

ORIGINAL**Distinct effects of anterior pyriform cortex and the lateral hypothalamus lesions on protein intake in rats**

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Abstract : Several specific locations in brain, including pyriform cortex and hypothalamus, are associated with regulation of food intake. Although lesions of these locations significantly alter food intake, their involvement in the selection of macronutrients is not well characterized. In this study, we examined distinct effects of anterior pyriform cortex (APC) and lateral hypothalamus (LH) lesions on protein intake in rats. The APC or LH of male adult rats were lesioned by treatment with kainic acid, and the rats were then given free access to two kinds of casein diets containing high (60%) and low (5%) protein. Total energy content of these diets was kept constant by changing the carbohydrate content. Following the APC lesions, body weight and food intake decreased, but returned to control levels on day 13 and day 4, respectively. APC lesions did not change the ratio of protein intake. In contrast, LH lesions disturbed body weight gain and the selection of a high protein diet for at least two weeks, although food intake returned to control levels by day 2. Our results suggest that LH, but not APC, may play an important role in the selection of protein intake in rats. *J. Med. Invest.* 54 : 255-260, August, 2007

Keywords : APC, food intake, LH, selection of macronutrients, rats

INTRODUCTION

Food choice determinants are still a matter of conjecture, but it is likely that rats select macronutrients according to their needs and to achieve optimal adaptation (1). Indeed in early studies, Richer, *et al.* observed that rats given separate sources of the three macronutrients (protein, fat, and carbohydrate) as well as various sources of vitamins and minerals chose adequate diets that led to normal growth and showed no symptoms of nutritional deficiencies (2). Moreover, several studies suggest that rats can se-

lect balanced diets and thrive well when given separate macronutrient sources (3, 4). Based on these investigations, it is presumed that the selections of individual nutrients as well as total energy intake are strictly regulated, albeit by unknown mechanisms.

There are many studies examining the effects of lesioning locations in brain that are associated with the regulation of feeding behavior and nutrient intake by sensing amounts and kinds of nutrients in diets. Especially, lesions of the lateral hypothalamus (LH) decrease body fat and increase the activity of the sympathetic nervous system in rats (5).

Abbreviations :

ANOVA, analysis of variance ; AP, anteroposterior to interaural line ; APC, anterior pyriform cortex ; IAA, indispensable amino acids ; LH, lateral hypothalamus ; VMH, ventromedial hypothalamus.

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Leung and Rogers reported that rats with lesions in certain areas of the anterior pyriform cortex (APC) chose an amino acid imbalanced diet over a protein-free diet, whereas intact controls selected a protein-free diet over an imbalanced diet (6). However, the involvement of these locations in macronutrient selection is still unclear.

The present study was designed to investigate the involvement of APC or LH on feeding behavior in rats. Following lesions of either APC or LH, rats were given free access to two kinds of casein diets containing high (60%) or low (5%) amounts of protein. We then measured body weight, food intake, and protein intake in the rats and compared these values with those in control (sham-operated) animals to assess the feeding behavior of rats after the lesions.

MATERIALS AND METHODS

Diets and animals

All male Sprague-Dawley rats (6 weeks old) were housed individually in cages, in a temperature controlled ($25 \pm 1^\circ\text{C}$) room under 12 h light/dark cycle (lights on from 8 : 00 to 20 : 00). Rats were maintained on a freely feeding, self-selection paradigm with two kinds of diets, high protein (60% casein) and low protein (5% casein), during the experiment period (from day -7 to day 15, lesions were performed on day 0). The compositions of the experimental diets are shown in Table 1. The rats were allowed free access to these diets daily from 9 : 00 to 21 : 00. Water was also available throughout the experiments.

The APC and LH lesions

Kainic acid (0.5 mg/0.5 ml saline) was bilaterally injected into APC [$n = 5$; anteroposterior to interaural line (AP) : +2.2 mm, lateral : +4.0 mm, dorsal : +7.5 mm] or LH ($n = 5$; AP : -3.3 mm, lateral : +1.3 mm, dorsal : +7.7 mm) in rats placed in a stereotaxic frame on day 0, as described previously (7). Briefly, injections were made via a 33-gauge injector using a guide cannula. Each injection needle was connected to a 10-ml syringe fitted to a microinjection pump. Control animals were injected with vehicle in the respective areas ($n = 5$ for each area). Rats did not show any neurological symptoms after the operation (data not shown). Daily food consumption and body weight of the rats were measured for 15 days, and the ratios of protein intake to total

macronutrients were calculated. After finishing the experiments, injection sites and brain lesions were confirmed on Cresyl violet-stained microscopy sections (data not shown).

All animal experiments in the present study were approved by The Committee for the Care and Use of Animals in The University of Tokushima Graduate School and performed by the Institutional Animal Care and Oversight Committee according to established guideline principles.

Statistical analysis

All data were statistically evaluated by one-way ANOVA using SPSS software (release 6.1; SPSS Japan Inc., Tokyo, Japan) and were expressed as mean \pm SD, $n = 5$ in each group. Individual differences between groups were assessed with Student's *t*-test. Differences were considered significant at $P < 0.05$.

RESULTS

Body weight

Lesioning APC resulted in a decrease in body weight in rats immediately after the surgery (Fig. 1A), which steadily returned to control levels by day 13. In contrast, lesioning LH resulted in a decrease in body weight gain immediately after surgery, which failed to return to control levels during the

Table 1. Composition of nutrients in experimental diets

	Low protein diet	High protein diet
	(%)	(%)
Casein ¹	5.0	60.0
α -Corn starch	55.7	19.0
Sucrose	27.8	9.5
Soy bean oil	5.0	5.0
Mineral mix (AIN 93) ²	3.5	3.5
Vitamin mix (AIN 93) ²	1.0	1.0
Cellulose	2.0	2.0
Total	100.0	100.0

¹Casein was purchased from Oriental Yeast (Osaka, Japan).

²American Institute of Nutrition diet 93 (17).

two weeks of the experiment (Fig. 1B).

Food intake

The APC and LH lesions resulted in similar profiles of daily food intake per 100 g body weight after the surgeries (Fig. 2). The APC and LH lesions induced temporary hypophagia compared with the

respective sham-operated groups. However, the food intakes in both the APC and LH lesion groups returned to control levels by day 4 and day 2, respectively.

Protein intake

Protein intake per 100 g body weight and the ra-

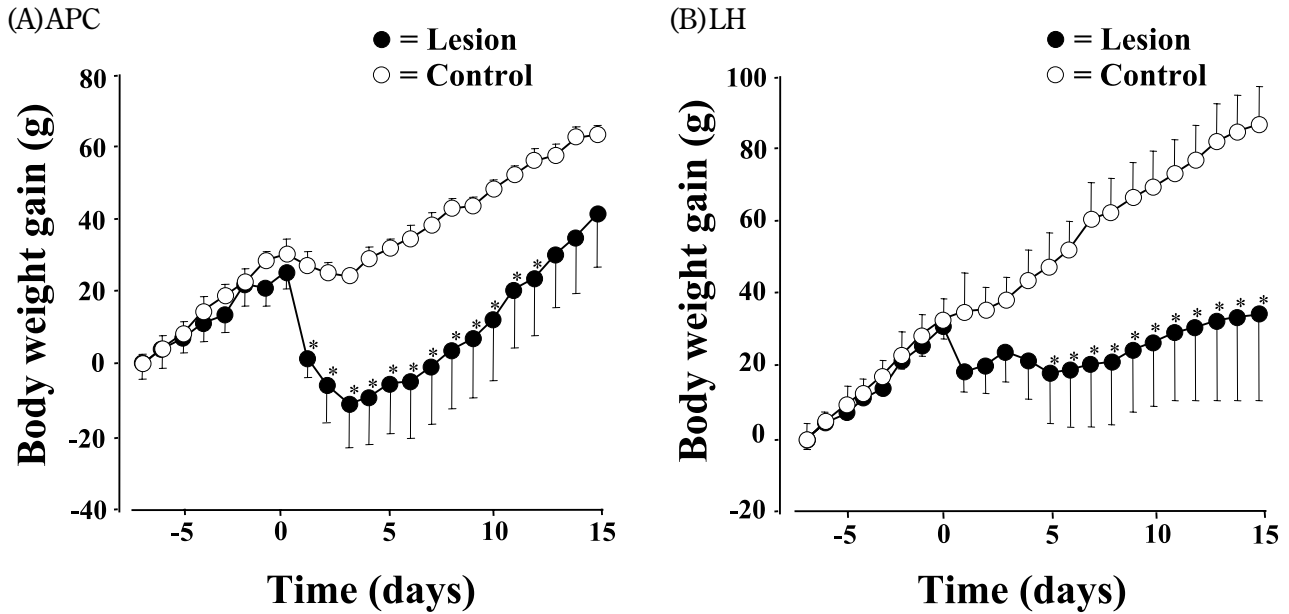


Fig. 1. Changes in body weight of the APC- and LH-lesioned rats. We measured body weight of rats before and after the APC (A) or LH (B) lesions, and that of their respective sham-operated (control) rats. Day 0 indicates the day of operation. Values are mean \pm SD, n = 5 rats per group. **P* < 0.05 significantly different from control.

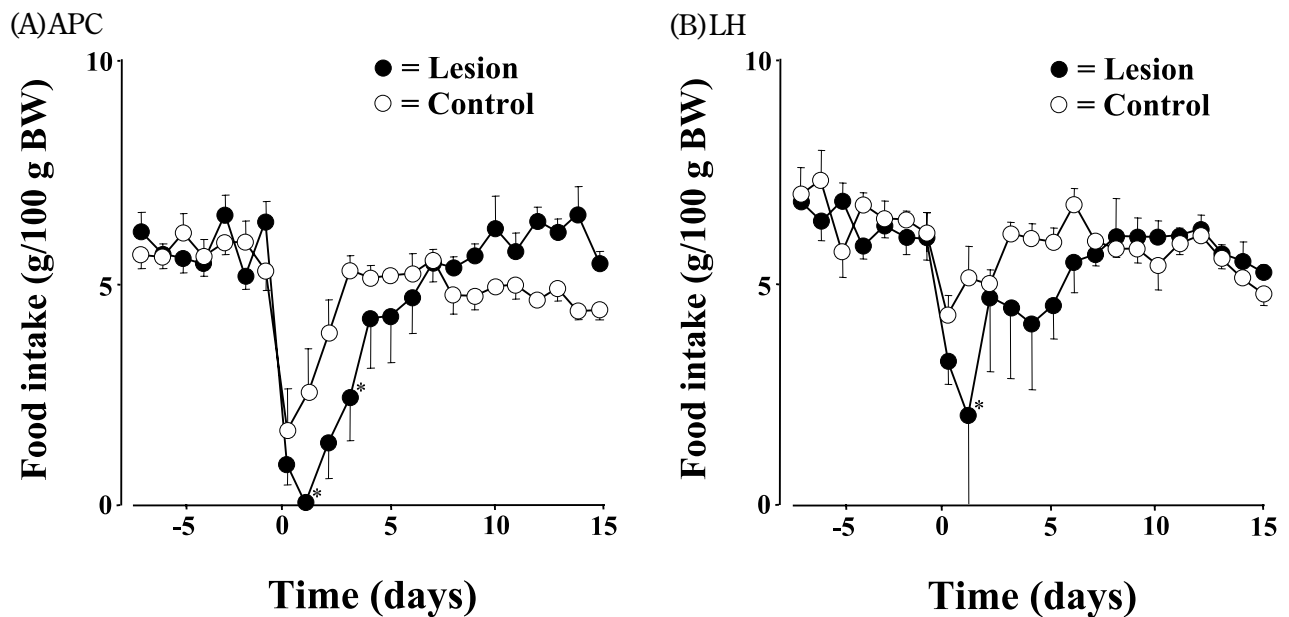


Fig. 2. Daily food intake in the APC- and LH-lesioned rats. We calculated daily food intake per 100 g body weight of rats before and after the APC (A) or LH (B) lesions and compared these values with those of the respective sham-operated (control) rats. Values are mean \pm SD, n = 5 rats per group. **P* < 0.05 significantly different from control.

ratio of protein intake to total energy intake are shown in Fig. 3. The APC and LH lesions significantly decreased protein intake, compared with that of the respective control groups. Protein intake of the APC lesion group returned to control levels in parallel with the restoration of food intake (Fig. 3A). The ratio of protein intake to total energy intake in the APC lesion group was constant before and after the

lesions (Fig. 3B), indicating that the protein intake of the APC lesion group changed in parallel with the food intake. By contrast, the protein intake and its ratio to total energy intake of the LH lesion group were significantly decreased (about 50% of each control value) for two weeks after the lesions (Fig. 3C and D), whereas food intake was restored to control levels by day 2 (Fig. 2B).

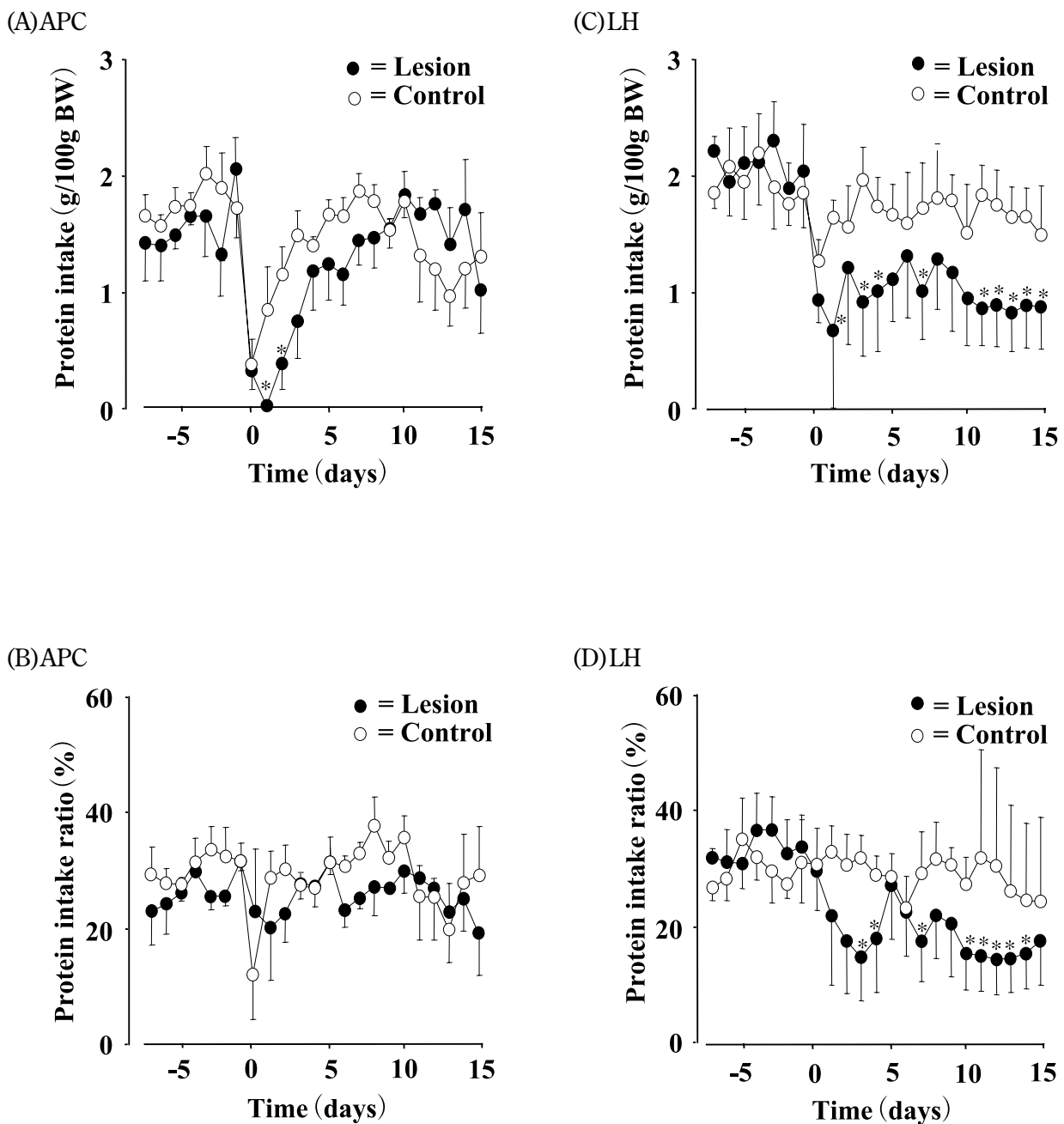


Fig. 3. Changes in protein intake in the APC and LH-lesioned rats.

We calculated daily protein intake per 100 g body weight and the ratio of protein intake to total energy intake of rats before and after the APC (A and B) and LH (C and D) lesions, and compared these values to those of the respective sham-operated (control) rats. Values are mean \pm SD, $n = 5$ rats per group. * $P < 0.05$ significantly different from control group.

DISCUSSION

Three main candidates in brain, the ventromedial hypothalamus (VMH), APC and LH, have been associated with regulation of food intake in animal models. For example, bilateral lesions of the VMH can produce obesity in animals (8, 9). Since bilateral lesions of the APC increase the intake of indispensable amino acid (IAA)-imbalanced diets (6), the APC has been implicated as the primary chemosensor responsible for detection of low levels of dietary IAA (10). The LH was also classically viewed as the "feeding center", because stimulation of this nucleus increases food intake, while its destruction attenuates feeding and causes weight loss (11). In our previous study, VMH lesions induced hyperphagia due to increases in fat and carbohydrate intakes, but not an increase in protein intake, suggesting that other brain regions may exist to regulate protein intake (manuscript in preparation). The present study is a serial investigation on determining the brain areas involved in protein intake. Therefore, we examined the effects of lesioning the APC or LH on protein intake in rats.

Rats in the LH lesion group preferentially chose the low protein diet, compared with the APC-lesioned or sham-operated rats. This finding was supported by the fact that the ratio of protein intake to total energy intake in the LH-lesioned rats was decreased (about 50% of each control value) for two weeks after lesioning. We suggest that the LH may play a more important role in regulating protein intake than the APC. Further experiments are necessary to test this hypothesis.

Several specific conditions, such as burns and bed-resting, were shown to stimulate protein degradation and require increased protein intake (12, 13). However, fat and carbohydrate intake are more sensitive to such stress conditions than protein intake (14). Mild chronic stress increases fat and carbohydrate intake, and severe acute stress decreases their intake, but protein intake is not affected by either condition (15). These findings lead us to consider that under stress conditions protein intake should be increased. It is the next important subject to find reagents stimulating LH activity. We are examining the effects of injecting various reagents, such as neuropeptide Y and leptin, into the LH on protein intake by using the present system, because microinjection of neuropeptide Y into the LH increased food intake in rats (16).

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REFERENCES

1. Webster AJ : Energy partitioning, tissue growth and appetite control. *Proc Nutr Soc* 52 : 69-76, 1993
2. Richter CP, Holt LEJ, Barelare BJ : Nutritional requirements for normal growth and reproduction in rats studied by the self-selection method. *Am J Physiol* 122 : 734-744, 1938
3. Kanarek RB : Determinants of dietary self-selection in experimental animals. *Am J Clin Nutr* 42 : 940-950, 1985
4. Smith BK, Andrews PK, West DB : Macronutrient diet selection in thirteen mouse strains. *Am J Physiol* 278 : 797-805, 2000
5. Bray GA : Autonomic and endocrine factors in the regulation of food intake. *Brain Res Bull* 14 : 505-510, 1985
6. Leung PM, Rogers OR : Importance of prepyriform cortex in food-intake response of rats to amino acids. *Am J Physiol* 221 : 929-935, 1971
7. Yamauchi A, Shizuka F, Yamamoto T, Nikawa T, Kido Y, Rokutan K, Kishi K : Amino acids and glucose differentially increased extracellular 5-hydroxyindoleacetic acid in the rat brain. *J Nutr Sci Vitaminol (Tokyo)* 41 : 325-340, 1995
8. Bray GA, York DA : Hypothalamic and genetic obesity in experimental animals an autonomic and endocrine hypothesis. *Physiol Rev* 59 : 719-809, 1979
9. Penicaud L, Larue-Achagiotis C, Le Magnen J : Endocrine basis for weight gain after fasting or VMH lesion in rats. *Am J Physiol* 245 : 246-252, 1983
10. Blevins JE, Dixon KD, Hernandez EJ, Barrett JA, Gietzen DW : Effects of threonine injections in the lateral hypothalamus on intake of amino acid imbalanced diets in rats. *Brain Res* 879 : 65-72, 2000
11. Williams G, Bing C, Cai XJ, Harrold JA, King PJ, Liu XH : The hypothalamus and the control of energy homeostasis : different circuits, different purposes. *Physiol Behav* 74 : 683-701, 2001

12. Gianni B, Beniamino C, Manuela S, Ariennna P, Stefania L, Viviana DM, Rocco B, Michela Z, Gianfranco G : Metabolic consequences of physical inactivity. *J Ren Nutr* 15 : 49-53, 2005
13. Pereira C, Murphy K, Jeschke M, Herndon DN : Post burn muscle wasting and the effects of treatments. *Int J Biochem Cell Biol* 37 : 1948-1961, 2005
14. Wang SW : Effects of restraint stress and serotonin on macronutrient selection : a rat model of stress-induced anorexia. *Eat Weight* 7 : 23-31, 2002
15. Moles A, Bartolomucci A, Garbugino L, Conti R, Caprioli A, Coccorello R, Rizzi R, Ciani B, D'Amato FR : Psychosocial stress affects energy balance in mice : modulation by social status. *Psychoneuroendocrinology* 31 : 623-633, 2006
16. Jolicoeur FB, Bouali SM, Fournier A, St-Pierre S : Mapping of hypothalamic sites involved in the effects of NPY on body temperature and food intake. *Brain Res Bull* 36 : 125-129, 1995
17. Reeves PG, Nielsen FH, Fahey GC Jr : AIN-93 purified diets for laboratory rodents : final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. *J Nutr* 123 : 1939-1951, 1993