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Toxicological Studies of Monosodium L-Glutamate in Rodents: Relationship Between Routes of Administration and Neurotoxicity

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It was reported that administration of monosodium L-glutamate (MSG) resulted in necrotic changes of neurons in the central nervous system (4,8,20,23) and in the retina (10,26) and induced elevation of some serum hormone levels (22) as acute effects in rodents. Physiological and behavioral abnormalities were demonstrated in rodents following neonatal administration of MSG; namely, stunting (15,20,28), obesity (14,15,17,20,28), precocious puberty (35), female sterility (20), changes in activity level (1,15,25,27), and learning deficits (3,27). However, the abnormalities were observed in animals given a high dose of MSG either parenterally or by forced intubation. Since MSG is used as a food additive, its safety should be evaluated by studies involving the intended condition of use, that is, *ad libitum* dietary feeding. To achieve this end, this study compares the effect of MSG given by parenteral administration or by forced intubation and in *ad libitum* dietary feeding with respect to neuropathological, biochemical, endocrinological, and behavioral effects in mice and rats.

It has been suggested that the repeated ingestion of subneurotoxic doses of MSG in early development might result in functional abnormalities upon reaching maturity (22). The long-range effects of MSG on growth and various physiological parameters were examined in rats given subneurotoxic doses of MSG during the neonatal and infancy stages.

ACUTE EFFECTS

Histological Changes in the Brain

Acute histological changes in the brain following MSG administration were studied in mice, the species most susceptible to damage.

Distribution of Brain Lesions

The regions of histological changes in the brain were examined in infant mice (ICR) injected with a high dose of MSG (4 g/kg body weight) (Fig. 1). The brain was fixed by perfusion with formalin solution and embedded in glycol methacrylate. Serial Nissl-stained sections were examined microscopically. The affected regions were the hypothalamic arcuate nuclei, subfornical organ, preoptic area, area postrema, dentate gyrus, and cerebral cortex (32). Degeneration of neurons and subsequent necrotic changes, such as pyknosis and kariolysis, were observed in these regions. Olney (23) found lesions in the medial habenula, as well as the regions described above. Reynolds et al. (29) also found lesions in the subcommissural organ, fornix, entopeduncular nuclei, amygdala, tectum, and cerebellum after similar treatment. The effects of MSG on the arcuate nuclei were studied, since these nuclei were most sensitive to MSG (29,32).

Relationship Between Hypothalamic Lesions and Plasma Glutamate Level

Parenteral Administration and Forced Intubation

There are few detailed, analytical studies of the relationship between the route of administration and hypothalamic lesions in animals of various ages. Our results on the lowest effective dose (LED) of MSG inducing hypothalamic lesions by various routes of administration in ICR mice are summarized as follows: by intraperitoneal injection (i.p.) and forced intubation (p.o.) in 10-day-old mice, 0.4 (32) and 0.7 g/kg, respectively; by subcutaneous injection (s.c.) and intubation in 23-day-old mice, 0.7 and 2.0 g/kg, respectively; and subcutaneously in adult mice, 1.2 g/kg. These results indicate that hypothalamic lesions are induced by lower doses of MSG by parental administration than by oral administration and that the LED increases with the age of the animals.

We studied in detail the relationship between the LED of MSG for hypothalamic lesions and plasma glutamate levels in infant (10-day-old), weanling (23-day-old), and adult (3- to 4-month-old) ICR mice. Plasma samples were deproteinized with sulfosalicylic acid and then analyzed by an amino acid analyzer (18). As shown in Table 1, the transient peak value of plasma glutamate at LED increased with age

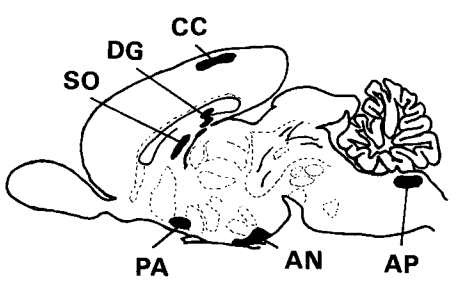


FIG. 1. Distribution of brain damage in 10-day-old mice injected intraperitoneally with 4 g/kg body weight of MSG. AN, arcuate nucleus; AP, area postrema; PA, preoptic area; SO, subfornical organ; DG, dentate gyrus; CC, cerebral cortex.

TABLE 1. *Relationship between dose of MSG for inducing hypothalamic lesions and plasma glutamate level in infant, weanling, and adult mice*

Age	Treatment of MSG: Route	Dose (g/kg)	No. of mice affected	Peak level of plasma glutamate (μmoles/100 ml)
Infant (10 days)	Nontreatment	—	0/8	17 ± 1[c]
	p.o.[a]	0.5	0/8	62 ± 6
	p.o.	0.7[d]	2/8	104 ± 18[d]
	p.o.	0.8	4/8	—
Weanling (23 days)	Nontreatment	—	0/8	10 ± 1
	s.c.[b]	0.5	0/8	278 ± 15
	s.c.	0.7[d]	2/8	385 ± 32[d]
	s.c.	1.0	6/8	760 ± 52
Adult (3–4 months)	Nontreatment	—	0/8	10 ± 1
	s.c.[b]	1.0	0/8	539 ± 25
	s.c.	1.2[d]	4/8	631 ± 30[d]
	s.c.	1.5	4/8	841 ± 105

[a] 10% aq. soln.
[b] 4% aq. soln.
[c] Mean ± SE.
[d] Lowest effective dose (LED) of MSG for inducing hypothalamic lesions and peak value of plasma glutamate at LED.

(19). The peak values in infant, weanling, and adult mice were about 6, 40, and 60 times resting levels, respectively. The increase in peak plasma glutamate with age may be related to the development of the blood brain barrier. Olney (21) reported that the peak concentration of plasma glutamate inducing hypothalamic lesions might be 20 times the resting level in infant mice. On the other hand, Stegink et al. (31) estimated that a hypothalamic lesion was induced when plasma glutamate rose to approximately 50 μmoles/dl, based on the plasma glutamate level after administration of hydrolyzed casein and fibrin and on the hypothalamic lesions induced by these hydrolysates (24) in 9- to 11-day-old mice.

The time course of plasma glutamate in infant, weanling, and adult mice was measured after administration of 1 g/kg of MSG by different routes: subcutaneous (aqueous solution), intraperitoneal (aq. soln.), forced intubation (aq. soln. or with diet), and free feeding in a single meal (Figs. 2 and 3) (18). Peak plasma glutamate after subcutaneous injection was highest in the infant mice and lowest in adult mice (Fig. 2). Lengthy retention of glutamate in plasma over base-line values was observed in infant animals. These results suggested that the hepatic capacity to metabolize glutamate developed with age. Peak plasma glutamate after forced intubation was much lower than after parenteral administration (s.c. and i.p.) in mice at any age. This may be due to the intestinal metabolism of glutamate.

MSG dissolved in milk (given to infants) or in soup (given to adults) resulted in lower glutamate levels than MSG given in aqueous solutions (Fig. 3). McLaughlan

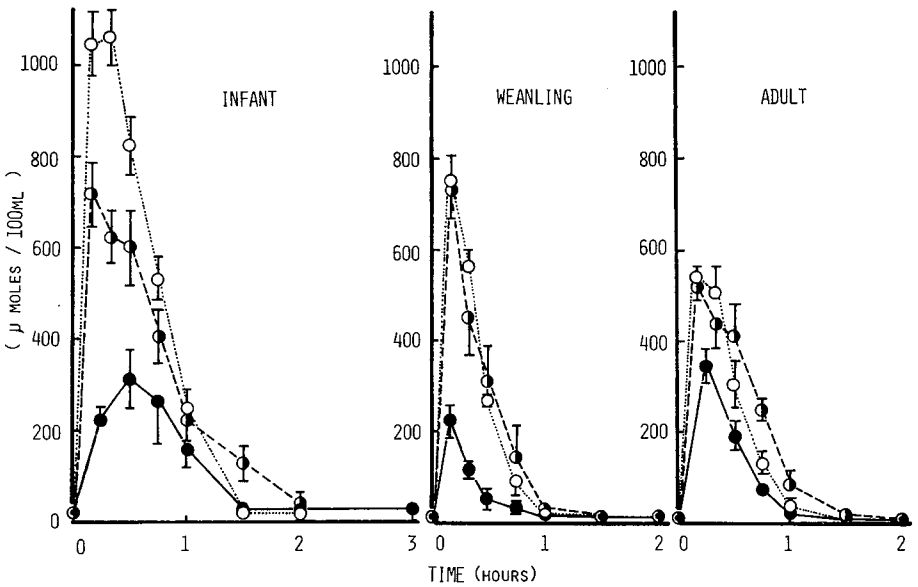


FIG. 2. Time course of plasma glutamate levels after administration of MSG. Infant, weanling, and adult mice were given a single dose of 1 g MSG/kg body weight by s.c. (○), i.p. (◑), or p.o. (●) administration. A 4% (w/v) MSG aq. soln. was used for the s.c. and i.p. routes, and a 10% (w/v) aq. soln. was used for the p.o. administration. Each point represents mean ± SE.

et al. (13) reported that the elevation of plasma glutamate levels in weanling rats following administration of 0.2 g/kg MSG with meat was slower than that in rats given the same dose in aqueous solution, but the peak values were similar in both treatments. In our studies, the peak in mice given 1 g/kg MSG in the diet was much lower than in mice given the same dose in aqueous solution. This discrepancy may be due to a difference in the dose of MSG and/or the kind of food mixed as vehicle.

Dietary Free Feeding

Figure 3 shows the plasma glutamate levels in weanling and adult mice fed a large amount of MSG (1 g/kg) in a commercial laboratory chow *ad libitum* in a single meal (15 to 30 min). In both weanling and adult mice, plasma glutamate levels were lower than after forced intubation, the value being around 40 μmoles/dl. The results indicate that the method of administration has a great influence on the plasma glutamate level and may affect the occurrence of hypothalamic lesions.

There are some reports on the histological effects of MSG following dietary feeding in rodents. Semprini et al. (30) fed a diet containing 1 and 2% MSG *ad libitum* to rats and mice during pregnancy and lactation. Histological examinations of the brains of offsprings at 0, 15, and 30 days of age were made, and no abnormalities were found. Huang et al. (5) reported that histological examination of the hypothalamus of young male rats fed diets containing 2, 4, and 6% MSG *ad*

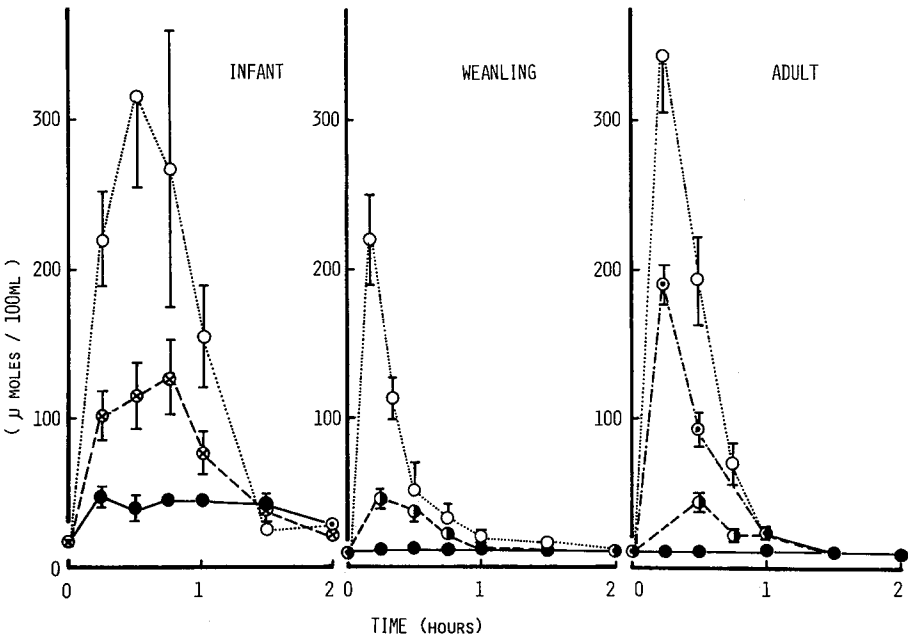


FIG. 3. Effects of MSG load by food accompaniment or dietary administration on time course of plasma glutamate levels in mice. Mice were given a single dose of 1 g MSG/kg body weight with or without food accompaniment by p.o. or dietary administration. Infant mice were given a 10% (w/v) MSG aq. soln. (○) or an infant formula containing 10% (w/v) MSG (⊗) or an infant formula only (●) by p.o. administration. Weanling mice were given a 10% (w/v) MSG aq. soln. (○) by p.o., or were fed with a basal diet containing 10% (w/w) MSG (◑) or a basal diet only (●) by dietary administration. Adult mice were given a 10% (w/v) aq. soln. (○) or a clear soup containing 10% (w/v) MSG (⊙) or a clear soup only (●) by p.o., or were fed with a basal diet containing 10% (w/w) MSG (◑) or a basal diet only (●) by dietary administration. Each point represents mean ± SE.

libitum for 80 days showed no necrosis of neurons. In addition, Wen et al. (36) reported that no abnormality of the hypothalamus was found in weanling rats fed diets containing 20 and 40 g MSG/100 g feed *ad libitum* for 5 weeks.

The effects of MSG administered by dietary feeding were studied. Mice were fed diets containing 5, 10, and 15% MSG or given a 5% aq. soln. of MSG *ad libitum* for 1 to 4 days during pregnancy or lactation or during the weaning stage (33). Since obvious necrosis of the hypothalamic neurons due to MSG disappears within 24 hr of administration of MSG, mice were killed every day within 2 to 3 hr of the end of the feeding period. Histological examination of the hypothalamus showed no necrosis of neurons in pregnant mice or their fetuses, or lactating females or their suckling mice. Weanling mice had no brain lesions following the ingestion of large amounts of MSG (20 to 30 g/kg/day) in diet or water (Table 2).

In order to determine why necrosis of the neurons was not induced in the dietary feeding experiment, feeding and drinking patterns and plasma glutamate levels were

TABLE 2. *Daily MSG intake in free-feeding mice*

Treatment	MSG intake (g/kg body weight/day)			
	Weanling[a]		Pregnant[b] females	Lactating[c] females
	Male	Female		
5% MSG diet	11	10	6	11
10% MSG diet	23	22	8	23
15% MSG diet	31	35	11	31
5% MSG soln.	20	18	10	21

[a] Mean value of 6 animals in total of 12 days.
[b] Mean value of 2 animals in total of 3 days.
[c] Mean value of 2 animals in total of 6 days.

examined. Figure 4 shows the amount of MSG ingested every 30 min by weanling and adult mice in one day. Mice were maintained in a room with a 12-hr light cycle. In the dark phase, mice ingested 1 to 3 g/kg of MSG per 30 min incessantly (6). Figure 5 shows the individual plasma glutamate levels. In the dark phase, the increased intakes of food and water seem to give higher plasma glutamate levels, but they remained much lower than those required to induce hypothalamic lesions. These low levels of plasma glutamate in mice fed large amounts of MSG in water or in the diet explain why brain lesions were not observed.

Acute Effects of MSG on Some Endocrine Functions

It has been reported that subcutaneous injections of high doses of MSG (1 g/kg) in adult male rats acutely raises serum luteinizing hormone (LH) and testosterone levels (22). It was postulated that these changes were induced by glutamate exerting its effect against the hypothalamic arcuate neurons.

We examined the acute effects of MSG administered parenternally on LH and testosterone levels in adult male Wistar rats. Serum LH was measured by double antibody radioimmunoassay and serum testosterone was measured by radioimmunoassay by the charcoal adsorption method (37). As shown in Fig. 6, the levels of serum LH and testosterone fluctuated in the light or dark phases after subcutaneous injection of MSG (1 g/kg), but did not rise immediately after injection. In the rats injected with NaCl, there was also a significant change 4 hr after injection. These fluctuations appeared to be within the range of circadian variation obtained with nontreated animals and were considered temporary, since serum LH levels the day after the MSG injection were comparable to those of nontreated rats (12).

It was determined whether these variations in hormone levels during the day were affected by free feeding of diets containing large amounts of MSG. Male rats were given diets containing 4 or 8% MSG for 8 days (average MSG intake was 2.8 or 5.2 g/kg/day). On the day 8, serum LH, testosterone, and glutamate were measured every 4 hr (Fig. 7). In control rats fed the basal diet, serum LH levels appeared to fluctuate, but no clear circadian variation was observed. Changes in LH and

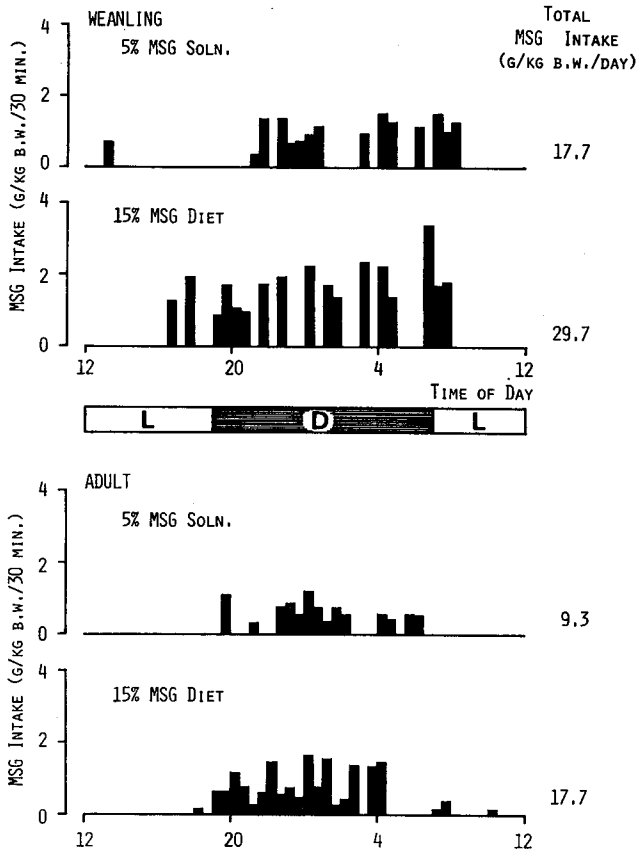


FIG. 4. A 24-hr pattern of MSG intake in weanling and adult mice. Each bar represents the cumulative value taken every 30 min. Mice were fed with diets containing 15% (w/w) MSG or were given 5% (w/v) MSG solution as drinking water *ad libitum*.

testosterone levels in a day were comparable to those reported by Kalra et al. (7). Significant changes of LH levels were partially noted in the rats fed with MSG in the diet, but seemed to be of no biological significance, because no dose-related response was observed and the changes were within the range of daily variation in control rats. Serum glutamate levels in the rats fed the 8% MSG diet increased in the dark phase, but no corresponding changes in serum LH or testosterone levels were observed. These results suggested that ingestion of a large amount of MSG with the diet had no substantial effect on these hormone levels.

LONG-RANGE EFFECTS

The long-range effects of MSG on growth, organ weight, reproductive function, and behavior were studied in rats (34). Wistar rats were given neurotoxic or

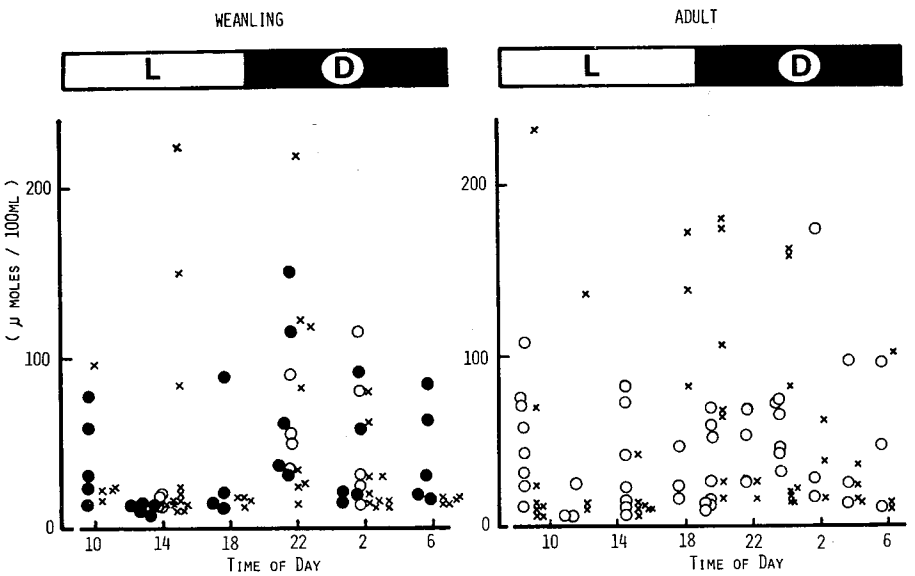


FIG. 5. A 24-hr variation of plasma glutamate concentrations in weanling and adult mice. Mice were fed with diets containing 10% (w/w) MSG (○) and 15% (w/w) MSG (●), or were given 5% (w/v) MSG solution as drinking water (×) *ad libitum*.

subneurotoxic doses of MSG in the neonatal and infant stage and were fed large amounts of MSG in the diet during weaning. Animals were housed in a room maintained at constant temperature (23 ± 2° C) and under controlled lighting (12-hr light and dark periods).

Growth

As shown in Table 3, daily subcutaneous injections of a high dose of MSG (4 g/kg body weight) to neonatal rats resulted in suppression of weight gain in males and suppression of development in body and tail length in both sexes. Autopsy at 3 months in females and 5 months in males revealed that their thigh bones were shorter than those of controls. Lee's index, which has been shown to correlate well with carcass fat (2), increased significantly in these rats, particularly in females. But obesity accompanied with a marked increase of body weight as reported in mice (20) was not observed for 16 weeks. Nikoletseas (17) and Redding et al. (28) also reported obesity without marked weight gain in rats.

In our experiment, the obese rats showed hypophagia in adulthood, as reported previously in mice (20). However, these animals were hyperphagic for 10 days immediately after weaning, which suggested an increase in the number of lipocytes in this stage.

Body weight gain and the development of body and tail lengths in rats injected subcutaneously with 4 g/kg of MSG daily for 10 days in the infant stage were

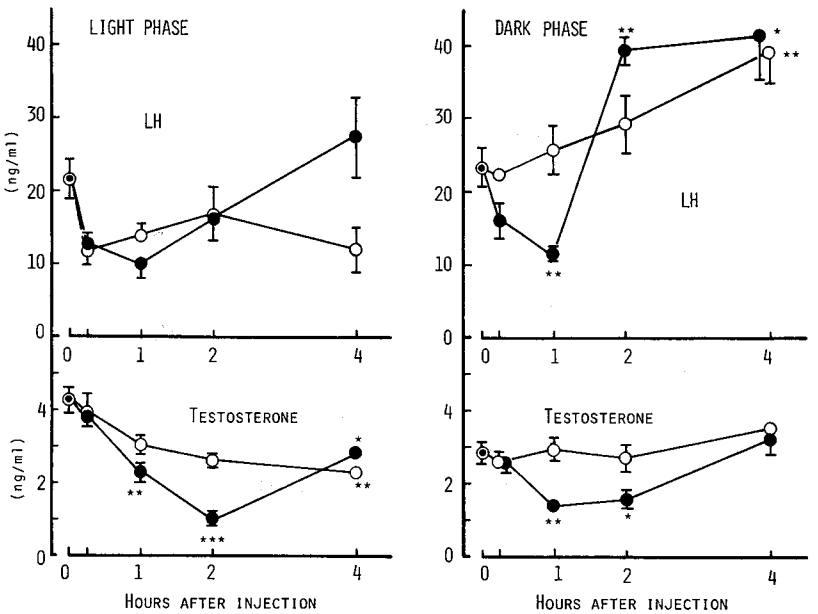


FIG. 6. Effect of subcutaneous injection of MSG or NaCl on serum LH and testosterone concentration in rats in the light and dark phases. Rats were given a single dose of 1 g MSG/kg or 0.35 g NaCl/kg 0.25, 1, 2, and 4 hr before sacrifice. LH and testosterone values were expressed as ng/ml serum using NIAMDD Rat LH-RP-1 and testosterone provided by Sigma Co. as standards. Each point represents mean ± SE. ⊙, control; ●, MSG; ○, NaCl; $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$ versus control (Student's t-test).

suppressed in comparison with the control. However, Lee's index indicated that these animals were not obese, but tended to be lean, unlike rats injected with MSG as neonates. Similar results were reported in rats treated with a single shot of 4 g/kg (15) or daily injection of 2 g/kg (9) in the neonatal stage. As shown in Fig. 8, neurons of the hypothalamic arcuate nuclei in rats injected with MSG in the neonatal stage disappeared almost completely, whereas considerably numbers of neurons were observed in rats injected in the infant stage. These results suggest that the effect of MSG on growth depends on the severity of hypothalamic damage.

The LED of MSG capable of inducing hypothalamic lesions in 2-day-old rats injected subcutaneously and in 10-day-old rats dosed by forced intubation were 0.4 and 1.4 g/kg, respectively. Daily subcutaneous injections of a subneurotoxic dose of MSG (0.2 g/kg) in the neonatal stage and daily administration by forced intubation of a subneurotoxic dose (0.5 g/kg) in the infant stage did not affect body weight, body length, tail length, or Lee's index. When weanling rats were further fed with diets containing 5% MSG *ad libitum* for 10 days after forced intubation as described above, these rats ingested about 8 g MSG/kg body weight/day. They did not present any sign of obesity or stunted growth, despite the ingestion of large amounts of MSG.

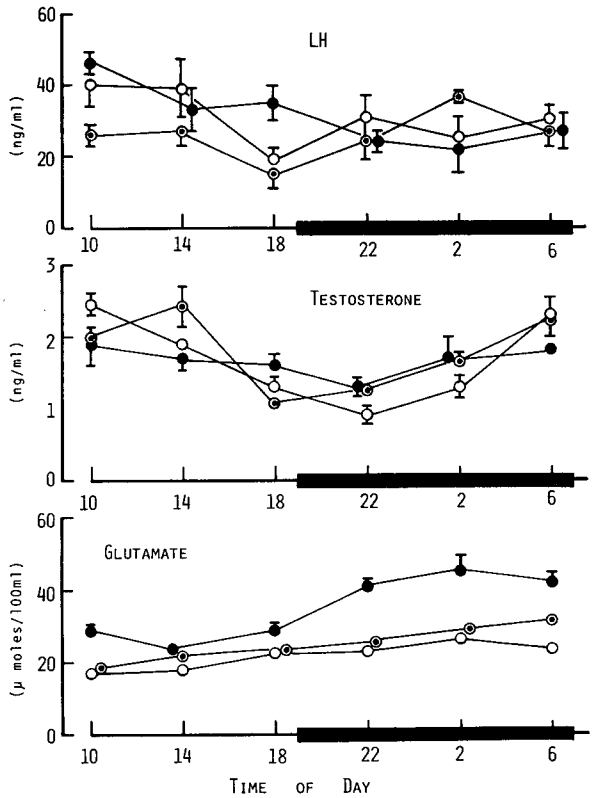


FIG. 7. Effect of dietary feeding of MSG on 24-hr changes of serum LH, testosterone, and glutamate concentration in rats. Each point represents mean ± SE. The solid bars along the abscissa represent the dark period: ○, control; ⊙, 4% (w/w) MSG diet; ●, 8% (w/w) MSG diet.

Organ Weight

Table 4 shows the weights of hormonal and other organs at autopsy of 3-month-old female rats and of 5-month-old male rats. In rats injected repeatedly with high doses of MSG in the neonatal stage, the weights of the anterior pituitary, adrenals, ovaries, uterus, testes, and seminal vesicle were significantly lower than in controls. On the other hand, in rats injected with high doses of MSG in the infant stage, organ weights—excepting the uterus, ovaries, and testes—were not significantly changed. Histological changes were not observed under light microscopy, even in the organs that decreased in weight.

It has been reported that daily injections of high doses of MSG in neonatal rodents resulted in a decrease in the weight of the pituitary, ovaries, uterus, adrenals, and testes (15,35). A significant decrease in weight of the pituitary was reported in mice (14) given a single shot of 4 g/kg in the neonatal stage, but Matsuyama et al. (11)

TABLE 3. *Body weight, body length, tail length, and Lee's index of rats at 16 weeks of age*

Group	Sex	Body weight (g)	Body length[a] (cm)	Tail length (cm)	Lee's index[b]
Experiment I (s.c., daily 2–11 days of age)					
Saline	M	547 ± 34[e]	25.0 ± 0.7	19.4 ± 0.5	0.325 ± 0.007
	F	317 ± 20	22.0 ± 0.5	17.2 ± 0.3	0.309 ± 0.006
MSG	M	526 ± 44	25.1 ± 0.3	19.5 ± 0.5	0.321 ± 0.008
0.2 g/kg	F	313 ± 21	22.1 ± 0.4	17.4 ± 0.3	0.308 ± 0.005
MSG	M	422 ± 37[f]	21.7 ± 0.6[f]	14.6 ± 1.5[f]	0.345 ± 0.007[f]
4 g/kg	F	300 ± 23	19.3 ± 0.5[f]	13.9 ± 0.6[f]	0.348 ± 0.012[f]
MSG	M	472 ± 39[f]	24.5 ± 0.6	18.4 ± 0.5[f]	0.317 ± 0.008[g]
4 g/kg[c]	F	273 ± 22[f]	20.9 ± 0.4[f]	16.5 ± 0.3[f]	0.310 ± 0.008
Experiment II (p.o., daily 10–19 days of age)					
Saline	M	536 ± 30	25.4 ± 0.4	19.2 ± 0.6	0.320 ± 0.008
	F	300 ± 13	21.7 ± 0.4	17.2 ± 0.2	0.309 ± 0.003
MSG	M	544 ± 30	25.3 ± 0.7	19.4 ± 0.3	0.323 ± 0.005
0.5 g/kg	F	300 ± 12	21.8 ± 0.4	17.5 ± 0.3	0.307 ± 0.007
MSG	M	513 ± 26	25.2 ± 0.3	19.2 ± 0.3	0.320 ± 0.006
0.5 g/kg[d]	F	304 ± 25	21.8 ± 0.8	17.5 ± 0.4	0.308 ± 0.006

[a] Nasoanal length.
[b] Lee's index = (body weight)$^{1/3}$/body length.
[c] Rats of this group were treated from 10 to 19 days of age.
[d] Rats of this group were additionally fed with diets containing 5% (w/w) MSG from 20 to 29 days of age after p.o. administration.
[e] Mean ± SD (N = 12).
[f] $p < 0.001$ vs saline group.
[g] $p < 0.05$ vs saline group.

failed to obtain these findings in similar experiments. Lengvári reported that there was no decrease in the weight of the pituitary, adrenal or thyroid gland, or gonads in rats injected with a single dose of 2 g/kg in the neonatal stage (9). As with the effects of MSG on growth, the effect of MSG on organ weights may depend on the severity of hypothalamic damage, which varies with the dose and age of the animals at the time of experimentation.

In rats repeatedly treated with subneurotoxic doses as neonates or infants and in rats fed large amounts of MSG in the diet during weaning, the organ weights were not changed compared with those of the controls.

Reproductive Function

The age at vaginal opening, an index of the onset of puberty, was noted, and vaginal smears were taken from 50 to 90 days of age in rats treated with MSG. Serum levels and pituitary contents of LH and follicle stimulating hormone (FSH) were measured in the morning and evening on the day of proestrus in 3-month-old rats. LH and FSH were measured by double antibody radioimmunoassay. As shown in Fig. 9, female rats injected repeatedly with high doses of MSG in the neonatal

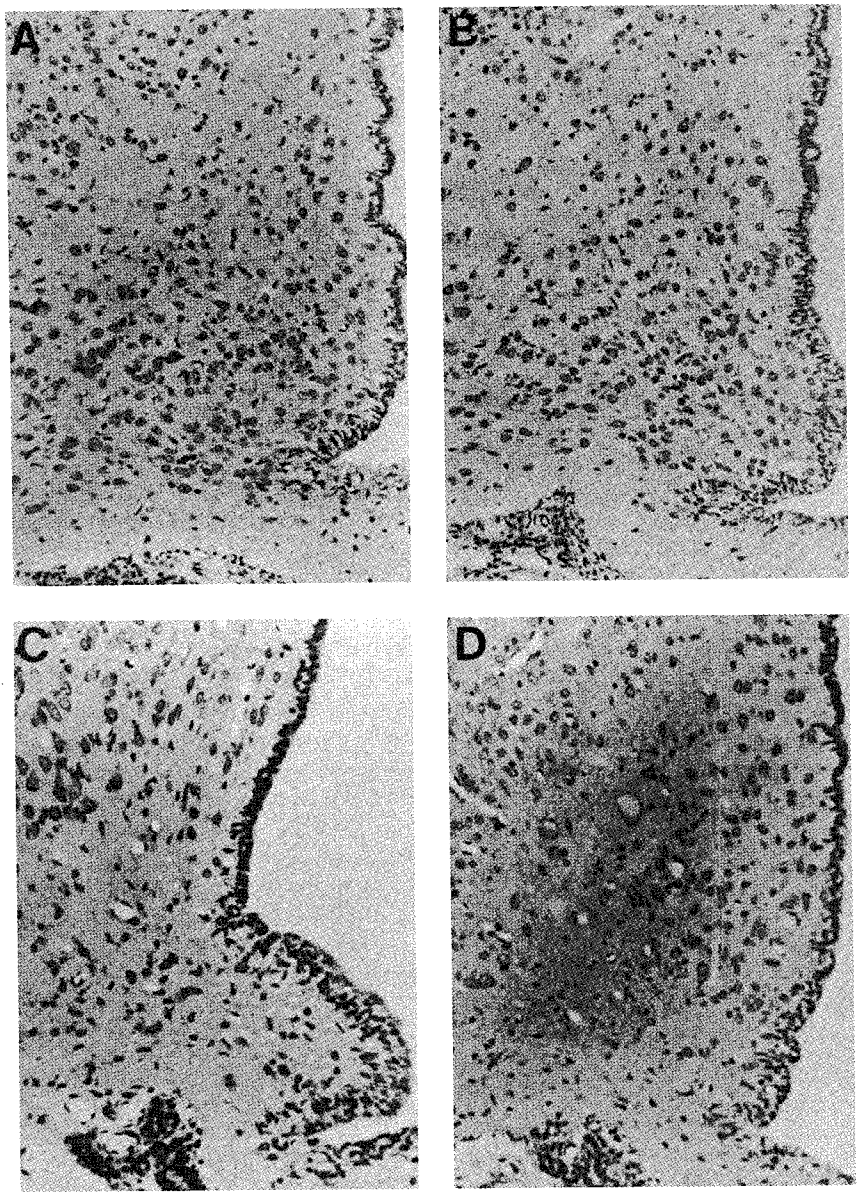


FIG. 8. Hypothalamic arcuate region of 5-month-old rats (× 200). **A:** Control rats were given daily saline from 10 to 19 days of age. **B:** After daily oral administration of 0.5 g MSG/kg from 10 to 19 days of age, rats were fed with large amounts of MSG in the diet from 20 to 29 days of age. No histological changes are seen. Compare with control rats. **C:** Rats were daily injected with 4 g MSG/kg from 2 to 11 days of age. Neurons in the arcuate nucleus disappeared almost completely, and the third ventricle was dilated. **D:** Rats were daily injected with 4 g MSG/kg from 10 to 19 days of age. A considerable number of neurons in the arcuate nucleus are observed.

TABLE 4. *Organ weight in adult rats*

Group	Sex	Anterior pituitary (mg)	Gonads (g)	Seminal vesicle (g)	Uterus (g)	Adrenals (mg)	Thyroid (mg)
Experiment I (s.c., daily 2–11 days of age)							
Saline	M[c]	8.4 ± 1.7[e]	2.93 ± 0.16	1.94 ± 0.44	—	56.8 ± 7.3	27.3 ± 5.4
	F[d]	11.6 ± 2.1	0.12 ± 0.02	—	0.91 ± 0.07	76.9 ± 10.0	—
MSG	M	8.5 ± 0.5	2.91 ± 0.10	2.05 ± 0.41	—	52.2 ± 5.3	22.0 ± 1.1
0.2 g/kg	F	12.3 ± 1.8	0.12 ± 0.02	—	0.88 ± 0.11	79.4 ± 6.7	—
MSG	M	4.4 ± 0.6[f]	1.34 ± 0.10[f]	1.21 ± 0.16[g]	—	42.4 ± 4.2[f]	20.9 ± 4.0
4 g/kg	F	7.5 ± 1.7[f]	0.05 ± 0.02[f]	—	0.63 ± 0.13[f]	49.1 ± 6.5[f]	—
MSG	M	7.7 ± 0.6	2.69 ± 0.09[f]	1.76 ± 0.30	—	54.1 ± 9.8	21.8 ± 5.1
4 g/kg[a]	F	12.0 ± 2.0	0.10 ± 0.07[g]	—	0.82 ± 0.09[g]	71.0 ± 12.7	—
Experiment II (p.o., daily 10–19 days of age)							
Saline	M	9.2 ± 0.8	3.04 ± 0.08	2.32 ± 0.19	—	56.2 ± 6.9	26.1 ± 3.2
	F	13.6 ± 1.4	0.12 ± 0.01	—	0.88 ± 0.08	81.2 ± 7.9	—
MSG	M	9.5 ± 0.9	3.04 ± 0.13	2.41 ± 0.47	—	54.9 ± 4.8	23.8 ± 4.1
0.5 g/kg	F	12.9 ± 2.7	0.12 ± 0.02	—	0.89 ± 0.10	85.0 ± 10.0	—
MSG	M	10.1 ± 0.8	2.93 ± 0.10	2.38 ± 0.27	—	54.5 ± 3.0	25.8 ± 3.4
0.5 g/kg[b]	F	13.9 ± 1.9	0.12 ± 0.01	—	0.86 ± 0.12	79.4 ± 6.8	—

[a] Rats of this group were treated from 10 to 19 days of age.
[b] Rats of this group were additionally fed with diets containing 5% (w/w) MSG from 20 to 29 days of age after p.o. administration.
[c] Five-month-old male rats ($N = 6$).
[d] Three-month-old female rats ($N = 12$).
[e] Mean ± SD.
[f] $p < 0.001$.
[g] $p < 0.01$.

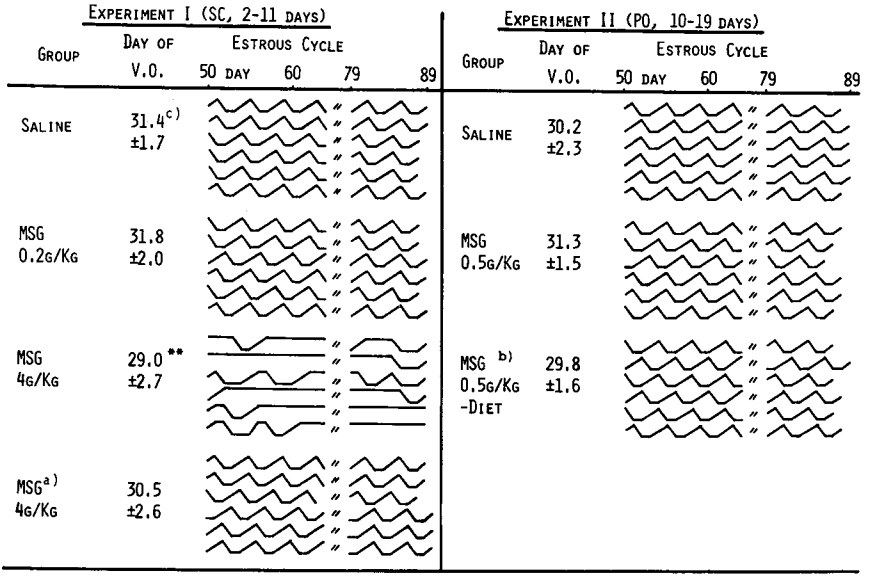
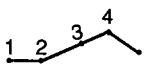


FIG. 9. Day of vaginal opening (V.O.) and estrous cycle in rats. Estrous cycles of 6 rats of each group are shown and illustrated in the following manner:



where 1 = diestrus-1, 2 = diestrus-2, 3 = proestrus, and 4 = estrus. a) Rats of this group were treated from 10 to 19 days of age. b) Rats of this group were additionally fed with diets containing 5% (w/w) MSG from 20 to 29 days of age after p.o. administration. c) Mean ± SD (N = 12). $^{**}p < 0.01$.

stage had early vaginal opening and irregular estrous cycles, i.e., continuous estrus or prolongation of estrus or diestrus. In these females, pituitary LH and FSH were significantly lower in the morning than in controls (Table 5). Mean serum LH and FSH in the morning and evening did not significantly differ from those in control animals. Among females of this treated group, rats with prolonged estrus had low serum levels (LH, 29 ng/ml; FSH, 198 ng/ml), indicating no surge of gonadotrophins in the evening.

Rats injected with high doses of MSG in infancy showed no differences in the day of vaginal opening with respect to the regularity of estrous cycles. Although the pituitary FSH content of these rats was significantly diminished, serum LH and FSH levels in the morning and evening did not differ from those in controls. The elevation of serum gonadotrophins in the evening in these rats indicated a normal preovulatory surge of LH. It is noticeable that the rats of this group with hypothalamic damage showed normal secretion of gonadotrophins. It has been reported that injection of high doses of MSG to neonatal rodents induces early vaginal opening (35), irregular estrous cycles (14), and a decrease in the pituitary

TABLE 5. *Serum level and anterior pituitary content of LH and FSH on proestrous day in adult female rats*

Group		Serum level (ng/ml) AM[a]	Serum level (ng/ml) PM[a]	Anterior pituitary content (μg/gland) AM[a]	Anterior pituitary content (μg/gland) PM[a]
Experiment I (s.c., daily 2–11 days of age)					
Saline	LH	20 ± 7[d]	627 ± 190	146 ± 17	90 ± 28
	FSH	136 ± 26[d]	450 ± 51	4.1 ± 1.6	2.1 ± 1.2
MSG	LH	20 ± 9	750 ± 166	155 ± 12	110 ± 35
0.2 g/kg	FSH	142 ± 16	405 ± 53	5.7 ± 1.6	2.8 ± 1.3
MSG	LH	13 ± 8	368 ± 400	86 ± 46[e]	74 ± 12
4 g/kg	FSH	155 ± 25	308 ± 197	1.5 ± 0.5[f]	1.5 ± 0.9
MSG	LH	23 ± 5	627 ± 169	126 ± 16	85 ± 26
4 g/kg[b]	FSH	145 ± 17	385 ± 56	2.1 ± 0.7[e]	1.9 ± 0.5
Experiment II (p.o., daily 10–19 days of age)					
Saline	LH	19 ± 4	773 ± 157	147 ± 47	101 ± 2.4
	FSH	152 ± 24	430 ± 69	5.3 ± 1.4	2.7 ± 1.7
MSG	LH	14 ± 9	837 ± 146	120 ± 52	102 ± 22
0.5 g/kg	FSH	138 ± 22	449 ± 44	4.8 ± 2.1	2.3 ± 0.8
MSG	LH	22 ± 10	797 ± 129	149 ± 32	112 ± 27
0.5 g/kg[c]	FSH	155 ± 22	396 ± 70	5.3 ± 1.4	2.2 ± 0.7

[a] Rats were sacrificed in the morning (10:30–11:30 a.m.) and evening (5:30–6:00 p.m.).
[b] Rats of this group were treated from 10 to 19 days of age.
[c] Rats of this group were additionally fed with diets containing 5% (w/w) MSG from 20 to 29 days of age after p.o. administration.
[d] Mean ± SD (N = 6), expressed as NIH-LH-RP-1 and NIH-FSH-RP-1.
[e] $p < 0.05$.
[f] $p < 0.01$.

LH content (28). However, Matsuyama et al. (11) and Lengvári (9) did not observe abnormalities in the onset of puberty or estrous cycle in rats repeatedly injected with 2 g/kg in the neonatal stage. These facts suggest that the effect of MSG on reproductive function depends on the severity of hypothalamic damage.

Rats given repeated subneurotoxic doses of MSG as neonates or infants and rats given large amounts of MSG in the diet during weaning showed no abnormalities in the onset of vaginal opening or estrous cycle (Fig. 9). Serum levels and pituitary contents of LH and FSH did not differ from those of controls (Table 5). The gonadotrophin secretion system functioned normally in these females.

As shown in Table 6, the pituitary LH content in males injected repeatedly with high doses of MSG in the neonatal stage was significantly lower than in controls, but serum LH and FSH levels did not differ. The later findings appeared to be consistent with the reports by Nemeroff et al. (16). Male rats injected with high doses of MSG in infancy, rats given subneurotoxic doses in the neonatal or infant stage, and those given large amounts of MSG in the diet during weaning did not show any differences in serum levels or pituitary content of LH and FSH compared with controls.

TABLE 6. *Serum level and anterior pituitary content of LH and FSH in adult male rats*

Group	Serum level (ng/ml)		Anterior pituitary content (μg/gland)	
	LH[a]	FSH[a]	LH[a]	FSH[a]
Experiment I (s.c., daily 2–11 days of age)				
Saline	26 ± 12	206 ± 13	47 ± 8	35 ± 7
MSG 0.2 g/kg	30 ± 8	213 ± 64	46 ± 10	38 ± 6
MSG 4 g/kg	18 ± 9	185 ± 28	34 ± 10[d]	34 ± 5
MSG 4 g/kg[b]	16 ± 5	195 ± 30	39 ± 6	34 ± 7
Experiment II (p.o., daily 10–19 days of age)				
Saline	33 ± 23	210 ± 20	51 ± 8	44 ± 12
MSG 0.5 g/kg	24 ± 6	210 ± 29	49 ± 6	42 ± 9
MSG 0.5 g/kg[c]	35 ± 11	234 ± 22	53 ± 5	39 ± 6

[a] Mean ± SD ($N = 6$), expressed as NIH-LH-RP-1 and NIH-FSH-RP-1.
[b] Rats of this group were treated from 10 to 19 days of age.
[c] Rats of this group were additionally fed with diets containing 5% (w/w) MSG from 20 to 29 days of age after p.o. administration.
[d] $p < 0.05$.

Behavioral Observations

Spontaneous motor activities (SMA) were measured by Animex under the usual housing conditions in male rats treated with MSG. As shown in Table 7, rats injected daily subcutaneously with high doses of MSG in the neonatal stage had significantly less total SMA in one day, decreased SMA in the dark phase, and a greater percentage of light-phase activity. In a 24-hr pattern of SMA (Fig. 10), these rats showed significantly less activity in the dark phase, except for the initial stage, and a significant increase of activity at later stages of the light phase. The decrease in total SMA in a day may be correlated with the decrease in dietary intake during the day in these obese animals. Similar decreases in total SMA in a day have been reported in rats (27) and mice (25). On the other hand, Araujo et al. (1) observed increased SMA in 2 hr in mice. The contradictory results are considered to be due to differences in the experimental conditions, such as the dose of MSG administered, apparatus employed, and time of measurement.

Daily subcutaneous injections of high doses of MSG during infancy, the administration of subneurotoxic doses in the neonatal or infant stages, and large amounts of MSG in the diet during weaning did not elicit any changes in total SMA in a day or in the 24-hr SMA pattern in rats when compared with controls.

Three-month-old male rats were exposed to an open field arena for 3 min, and center latency and ambulation scores were recorded. Observations were carried out

TABLE 7. *SMA in a day measured by Animex in 3-month-old male rats*

Group	SMA (× 10^3 counts)[a] Total	Light phase / Dark phase	% Light-phase activity
Experiment I (s.c., daily 2–11 days of age)			
Saline (N = 9)	31.8 ± 4.7[d]	6.3 ± 1.9 / 25.5 ± 4.6	19.8 ± 6.3
MSG 0.2 g/kg (N = 4)	30.7 ± 2.7	6.1 ± 1.6 / 24.5 ± 1.8	19.9 ± 4.3
MSG 4 g/kg (N = 9)	21.8 ± 7.3[e]	6.9 ± 1.9 / 14.9 ± 4.2[e]	32.2 ± 8.7[f]
MSG[b] 4 g/kg (N = 4)	29.2 ± 2.6	5.4 ± 1.1 / 23.9 ± 1.6	18.3 ± 2.1
Experiment II (p.o., daily 10–19 days of age)			
Saline (N = 4)	27.1 ± 2.1	6.1 ± 1.0 / 21.0 ± 2.4	22.5 ± 4.4
MSG 0.5 g/kg (N = 4)	28.3 ± 3.2	5.7 ± 1.6 / 22.6 ± 2.5	19.9 ± 4.3
MSG[c] 0.5 g/kg (N = 4)	28.0 ± 3.4	5.9 ± 2.0 / 22.1 ± 2.0	20.8 ± 4.8

[a] Activity was measured in a pair of rats under the illumination schedule of 12 hr light/12 hr dark.
[b] Rats of this group were treated from 10 to 19 days of age.
[c] Rats of this group were additionally fed with diets containing 5% (w/w) MSG from 20 to 29 days of age after p.o. administration.
[d] Mean ± SD.
[e] $p < 0.001$.
[f] $p < 0.01$.

from 1:00 to 5:00 p.m. on two consecutive days. In this open field test, prolongation of center latency and increased ambulation were observed in rats injected daily subcutaneously with high doses of MSG in the neonatal stage (Table 8). These changes suggested that the emotionality of these rats was affected. As for the increase in ambulation, the time when the open field test was carried out coincided with the time of increased SMA (Fig. 10). In other groups treated with MSG, no difference was observed in the open field test compared with the control group.

Nemeroff et al. reported that self-mutilation (tail autoingestion) was observed in 14% of males and 88% of females injected daily with high doses of MSG in the neonatal stage (15). Tail mutilation was observed in a few cases among rats injected subcutaneously with high doses of MSG as neonates.

Berry et al. (3) reported less ability to learn in the water maze test only in rats

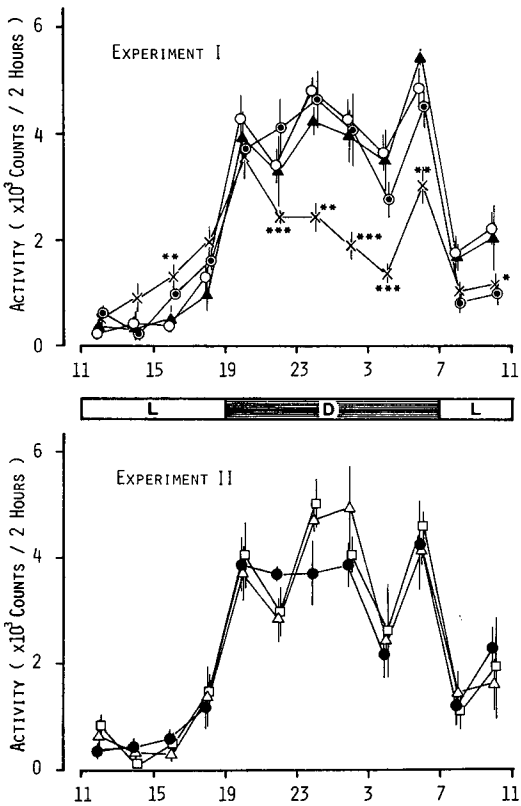


FIG. 10. A 24-hr pattern of SMA in 3-month-old male rats. Activity in a pair of rats was measured by Animex. Each point represents the mean ± SE of cumulative values taken every 2 hr. Rats were daily injected with saline (○), 0.2 g MSG/kg body weight (▲) and 4 g/kg (×) from 2 to 11 days of age, and 4 g/kg from 10 to 19 days of age (⊙), subcutaneously (Experiment I). Rats were daily given saline (●) and 0.5 g MSG/kg (△) from 10 to 19 days of age, orally. After this treatment with MSG, rats were fed with diets containing 5% (w/w) MSG from 20 to 29 days of age (□) (Experiment II). $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$.

given repeated subcutaneous injections of high doses of MSG in the neonatal stage. Pradhan et al. (27) reported a deficiency in discriminatory learning in a T-maze test in rats given a high dose of MSG repeatedly by forced intubation in the neonatal stage, but their learning ability in a fixed-ratio food reinforcement schedule was not affected. In a Lashley III maze in our experiments, there were no differences in the number of errors or in running time among all groups tested.

To check neuromuscular ability, the rotating-rod test and the inclined-plane test were carried out in 1- and 3-month-old rats. There were no differences among all groups in either test. No abnormalities in corneal reflexes or pinna reflexes were observed in any group.

TABLE 8. *Center latency and ambulation scored in 3-min period by open field test in 3-month-old male rats*

Group	Center latency (sec)		Ambulation	
	1st day	2nd day	1st day	2nd day
Experiment I (s.c., daily 2–11 days of age)				
Saline	16.3 ± 3.3[c]	9.0 ± 2.2	16.4 ± 3.7	28.5 ± 4.4
MSG 0.2 g/kg	20.9 ± 5.3	7.5 ± 1.2	20.0 ± 2.1	30.8 ± 2.6
MSG 4 g/kg	15.3 ± 2.5	24.5 ± 6.1[d]	27.3 ± 3.0[d]	31.4 ± 4.2
MSG 4 g/kg[a]	39.3 ± 20.4	26.3 ± 11.9	17.4 ± 3.8	24.0 ± 3.8
Experiment II (p.o., daily 10–19 days of age)				
Saline	20.5 ± 8.7	10.1 ± 1.3	21.0 ± 3.5	28.8 ± 2.1
MSG 0.5 g/kg	21.8 ± 5.1	18.1 ± 7.2	18.9 ± 3.9	24.9 ± 3.7
MSG 0.5 g/kg[b]	10.1 ± 2.3	11.5 ± 1.5	17.0 ± 2.8	21.3 ± 3.4

[a] Rats of this group were treated from 10 to 19 days of age.
[b] Rats of this group were additionally fed with diets containing 5% (w/w) MSG from 20 to 29 days of age after p.o. administration.
[c] Mean ± SE ($N = 8$).
[d] $p < 0.05$.

SUMMARY

The LED of MSG inducing lesions in the hypothalamic arcuate nuclei and plasma glutamate levels at LED increased with age in mice.

Plasma glutamate was raised less after oral administration of MSG than after parenteral injection. When mice were given MSG orally with food, plasma glutamate rose less than after injections of similar doses.

Weanling, pregnant, and lactating mice fed large amounts of MSG in the diet (10 to 30 g/kg body weight/day) did not develop hypothalamic lesions. Their fetuses and the newborn mice were also unaffected. Plasma glutamate levels in mice fed large amounts of MSG in the diet were much lower than those required to induce brain damage.

Fluctuations of serum LH and testosterone levels in rats fed MSG in the diet were within the normal daily range of control animals, and dietary feeding of large amounts of MSG had no substantial effects on these hormone levels.

Disturbances in growth and reproductive function and some behavioral abnormalities were observed in rats injected repeatedly with high doses of MSG as neonates or infants. These disturbances and abnormalities were more marked when the MSG had been injected during the neonatal period. When neonatal and infant rats were repeatedly given subneurotoxic doses and when weanling rats were fed

diets containing large amounts of MSG, no adverse effects were seen in the mature animals.

From these results it can be concluded that MSG, a food additive, does not cause any acute or long-range adverse effects following *ad libitum* feeding in rodents.

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