Original Article *J. Clin. Biochem. Nutr.*, **44**, 247–252, May 2009

Effect of Procainization of Ventromedial Nucleus of Hypothalamus on the Feeding Behavior of Rats

Rajesh Yadav, Manjula Suri, Rashmi Mathur, and Suman Jain*

Department of Physiology, All India Institute of Medical Sciences, Ansari Nagar, New Delhi-110029, India

Received 8 April, 2008; Accepted 19 November, 2008

Summary **Bilateral lesions in the Ventromedial nucleus of hypothalamus (VMH) cause hyperphagia and a preference for high lipid, high carbohydrate diet. Reversible lesion by procaine microinfusion produces a decrease in serum glucose and immunoreactive insulin levels. In the present study the effect of procaine microinfusion on feeding behavior and taste preference was observed. 5 h and 24 h food intake, water intake and weekly body weight of the rats was measured. Three bottle preference test was used to study the diet preferences. The 24 h food intake was found to be significantly more on 1st, 2nd and 3rd day (41 ± 6.03, 38.83 ± 6.17 and 33.66 ± 5.88 g/day, respectively) of procaine injection. There was also a significant increase in food intake at 0.25 h (4.166 ± 2.04 g) and 1 h (5 ± 0 g) as compared to saline group (0 ± 0 g at 0.25 h and 0.83 ± 2.04 g at 1 h). Post procaine water intake and body weight for seven days was not statistically significantly when compared to pre-lesion values. In the three bottles preference test, after procaine microinfusion there was a significantly increased preference for 20% sucrose and 0.15% saccharin than quinine and citric acid. The results suggest that bilateral procainization of VMH produces a transient increase in food intake and enhance preference for sweet tasting substances.**

Key Words**: food intake, water intake, body weight, preference, lesion**

Introduction

Ventromedial nucleus of hypothalamus (VMH) has been designated as the satiety center [*1*]. Its stimulation causes cessation of eating in the animals, whereas bilateral lesions results in hyperphagia, polydypsia, increase in body weight and finickiness [*2*, *3*]. VMH lesioned rats also show preference for high lipid, high carbohydrate diet vis-a- vis a preference for sucrose over other solutions [*2*, *4*]. If the food supply is abundant the syndrome of hypothalamic obesity is observed in these VMH lesioned rats. Lesion studies have been done by passing direct current or injecting chemicals, leading to an irreversible, complete neuronal and or fiber tract loss [*5*]. The effects of progressive lesion or recovery can be studied by utilizing anesthetic agents.

Reversible lesion of VMH, made by procaine microinfusion, results in a decrease in serum glucose and immunoreactive insulin levels with no change in the rate of gastric emptying and blood corticosterone levels [*6*, *7*]. Procaine is a local anesthetic, that produces temporary and reversible lesion and its effect has been shown to be elicited within 2–5 min and lasts for approximately 1 h [*8*]. Serum glucose as well as insulin levels are known to modulate food intake [9]. Chronic infusion of glucose in rats [*10*] or intracerebroventricular administration of insulin [*11*] in baboons has been shown to decrease food intake and body weight. Therefore the objective of the present study was to observe the effect of procaine microinfusion in VMH on 5 and 24 h food intake, water intake, daily body weight and on taste preferences.

*To whom correspondence should be addressed.
Tel: 91-11-26594812 Fax: 91-11-26588641
E-mail: sumanjain10@gmail.com

Materials and Methods

Animals

Adult male wistar rats ($n = 6$) weighing between 200–250 g were housed in individual cages under a 14:10 h light : dark cycle in a temperature controlled room (25 ± 2°C) and had free access to laboratory food pellets (Ashirwad Industries , India) and fresh tap water. The study was ethically cleared by the Institutional ethics committee.

Surgery

Rats were anaesthetized with intraperitoneal injection of ketamine (50 mg/kg), supplemented by ether if necessary. The head was fixed in the stereotaxic frame. The guide cannula (21 gauge, length 6 mm, outer diameter 0.8 mm, inner diameter 0.6 mm) was aimed at VMH, but implanted 3 mm above the VMH i.e. in the mediodorsal thalamus. The cannula was firmly secured to the skull bone with dental cement. Stylet was placed inside the guide cannula. Skin was pulled loosely around the dental cement and sutured with silk thread. Rats received post-operatively injection of gentamycin (50 mg/kg body weight/day for 5 consecutive days) intramuscularly and body temperature was maintained. During the day of the experiment, the injector cannula (steel capillary tube 26 gauge, 12 mm OD) was lowered into the ventromedial hypothalamic nucleus (AP –2.8, V 9 mm and ML 0.5 mm) [*12*], via the guide cannula. Rats received a slow, bilateral infusion of either 5% procaine or physiological saline (1 μl over a min) into the VMH through a slow injector.

Behavioral tests

1. Daily food intake Adult male rats were housed individually in separate polypropylene cages. They were given measured food pellets in the spill proof food containers. The left over food in the container was weighed every 24 h between 10:00 to 11:00 h. Care was taken to see that enough food was provided so that there was some food left over after the rat had eaten ad libitum.

2. Daily water intake Adult male rats were housed individually in separate polypropylene cages. They were given measured water in the spill proof water container. The left over water was measured every 24 h between 10:00 to 11:00 h. Care was taken to see that there was some water left after the rat had drunk ad libitum.

3. Daily body weight The body weight of each animal was recorded daily between 10:00 to 11:00 AM using weighing balance.

4. Three solution preference bottle tests Three bottles made up of plastic were named right, middle and left. In the right and left bottle 25 ml of taste solution was provided and 100 ml of water was provided in middle bottle. All bottles were inserted into rat cage with gap of 2 cm. Bottles were placed into cage according to sequence Solution—Water—Solution.

To each rat following taste solutions (one at a time) were presented along with water: 20% sucrose, 2 mM saccharine (0.15%) 50 mM citric acid (1.05%), 300 mM quinine hydrochloride (0.0235) and 75 mM sodium chloride (0.9%). 30–50 gm food was also given along with taste solution and water. Taste preference test was done on alternate days and observation was made upto 5 h at 0.25 h, 1 h, 2 h, 3 h, 4 h and 5 h. The order of tastants presented was as mentioned above and was maintained in both the saline/procaine groups. Three bottle tests were done before and after procaine injection.

Daily food intake, water intake and body weight was recorded for seven days after surgery. After stabilization of food and water intake, procaine was injected in VMN through guide cannula and food intake was recorded after 15 min. of injection and thereafter every 1 h for 5 h and then daily food intake was recorded upto 7 days. The same procedure was repeated with saline infusion at VMH after one week. Taste preference test was conducted after a gap of one week using freshly prepared solutions.

Sacrifice and histology

After 21 days of operation the rats were anaesthetized with ether and perfused first with 100 ml of saline (0.9%) and then with 10% formaldehyde to fix the brain tissue. The brain was removed from the skull and processed for histological confirmation of the site of the procaine injection. Formalin-fixed paraffin sections were cut at 4 microns from blocks set at the appropriate angle and every fifth section through the region of the implanted cannula were stained with hematoxylin-eosin.

Data analysis

The basal 24 h food intake/water intake/body weight of the rats was recorded for five days and then the average of these intakes were compared with the daily intakes post procaine or post saline injections, using non-parametric Wilcoxon signed rank test. The results of the food intake recorded after procaine/saline injections at 0.25 h, 1 h, 3 h and 5 h as well as the taste preferences were compared using Wilcoxon signed rank test.

Results

On histological confirmation of the site of lesion, the injector cannula was found to be in the ventromedial nucleus of hypothalamus in 7 out of 8 rats lesioned (Fig. 1). The results of these seven rats were therefore analyzed and presented here.

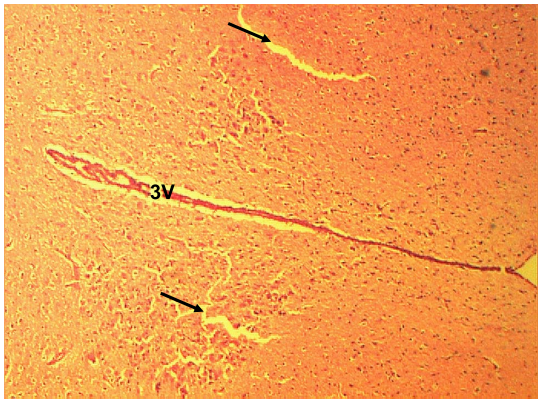

Fig. 1. Fig. 1 shows the site of cannula (marked by arrows) bilaterally in VMH at magnification 10X. The sections were stained using haemotoxylin-eosin. 3V = third ventricle.

Table 1. Table 1 shows the effect of bilateral procaine injection into the ventromedial nucleus of hypothalamus on the 5 h food intake in grams (Mean ± SD, $n = 7$).

Time (h)	Food Intake (g)		p value
	Saline injection	Procaine injection	
0.25	0 ± 0	4.166 ± 2.04	0.015
1	0.83 ± 2.04	5 ± 0	0.015
2	0 ± 0	0.83 ± 2.04	0.69
3	0.83 ± 2.04	2.166 ± 3.48	0.58
4	3.33 ± 2.58	3 ± 3.46	0.93
5	1.66 ± 2.58	3.33 ± 2.58	0.39

The p values in the table refer to the comparison between the saline and procaine group at each time period. A statistically significant increase in the food intake was observed in the procaine injected group with respect to saline, at 0.25 h and 1 h of procaine injection. $p<0.05$ is statistically significant.

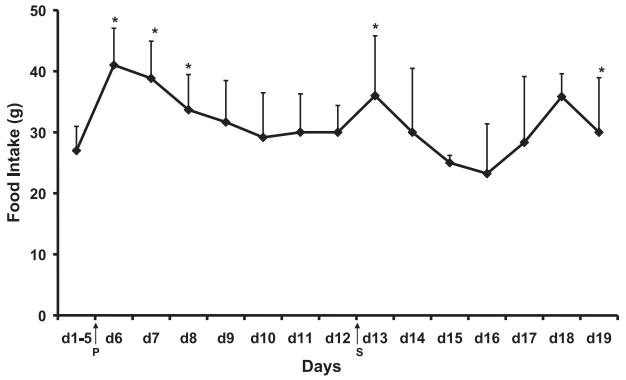

Fig. 2. Fig. 2 shows the effect of injecting procaine bilaterally into the VMH on the 24 h food intake (gm) (Mean ± SD, $n = 7$). P and S represents the time when procaine and saline respectively were injected into the VMH. A significant increase in the food intake was observed for three days following procaine injection.* indicates comparison between the postinjection and preinjection basal values.* represents $p<0.05$.

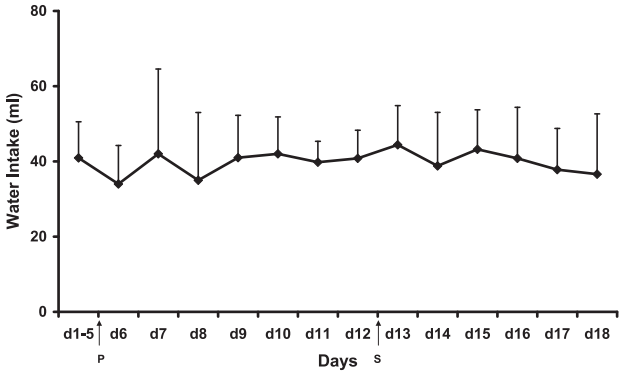

Fig. 3. Fig. 3 shows the effect of injecting procaine bilaterally into the VMH on the 24 h water intake (ml) (Mean ± SD, $n = 7$). P and S represents the time when procaine and saline respectively were injected into the VMH. No statistically significant difference in the 24 h water intake was observed following procaine or saline injection.

Effect of procaine microinfusion on food intake, water intake and body weight

The 24 h food intake was found to be significantly ($p<0.01$) more on 1st, 2nd and 3rd day (41 ± 6.03, 38.83 ± 6.17 and 33.66 ± 5.88 g/day, respectively) of procaine injection as compared to pre-lesion state (27 ± 3.96) (Fig. 2). Post saline injection there was an increase in food intake on 7th day (35.83 ± 3.76 g/day) (Fig. 2). While recording for 5 h food intake it was observed that the rats remained quiet after saline infusion, but immediately after procaine microinfusion, within few min. rats started to eat. The rats showed significant increase in food intake at 0.25 h (4.166 ± 2.04 g) and 1 h (5 ± 0 g) as compared to saline group (0 ± 0 g at 0.25 h and 0.83 ± 2.04 g at 1 h) (Table 1). Increase in food intake was observed again after 3 h onwards of procaine injection but it was not statistically significant.

The daily 24 h and 0.25 h, 1, 3 water intake after procaine/saline injections water intake was also recorded. Post procaine and post saline water intake for seven days was not statistically significantly when compared to pre-lesion values (Fig. 3). However, post procaine micro-infusion, there was a trend for an increase in the water intake for 5 h (1.66 ± 1.75 g at 0.25 h, 1.5 ± 1.87 g at 1 h, 1.16 ± 0.98 g at 2 h, 1.66 ± 1.21 g at 3 h, 1 ± 2 g at 4 h, 0.66 ± 0.81 g at 5 h) in comparison to saline group (1.166 ± 0.75 g at 0.25 h, 2.5 ± 2.25 g at 1 h, 2.83 ± 2.13 g at 2 h, 2.16 ± 2.04 g at 3 h, 1.83 ± 2.56 g at 4 h and 1.83 ± 2.13 g at 5 h). The values

Table 2. Table 2 shows the effect of bilateral procaine injection into the ventromedial nucleus of hypothalamus on the 5 h water intake (Mean ± SD, $n = 7$).

Time (h)	Water intake (ml)		p value
	Saline injection	Procaine injection	
0.25	1.66 ± 1.75	1.16 ± 0.75	0.93
1	1.50 ± 1.87	2.5 ± 2.25	0.48
2	1.16 ± 0.98	2.83 ± 2.13	0.18
3	1.66 ± 1.21	2.16 ± 2.04	0.589
4	1 ± 2	1.83 ± 2.56	0.589
5	0.66 ± 0.81	11.83 ± 2.13	0.31

The p values in the table refer to the comparison between the saline and procaine group at each time period. The p values show no change in the water intake in the procaine injected group with respect to saline. $p<0.05$ is statistically significant.

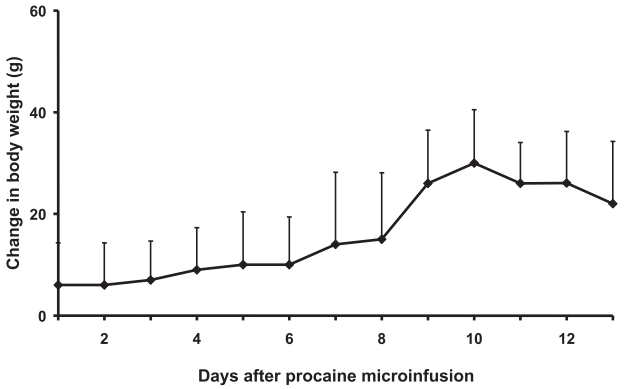

Fig. 4. Fig. 4 shows the effect of injecting procaine bilaterally into the VMH on the change in body weight (gm) (Mean ± SD, $n = 7$) from the three basal preinjection values. No statistically significant difference in the body weight was observed following procaine or saline injection.

were not statistically significantly different (Table 2).

To study the effect of procainization of VMH on body weight, the change in the body weight following procaine/saline injections from the basal pre-lesion was calculated. Although a gradual increase in the body weight was observed over the days but the data post-procaine and post-saline micro-infusions shows no statistically significant change in body weight (Fig. 4).

Effect of procaine microinfusion on taste preference

The taste preference was studied by three bottle taste preference tests, wherein the taste solutions were provided in left and right bottles and water in middle bottle. It was observed that the rats had a tendency to drink more sucrose from the bottle placed on the left side (12 + 7.70 ml and 11 + 6.34 ml) as compared to the right side (7 + 5.09 ml and 8.5 + 5.80 ml, respectively) (Table 3) in saline as well as in procaine injected group. However this was not statistically significant. On the other hand, in the saccharin and citric acid group the rats had a tendency to drink more from the bottles of the right side. In the quinine group no side preference was observed.

When the taste preference amongst the taste solutions within the group was analyzed it was observed that even before procainization there was a tendency to drink more of sucrose and saccharin but it was not statistically significant (Table 3). However, this behavior of the rats became more significant ($p<0.01$) after procainization. The total amount of sucrose and saccharine ingested (from all the bottles) by the rats was 20 ± 4.24 ml and 20 ± 5.65 ml, respectively, as compared to 8.75 ± 1.89 ml for citric acid, and 8.25 ± 1.88 ml for quinine.

Discussion

Procainization of ventromedial nucleus of hypothalamus significantly increased the food intake at 15 min and 1 h and the 24 h food intake was increased for 3 days. There was an enhanced preference for 20% sucrose and 0.15% of 2 mM saccharin solutions following procainization of VMH. No

Table 3. Table 3 shows the effect of bilateral procaine injection into the ventromedial nucleus of hypothalamus and the side preference on the intake of four taste solutions viz. 20% sucrose, 2 mM saccharine, 50 mM citric acid and 300 mM quinine hydrochloride (Mean ± SD, $n = 7$).

Test solution ingested (ml)	Without procaine (left side)	With procaine (left side)	p value	Without procaine (middle)	With procaine (midle)	p value	Without procaine (right side)	With procaine (rightside)	p value
Sucrose (ml)	12 ± 7.70	11 ± 6.34	0.716	2.25 ± 0.95	4 ± 2	0.141	7 ± 3.55	9.25 ± 5.49	0.71
Saccharin (ml)	8 ± 6.05	8.5 ± 3.41	1	4.25 ± 1.25	4.5 ± 1.29	0.317	17.5 ± 9.8	11.5 ± 4.72	0.27
Citric acid (ml)	6.00 ± 3.59	4.25 ± 1.5	0.58	7.5 ± 4.65	7.7 ± 3.86	0.85	7.5 ± 1.91	4.5 ± 0.58	0.66
Quinine (ml)	5.75 ± 1.25	5.0 ± 2.16	0.063	5.5 ± 0.577	4.5 ± 1.5	1	5 ± 0.81	3.25 ± 0.5	0.109

The p values in the table refer to the comparison between the without procaine and with procaine group in each taste solution. No statistically significant effect of procaine microinfusion was observed. $p<0.05$ is statistically significant.

statistically significantly increase in the water intake and body weight was observed. As procaine is a short acting anesthetic, when it is injected into VMH, it causes lesion by blocking the generation and conduction of nerve impulses [*8*]. VMH is satiety center, which give the information of fullness and functions by inhibiting the feeding centre. The feeding centre is chronically active and its activity is transiently inhibited by activity in the satiety centre after the ingestion of food [*13*, *14*]. Moreover VMH has been shown to maintain a tonic excitatory influence over hepatic glucose output (presumably via sympathetic nervous system). Therefore following procainization of VMH there is a fall in serum glucose concentration and decline in insulin [*6*]. This leads to hyperphagia and increase in food intake, due to decrease in the activity of glucostats present in the VMH and thereby loss of the inhibition of the feeding centre neurons. Insulin also regulates the food intake, it acts on insulin receptor in the hypothalamus to inhibit eating. Insulin receptor in the orexigenic neurons of the arcuate nucleus inhibit the release of Neuropeptide Y (NPY) [*9*, *15*], and insulin receptor in the anorexigenic neurons stimulate alpha-melanocyte stimulating hormones production, thereby decreasing food intake and thermogenesis. Further increase in food intake was observed again at 3 h onwards in both saline and procaine injected groups which may be due to the fact that rats are nocturnal animals and they resume feeding in the afternoons generally around 3:00 PM., which was the time for our observations.

Procainization of VMH increased the water intake but it was not statistically significant. After VMH lesion polydipsia is reported. One of the factors contributing to increased water intake may be marked increase in food intake as drinking is regulated by plasma osmolality and extracellular fluid volume [*16*]. In the present study, although an increase in food intake was observed for 1–3 days, a trend for increased water intake was observed for only 5 h after procainization.

There was no statistical significant difference between the pre and post lesion body weight, although a gradual increase in the body weight was observed. Body weight is determined by the balance between caloric intake and energy expenditure, which is regulated on a day to day and longer term basis. The lipostat theory postulates a mechanism that inhibits eating behavior and increase energy consumption whenever body weight exceeds a certain value [*16*]. The inhibition is relieved when body weight drops below the set point. This theory predicts that a feedback signal originating in adipose tissue influence the brain center that control eating behavior and activity. The size of the body fat depots plays a key role in regulating appetite [*9*]. In VMH lesioned rats defective neural control i.e. sympathetic discharge, to brown fat has been shown to contribute to the obesity [*17*, *18*]. In addition, the increased heat generated by food intake in normal animals may contribute to satiety, and this is reduced or absent in VMH lesioned rats. In the present study, as the lesions were produced by procaine that produces temporary reversible short lasting lesions, we did not observe any change in the body weight.

In taste preference studies the rats had left spout side preference for sucrose and right spout side preference for saccharine. Previous studies have shown that some strains of mice have preferences for the left spout when given a two bottle choice [*19*]. The procaine injected rats also had more preference for sucrose, which is a sweet tasting solutions, over quinine and citric acid, which is also reported in literature that VMH lesioned rats become finicky and have more preference for high lipid and high carbohydrate solutions [*4*].

Conclusion

Bilateral procainization of ventromedial nucleus of hypothalamus produces a transient increase in daily food intake and enhances preference for sucrose and saccharin i.e. sweet tasting substances.

Acknowledgement

We acknowledge Institute Research Grant, All India Institute of Medical Sciences, New Delhi for funding this project and Biostatistics department, All India Institute of Medical Sciences for statistical analysis.

Abbreviations

VMH, Ventromedial nucleus of hypothalamus; min, Minute; h, Hour; NPY, Neuropeptide Y.

References

[1] Brobeck, J.R.: Mechanism of the development of obesity in animal with hypothalamic lesions. *Physiol. Rev.*, **26**, 541–559, 1946.

[2] Ganaraja, B. and Jeganathan, P.S.: Effect of basolateral amygdale and ventromedial hypothalamic lesions on ingestion & taste preference in rat. *Indian J. Med. Res.*, **112**, 65–70, 2000.

[3] King, B.M.: The rise, fall, and resurrection of the ventromedial hypothalamus in the regulation of feeding behavior and body weight. *Physiol. Behav.*, **87(2)**, 221–244, 2006.

[4] Levison, M.J., Frommer, G.P., and Vance, W.B.: Palatability and caloric density as determinants of food intake in hyperphagic and normal rats. *Physiol. Behav.*, **10(3)**, 455–462, 1973.

[5] Dube, M.G., Kalra, S.P., and Kalra, P.S.: Low abundance of NPY in the hypothalamus can produce hyperphagia and obesity. *Peptides*, **28(2)**, 475–479, 2007.

[6] Duggan, J.P., Storlien, L.H., Kraegen, E.W., and Booth, D.A.: Effect of procaine injection into the ventromedial hypothalamic area (VMH) of the rat on serum insulin, glucose and corticosterone and gastric emptying rate. *Physiol. Behav.*, **43**, 29–33, 1988.

[7] Berthoud, H.R. and Jeanrenaud, B.: Changes of insulinemia and glycemia and feeding behavior induced by VMH-procainization in the rat. *Brain Res.*, **174**, 184–187, 1979.

[8] Nowacka, A., Jurkowlaniec, E., and Trojniar, W.: microinjection of procaine into the pedunculopontine tegmental nucleus suppresses hippocampal theta rhythm in urethane anesthetized rats. *Brain Res. Bull.*, **58(4)**, 377–384, 2002.

[9] Dhillo, W.S.: Appetite regulation: an overview. *Thyroid*, **17(5)**, 433–445, 2007.

[10] Gilbert, M., Magnan, C., Turban, S., Andre, J., and Guerre-Millo, M.: Leptin receptor-deficient obese Zucker rats reduce their food intake in response to a systemic supply of calories from glucose. *Diabetes*, **52**, 277–282, 2003.

[11] Woods, S.C., Lotter, E.C., McKay, L.D., and Porte, D. Jr.: Chronic intracerebroventricular infusion of insulin reduces food intake and body weight of baboons. *Nature*, **282**, 503–505, 1979.

[12] Paxinos, G. and Watson, C.: *The rat brain in stereotaxic coordinates*. Sydney Academic Press, 1985.

[13] Oomura, Y., Kimura, K., Ooyama, H., Maeo, T., Ikl, M., and Kuniyoshi, N.: Reciprocal activities of the ventromedial and lateral hypothalamic area of cats. *Science*, **143**, 484–485, 1964.

[14] Oomura, Y.H., Ooyama, M., Yamamoto, T., and Faka, F.: Reciprocal relationship of the lateral and ventromedial hypothalamus in the regulation of food intake. *Physiol. Behav.*, **2**, 97–115, 1967.

[15] Valassi, E., Scacchi, M., and Cavagnini, F.: Neuroendocrine control of food intake. *Nutr. Metab. Cardiovasc. Dis.*, **18(2)**, 158–168, 2008.

[16] Ganong, W.F.: Central regulation of visceral function, *in Review of Medical Physiology*, ed. By Ganong, W.F., McGraw Hill Publishers, pp. 232–255, 2005.

[17] Hogan, S., Himms-Hagen, J., and Coscina, D.V.: Lack of diet-induced thermogenesis in brown adipose tissue of obese medial hypothalamic-lesioned rats. *Physiol. Behav.*, **35(2)**, 287–294, 1985.

[18] Vander Tuig, J.G., Kerner, J., and Romsos, D.R.: Hypothalamic obesity, brown adipose tissue, and sympathoadrenal activity in rats. *Am. J. Physiol.*, **248**, E607–E617, 1985.

[19] Bachmanov, A.A., Rreed, D.R., Beauchamp, G.K., and Tordoff, M.G.: Food intake, water intake, and drinking spout side preference of 28 mouse strains. *Behav. Genet.*, **32(6)**, 435–443, 2002.