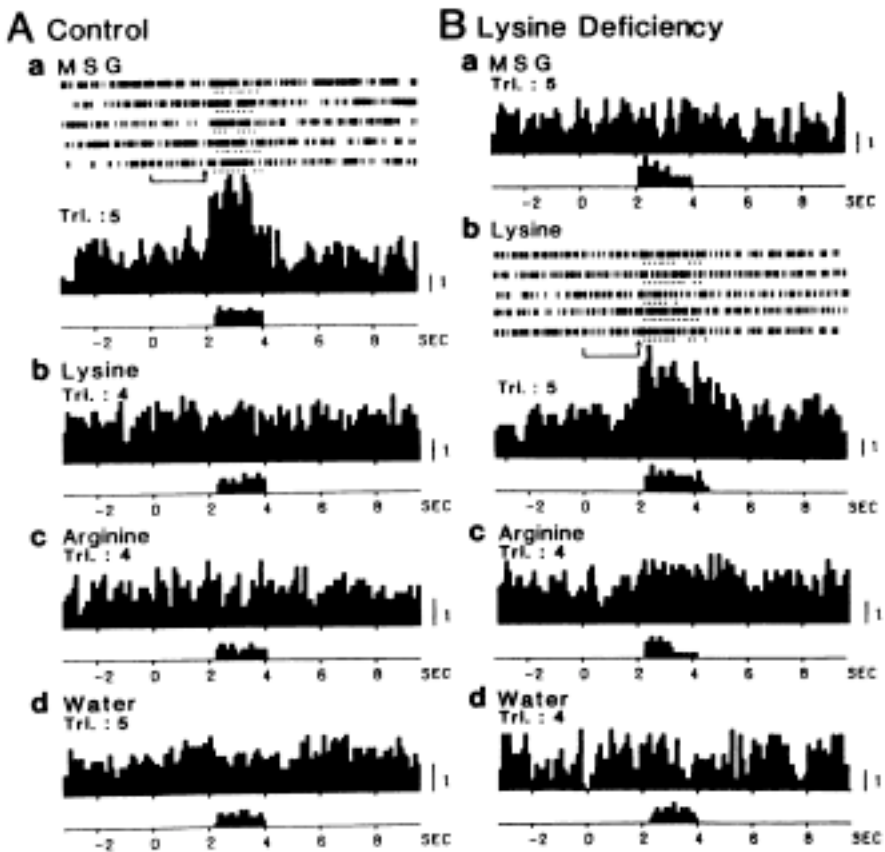


Figure 1 - Two examples of solution-specific neurons: one that responded only to MSG in control and one that responded only to Lys when the diet was Lys-deficient. The neuron in (A) was excited during MSG licking only and the neuron in (B) during Lys licking only. Horizontal brackets: cue tone period. Arrowheads: presentation of tube for licking. (With permission from Elsevier Science Ltd., 1996: "Perception, Memory and Emotion" ed.: T. Ono et al., 1996)

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to associate a specific acoustic cue tone with a specific solution. Following 24 hours of water deprivation, every solution represented a reward. Single neuron responses of the LH neurons to cue tones were classified into two types, non-differential and differential. The neurons that responded non-differentially to cue tones responded non-differentially to solutions as well. However, the number of neurons that responded differentially to the cue tone associated with L-arginine (an AA characterized by preferable sweet taste) and MSG (*umami*) were larger in control animals; the number responsive to the cue tone associated with Lys were larger in Lys-deficient rats (see Figure 1). Although it is difficult to find out if MSG-specific neurons were the same as Lys-specific ones, MSG-specific neurons appeared only in controls and Lys-specific neurons only in Lys-deficient rats. These results indicated that LH neurons are related to the integration of auditory and taste stimuli with reward. As the LH neurons responded non-differentially to Lys solution when the rats were fed a normal diet, they were characterized by a high degree of neural plasticity, similar to that found during learning in other brain areas (e. g., short- and long-term potentiation in the hippocampus).

## ***Brain Studies: Circadian Microdialysis Recording***

We have hypothesized that, besides activin A, there is at least one other neurochemical substance responsible for the qualitative changes recorded by the MRI (see sub-section 5) in the hypothalamus of Lys-deficient rats. Because of the role that the hypothalamic monoamines play in food-intake regulation, monoamine release was measured in non-stressed rats. To make these measurements, a new apparatus that allowed circadian microdialysis recording in freely eating, drinking and moving rats was built (for technical details, see 10; for a brief description of the experimental procedures, see Figure 2). Significant circadian oscillations in the VMH norepinephrine (NE) release were found in normally fed rats (see Figure 2). The involvement of the VMH in general food intake regulation indicates that the observed oscillations could be considered to be diet related, not light related. Indeed, >85% of the rats' food and fluid intakes occurred during the dark phase. VMH NE oscillations were depressed in rats fed a Lys-deficient diet, with the changes appearing already within the first 24 hours after the initial introduction of

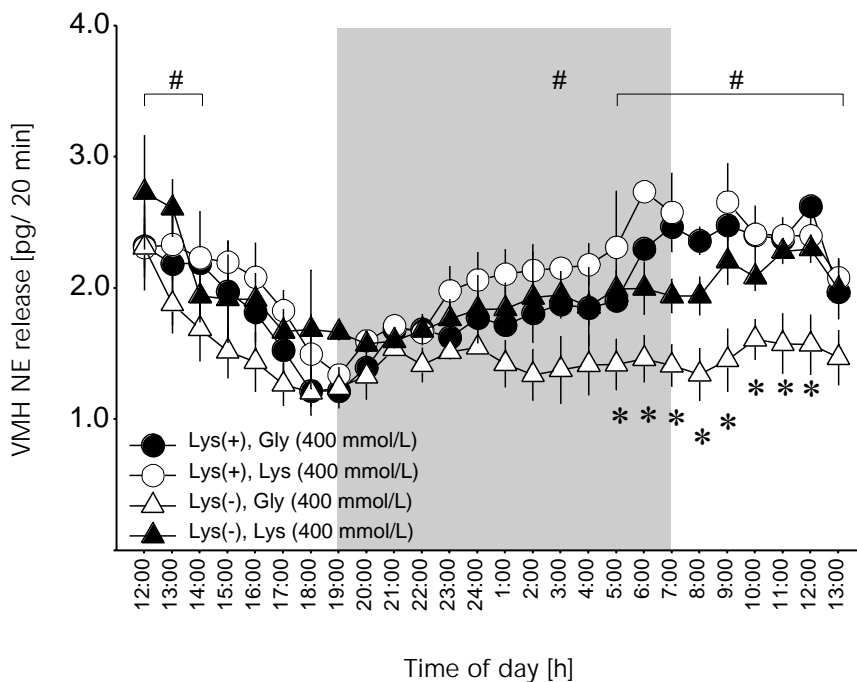


Figure 2 - Circadian pattern of norepinephrine (NE) release in the ventromedial hypothalamus (VMH) of male rats fed control diets and Lys-deficient diets. Rats were fed either normal [Lys(+)], or Lys deficient [Lys(-)] powdered diet and provided with distilled water, Gly (400 mmol/L), or Lys solution (400 mmol/L). Results from the groups that received Gly-solution and those that received distilled water did not differ significantly. Therefore, data from the distilled water-drinking group are not shown. Dialysis probes were inserted into the VMH at 08:00. Immediately thereafter, rats were placed into operant boxes and provided with diet and drink. Recording started at 12:00 (4 h after probe insertion) and finished at 14:00 on the next day. The dark phase (19:00 – 07:00) is indicated by the shaded portion. Values are means  $\pm$  SEM of 3 or 4 rats. \*, Significantly different from Lys(+), Gly group,  $p < 0.05$  (2-way ANOVA followed by Duncan's multiple range test) #, Significantly different from 19:00 time point in Lys(+), Gly group,  $p < 0.05$  (1-way ANOVA followed by unpaired t-test applied to Lys(+), Gly group). (With permission from J. Nutrition, Smriga et al., 2000)

a Lys-deficient diet (see Figure 2). As the intakes of normal and Lys-deficient diets did not significantly differ during the first 24 hours of the experiment, it was concluded that the significant depression of the VMH NE release in Lys-deficient rats was triggered by metabolic, rather than cephalic, inputs. A possibility of involvement

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of hepatoportal Lys sensors in the early recognition of an AA deficiency has been described above (7). The post-ingestive signals from the hepatoportal area may reach the hypothalamus directly, or via the brain chemosensor center (anterior piriform cortex) (3) that relays the signal into the VMH, triggering rapid decline in the VMH NE oscillations and, as a result, the hypophagia that characterizes later stages of Lys deficiency.

Taken together, these results clearly supported our initial theory, showing that the brain is the initial regulatory site in the recognition of Lys deficiency. (It is activated even before taste preference changes occur.) Interestingly, no changes in the LH NE release were found, although a strong depression of VMH NE release persisted during the whole course of Lys deficiency (1 week) and recovered immediately after Lys was provided to the deficient subjects (11).

## ***Conclusion***

A risk of deficiency in an AA, Lys, exists not only in poor developing regions, where diets are based solely on cereals, but also within elderly populations in developed countries. Experimentally, Lys deficiency can be modeled in rats by feeding the animals a Lys-deficient diet. Deficient rats learn to avoid a Lys-deficient diet and to prefer a previously aversive (bitter) Lys solution. Unlike the NaCl deficiency, this process takes several hours to develop fully, thus visceral, rather than cephalic inputs seem to be operational in the initial recognition of this particular dietary deficiency. We have identified several such factors that include circulating levels of activin A and plastic changes in the vagal Lys sensors. Together with significant declines of the plasma Lys, these factors form an overlapping array of afferent signal pathways. However, the primary regulatory centers during the initial phase of Lys deficiency were identified in the lateral (LH) and the ventromedial (VMH) areas of the hypothalamus. First, the firing of LH neurons plastically changed during the deficiency, implying an innate ability to adjust the neural activity and

nutritional needs. Second, VMH NE and activin A were identified as two of the neurochemical substrates responsive to Lys deficiency. Together, these brain mechanisms triggered taste-preference changes that helped in recognition and avoidance of a Lys-deficient diet. Considering the fundamental role of the nutritional AA balance, it is possible that other brain mechanisms for recognition and subsequent responses to Lys deficiency will soon be discovered.

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## Biography



### **Kunio Torii**

Dr. Kunio Torii received a DVM in animal physiology from the Tokyo University, Faculty of Agriculture, Department of Veterinary Science in 1971. He has been working at the Central Laboratories, Ajinomoto Co., Inc. Japan. He received a Ph.D. in Animal Nutrition from the Tokyo University (Department of Agricultural Chemistry). He also studied abroad at the Monell Chemical Senses Center, in the University of Pennsylvania, USA from 1977 to 1979 as a visiting scientist. In addition, he was a director for Torii Nutrient Stasis Project ERATO (Exploratory Research for Advanced Technology) and JST (Japan Science and Technology Corporation) from 1990-1995, and then became a basic research advisor of this agency. He is a council member of several other international societies, i.e., American Society for Neuroscience, American Society for Chemical Senses, e.t.c. On October 1999, he organized the 33rd annual meeting of the Japanese Society of Taste and Olfaction in Tokyo as the president. He gives special lectures at more than 20 universities in Japan every year to educate young students to become creative scientists in the near future.

Dr. Torii's researches focus on the central mechanism of homeostatic control by the brain and adaptive functions to prolonged marginal malnutrition and/or metabolic disorders with neuronal plasticity such as L-lysine deficiency, exercise and diabetes mellitus (type I and II).

