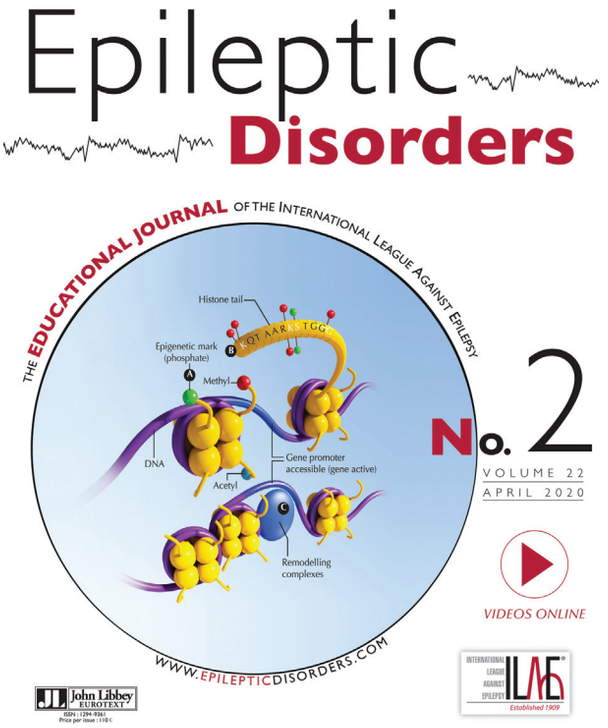
John Libbey EUROTEXT

The key scientific and medical informations

www.jle.com

Find the contents of this issue hereafter:

http://www.john-libbey-eurotext.fr/fr/revues/medecine/epd/sommaire.md?type=text.html



Montrouge, 05/01/2020

Hasan Raci Yananlı

You will find below the electronic reprint of your article (pdf format):

The effect of oral administration of monosodium glutamate on epileptogenesis in infant rats

published in:

Epileptic Disorders, 2020, Volume 22, Numéro 2

John Libbey Eurotext

This electronic reprint is provided for your exclusive use, and can't be communicated but for scientific and individual purposes. It mustn't, under any circumstances, be used or distributed for advertising, promotional or commercial purposes.

© John Libbey Eurotext, 2020

Original article

Epileptic Disord 2020; 22 (2): 195-201

The effect of oral administration of monosodium glutamate on epileptogenesis in infant rats

Mahluga Jafarova Demirkapu [1,a], Hasan Raci Yananlı [2,a], Elif Akşahin [2], Ceren Karabiber [2], Pınar Günay [2], Arda Kekilli [2], Betilay Topkara [3]

[1] Tekirdag Namık Kemal University, School of Medicine, Department of Pharmacology Tekirdag,
[2] Marmara University School of Medicine, Department of Pharmacology, Hastane Yolu Street number 5, 34854 Maltepe Istanbul,
[3] Koç University School of Medicine Istanbul, Turkey
[a] Authors contributed equally

Received May 03, 2019; Accepted December 07, 2019

ABSTRACT – *Aim*. Glutamate is an excitatory neurotransmitter that is widely distributed throughout the brain. An increase in glutamate concentration or sensitivity of glutamate receptors triggers neurodegenerative diseases, epilepsy in particular. Monosodium glutamate is a substance added to foods to enhance flavour. We investigated the effect of monosodium glutamate on epileptogenesis, as well asheight and weight, in rats that were just weaned.
Methods. Twenty-four male and female 21-day-old Wistar Albino rats were divided into two groups: one with monosodium glutamate added to the drinking water, and a control in which NaCl was added to the drinking water. The electrical stimulation threshold values were determined in animals to which the hippocampal kindling process was applied, and the stimulations at these threshold values were invariably applied to the animals until they were kindled.
Results. The electrical stimulation threshold values of the monosodium glutamate group did not statistically change, whereas the number of required stimulations for kindled rats was significantly lower compared with the control group.
Conclusion. These results reveal that long-term oral administration of glutamate salts causes an increase in excitability in the central nervous system during ontogenetic development.

Key words: epilepsy, glutamate, hippocampus, kindling

Correspondence:
Hasan Raci Yananlı
Marmara Üniversitesi - Medical School Pharmacology,
Maltepe Başıbüyük 34854 Istanbul,
34854 Turkey
<hasanyananli@yahoo.com>

doi:10.1684/epd.2020.1156

Monosodium glutamate (MSG), which is used for the flavouring of foods, was first isolated by Kikunae Ikeda in the early 1900s from dried konbu and was used in traditional Japanese soup (Ikeda, 2002). MSG was also used in Far East cuisine and has also been commonly used in Western countries over the years (Bellisle *et al*., 1991). MSG is an L-glutamate (L-GLU) derivative, which is soluble in water. When specific tongue receptors are stimulated, MSG is tasted as an umami taste,

John Libbey Eurotext, 2020

the fifth basic taste. The receptors that mediate the effects of MSG on the gastrointestinal system arefound in the stomach (Kondoh and Torii, 2008).

GLU, a non-essential amino acid, is the main excitatory neurotransmitter of the central nervous system (CNS) (Schousboe, 1981). At high concentrations, it is distributed in the cerebral cortex, cerebellum, and hippocampus in the brain. In addition to its physiological importance, it plays a key role in the pathophysiology of temporal lobe epilepsy (TLE) (Engel *et al*., 1997). TLE is the most common type of partial epilepsy in humans and in some cases, insufficient drug treatment causes a serious health problem (Eid *et al*., 2004).

In some studies, parenteral or oral administration of MSG has led to contradictory results. Parenteral administration of MSG has been shown to cause damage, especially in the hypothalamus, as well as increased short stature, metabolic derangement, and weight gain (Diniz *et al*., 2004; Fernandez-Tresguerres Hernández, 2005; Matyskova *et al*., 2008). Low concentrations of orally administered MSG have been shown to increase saturation, regulate appetite, and decrease sodium consumption without changing taste (Bellisle, 2008; Masic and Yeomans, 2014). However, high concentrations of MSG given orally caused metabolic disorders, depending on nutrition (Diniz *et al*., 2005). In another study, it was indicated that both oral and parental administration led to voracious behaviour (Hermanussen *et al*., 2006).

In previous studies, it was demonstrated that MSG caused tonic and clonic convulsions after high-dose and parenteral administration in adult rats, and reduced the convulsion threshold in newborn rats when taken orally in late pregnancy (Yu *et al*., 1997; González-Burgos *et al*., 2004). In a recent study, it was observed that epileptiform activity, induced by 4-aminopyridine, significantly increased in rats exposed to parental administration of MSG in the neonatal period (Hernandez-Ojeda *et al*., 2017).

In this study, we added MSG to the drinking water of rats that were just weaned and had not completed their development with an aim to investigate its role in the onset and spread of temporal lobe seizures via the hippocampal kindling model, as well as effects on weight gain and nasal-anal length (NAL).

Materials and methods

Animals and experimental design

Twenty-four Wistar Albino rats (six females and six males in each group, age 21 days, 75-95 g) supplied from Marmara University Animal Center (DEHAMER) were used in the study. All experimental procedures were approved by the local ethics committee, MUHDEK (Approval No. 46.2013.mar). The rats were housed with a reversed 12-hour light/dark cycle at 21 ± 3°C and 50 ± 5% humidity. There was unlimited access to standard rat chow.

The infant rats were weighed and divided into two groups, the MSG group and control group, and 1.0 g/L MSG (Sigma-1446600) and 1.0 g/L NaCl were added to the drinking water of the rats in the MSG group and control group, respectively. In order to prevent possible low consumption and mask the bad taste, 24 g/L sucrose was added to the water of both groups and the rats were provided with 24-hour unlimited access to this water. The weight of the animals was recorded from the beginning to the end of the experiment. On the 30th day of the experiment, their first NAL measurements were taken and stereotaxic surgery was conducted under anaesthesia. According to the group, water with MSG or NaCl was continued, and the animals were allowed to recover for one week.

On the 37th day of the experiment, when the animals were aged two months, the kindling procedures were started and continued until the rats were kindled. At the end of the experiment, the last NAL and weight measurements were taken under high-dose sodium thiopental anaesthesia. The animals were decapitated and their brains were removed for histological analysis. Only the results of rats with confirmation of the targeted brain region were used.

Kindling

On the 30th day of the experiment, standard stereotaxic surgery was conducted on the animals under 75-mg/kg ketamine and 10-mg/kg xylazine (i.p) anaesthesia. Two stainless steel screws with attached insulated wire were implanted into the left frontal and left parietal cortex for EEG recording. An electrical stimulation electrode (MS303/1 twisted, Plastic's One Inc., Roanoke, VA, USA) was implanted into the right hippocampus (AP -3.8 mm; ML -2.2 mm; DV-2.3 mm from the bregma; Paxinos and Watson, 1998). After a one-week recovery period, on the 37th day of the experiment, basal EEG records were obtained for one hour and electrical stimulation threshold values were determined. Animals with a threshold of over 500 μA were removed from the experiment. Rectangular constant current pulses were applied to the animals twice a day for 2 ms at their determined threshold values. EEG was recorded for one hour in total, 30 minutes before and after each stimulation. Electrical stimulations were applied according to Racine's scale (RS) to reach Stage 3 and display three consecutive Stage 5 seizures (Racine, 1972). Those thatreached Stage 5 up to the 25th stimulus were considered kindled. EEG records were

John Libbey Eurotext, 2020

Table 1. The effect of MSG in the drinking water on weight gain in rats.

Day of experiment	Group	Sex	mean ± SEM	*n*	*p*
1st	NaCl	female	82.5±1.8	6	0.18
	MSG:	female	86.6±2.3	6	
	NaCl	male	88.5±1.5	6	0.19
	MSG	male	84.3±2.6	6	
37th	NaCl:	female	155±0.9	6	0.03
	MSG	female	163.6±3.3	6	
	NaCl	male	190.3±4	6	0.59
	MSG	male	194.5±6	6	

evaluated using a BioAmp ML 136 amplifier and Chart v7 program (PowerLab8S ADI Instruments, Oxfordshire, UK).

Data analysis

All data are expressed as mean ± standard error of the mean (SEM). GraphPadPrism 5.03 software was used for the analysis of the data. The groups were compared using the unpaired two-tailed t (normally distributed) and Mann Whitney U (non-normally distributed) tests. For all statistical calculations, significance was considered at $p<0.05$.

Results

Evaluation of weight

The weight of the animals was recorded throughout the experiment and their weight on the first day and 37th day (the first day of the kindling process) of the experiment were compared. Although the weight of the male rats in both MSG and control groups was not significantly different ($p>0.05$), the weight of female rats in the MSG group was significantly increased compared with the control group (*table 1, figure 1*).

Evaluation of nasal-anal length (NAL)

NALs measured just before the kindling process were compared between males and females in both groups. No significant difference was found between the NALs of male and female rats in the MSG and control group (*table 2, figure 2*).

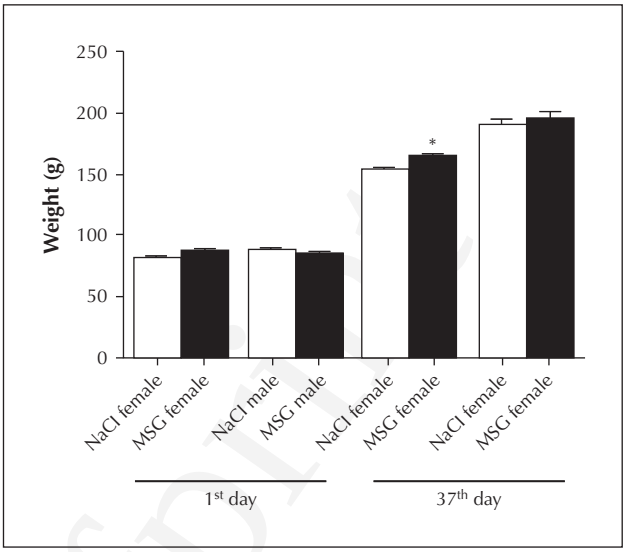


Figure 1. The effect of MSG in the drinking water on weight gain in rats. The weight of animals on the first day of the experiment, before randomisation (left columns), and on the first day of the kindling process (on 37th day of the experiment, right columns) were compared using the unpaired two-tailed t-test. Instead of MSG, an equivalent volume of NaCl was added to the water of the control group. All data are expressed as mean ± SEM. *$p<0.05$.

Table 2. The effect of MSG in the drinking water on NAL in rats.

Group	Sex	mean ± SEM	*n*	*p*
NaCl	female	18.67 ± 0.25	6	1
MSG	female	18.67 ± 0.24	6	
NaCl	male	20.02 ± 0.34	6	0.2
MSG	male	19.45 ± 0.23	6	

The effect of oral MSG administration on the kindling process

No significant differences were found in the electrical stimulation threshold values, determined on the first day of the kindling process, between the MSG group and the control group (*table 3, figure 3*).

A statistically significant difference was found in the number of stimulations required to reach Stage 3 and Stage 5 based on the RS in the MSG group compared with the control group ($p<0.05$) (*table 4, figure 4*).

Discussion

In this study, we investigated the effects of orally administered MSG on height and weight as well as on

John Libbey Eurotext, 2020

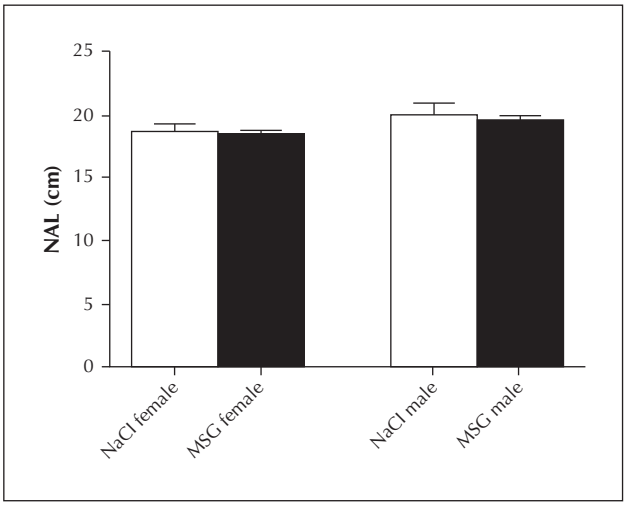


Figure 2. The effect of MSG added to drinking water on NAL in rats. NALs measured before the kindling process were compared using the unpaired two-tailed t-test. Instead of MSG, an equivalent volume of NaCl was added to the water of the control group. All data are expressed as mean ± SEM.

Table 3. The effect of MSG in the drinking water on the electrical stimulus threshold values determined before the kindling process, started on the 37th day of the experiment, in rats.

Group	mean ± SEM	*n*	*p*
NaCl	337.5 ± 45.22	10	0.25
MSG	270.0 ± 34.92	10	

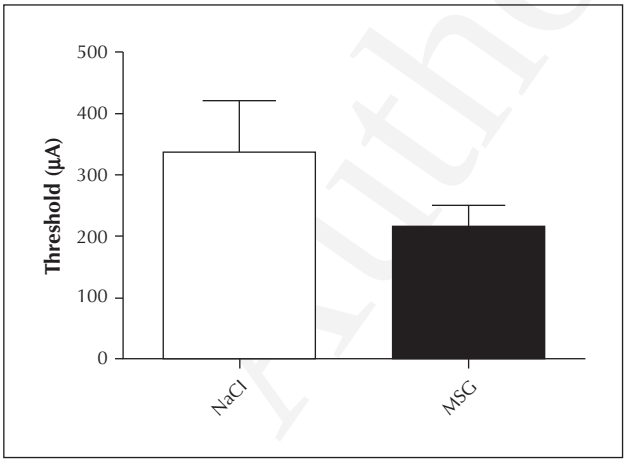


Figure 3. The effect of MSG added to the drinking water on the electrical stimulus threshold values determined before the kindling process, which was started on the 37th day of the experiment, in rats. The electrical stimulus threshold values of MSG and control groups were compared using the unpaired two-tailed t-test. Instead of MSG, an equivalent volume of NaCl was added to the water of the control group. All data are expressed as mean ± SEM.

Table 4. The effect of MSG in the drinking water on the number of stimulations in rats. The number of stimulations required for the MSG and control groups were compared using the Mann-Whitney U test.

Group	Sex	mean ± SEM	No. rats reaching Stage 5 (total *n*=10)	*p*
NaCl	female	18± 1.8	4	0.02
MSG	female	12.7± 2.3	4	
NaCl	female	19.5± 1.5	4	0.02
MSG	female	13.7± 2.6	4	

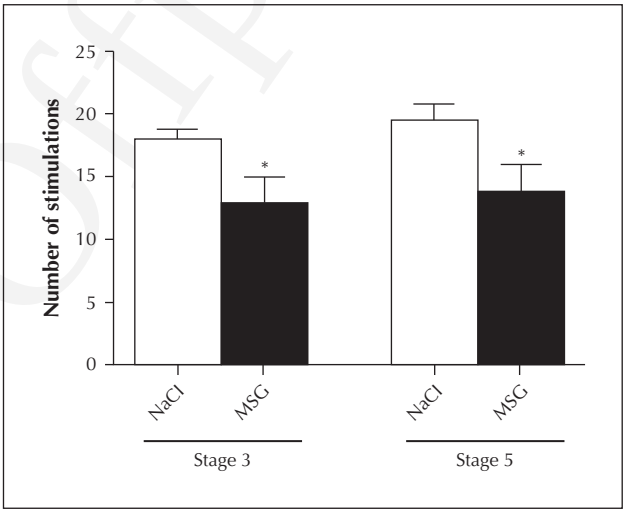


Figure 4. The effect of MSG added to the drinking water on the number of stimulations required to reach Stage 3 and Stage 5 based on the RS in rats. The number of stimulations required for the MSG and control groups were compared using the Mann-Whitney U test. Instead of MSG, an equivalent volume of NaCl was added to the water of the control group. All data are expressed as mean ± SEM. $^{**}p<0.01$.

the CNS. Although oral administration of MSG did not affect the height of either sex, it increased weight gain of the female rats (*figure 1*).

Previous studies have demonstrated that only orally administered MSG was effective through binding to specific receptors in the gastrointestinal tract. These receptors are taste-mGluR4 (Chaudhari *et al.*, 2000) and T1R family receptors, which create a heterodimeric structure (Li *et al.*, 2002), located in taste buds on the tongue. Although MSG produces umami taste with specific tongue receptors, it stimulates the vagus nerve, probably via increased production of bioactive substances such as nitric oxide and serotonin

Author offprint

by activating the specific stomach receptors (Kondoh and Torii, 2008). In light of this knowledge, exogenous GLU, probably via afferent vagus nerve fibres, stimulates the lateral hypothalamic area (LHA), which is part of the autonomous nervous system and plays a critical role in the regulation of eating and drinking behaviour, in addition to the insular cortex and basal ganglia, and increases weight gain in animals. Thus, in the event of total or partial vagotomy, food intake and weight gain decreases (Kondoh *et al*., 2000). In one study, a decrease in synaptic plasticity (long-term potentiation) in the hippocampal CA1 region occurred in rats fed a high-fatdiet for an extended period, which was associated with weight gain in the animals (Hwang *et al*., 2010). In other words, a decrease in hippocampal synaptic plasticity may be a facilitative cause of weight gain by affecting the hypothalamus, which is more sensitive to these effects. However, the fact that weight gain occurred only in female rats in our experiment makes us consider that another mechanism may also play a role in weight gain. Oestrogen and GLU have a bidirectional relationship. Whereas oestrogen reduces blood and brain GLU concentrations, GLU stimulates the production of oestrogen and increases its blood concentrations (Zlotnik *et al*., 2011). In our study, oral consumption of GLU may have induced weight gain in female rats by making metabolic changes through oestrogen.

Another, and perhaps more important, finding of our study was that the MSG group reached Stage 3 based on the RS faster than the control group although equivalent stimulation thresholds were applied to the animals (*figure 4*). Stage 3 is an indicator that epileptic activity covered the brain hemisphere where stimulation was applied and is a significant stage in epileptogenesis (Racine, 1972). An increase in GLU concentration, which is the main excitatory neurotransmitter of the CNS, causes oxidative stress while overstimulation of its receptors causes neuronal apoptosis and necrosis mediated by increasing intracellular Ca^{2+} (Patel *et al*., 1996). Neuronal damage may cause neurodegenerative diseases in addition to the emergence of epileptic seizures (Gill *et al*., 2000). However, it is commonly held that the MSG concentration added to foods does not affect brain GLU concentrations (Fernstrom, 2018). Our study reveals a significant finding in this regard: even oral consumption of GLU salts speeds up the emergence of epileptogenesis and facilitates animals to be kindled (*figure 4*).

Kindled or Stage 5 according to the RS demonstrates that the stimulation does not stay in the hemisphere where it is applied, but spreads to the entire brain leading to generalized seizures (Racine, 1972). Although it would seem unlikely that GLU, taken at low concentrations over a short period, could cross the blood-brain barrier and become more concentrated in the brain, a high dose and/or repeated exposure of GLU may have caused neurotoxicity upon crossing the barrier. Oral administration of MSG has been shown to lead to behavioural changes that could be expressed as anxiogenic behaviour (Narayanan *et al*., 2010). Moreover, some studies have demonstrated that exogenous MSG could cause damage in the brain and organs such as the kidney and liver by creating oxidative stress or/and an excitotoxic effect (Eweka and Om'Iniabohs, 2008). These effects, emerging after oral administration, may be due to the fact that MSG can cross the blood-brain barrier when administered at high concentrations over extended periods or can cause oxidative stress via receptors in the periphery. Additionally, we can consider that this effect of exposure to MSG at an early stage of life may be because the CNS is more sensitive and the blood-brain barrier is more permeable, as development is not yet complete (Fernandez-Tresguerres Hernández, 2005). Orally administered GLU speeds up epileptogenesis and facilitates kindling that may arise from an affected hippocampus which plays a critical role in epilepsy, similar to vagus nerve afferent fibres affecting the LHA, leading to weight gain. As another hypothesis, orally administered GLU may have caused an increase in sensitivity to GLU in the hypothalamus as a result of changes in the periphery, creating oxidative stress.

In previous studies, it was observed that parenterally administered MSG facilitates the kindling process in various types of models (Castellanos *et al*., 1998; Mori *et al*., 1989). This is due to glutamate being an excitatory amino acid. Moreover, the preferred routes of administration in these models cannot be used in humans. In our study, unlike other studies, we used a model whereby MSG is administered as a food additive. We started to give MSG to rats orally on the day they were just weaned (21stday), equivalent to an approximately two-year-old human. In terms of CNS development, the end of the second month in rats corresponds to the age of 12 in humans.

In conclusion, although orally administered GLU salts are known to be well metabolized and only a small amount enterssystemic circulation, its extended use may cause the emergence of hyperexcitability in the brain and facilitate neurodegenerative disorders, particularly epilepsy. Therefore, it would not seem appropriate to consume such food additives during a period when the development of the blood-brain barrier and CNS is not yet complete. □

Disclosures.
None of the authors have any conflict of interest to declare.

John Libbey Eurotext, 2020

References

Bellisle F. Experimental studies of food choices and palatability responses in European subjects exposed to the Umami taste. *Asia Pac J Clin Nutr* 2008; 17(Suppl 1): 376-9.

Bellisle F, Monneuse MO, Chabert M, Larue-Achagiotis C, Lanteaume MT, Louis-Sylvestre J. Monosodium glutamate as a palatability enhancer in the European diet. *Physiol Behav* 1991; 49(5): 869-73.

Castellanos CA, Roldan G, Gómez C, *et al*. Enhancement of pentylenetetrazol-induced kindling in mice treated neonatally with monosodium glutamate. *Proc West Pharmacol Soc* 1998; 41: 53-4.

Chaudhari N, Landin AM, Roper SD. A metabotropic glutamate receptor variant functions as a taste receptor. *Nat Neurosci* 2000; 3(2): 113-9.

Diniz YS, Fernandes A, Campos KE, Mani F, Ribas BO, Novelli ELB. Toxicity of hypercaloric diet and monosodium glutamate: oxidative stress and metabolic shifting in hepatic tissue. *Food Chem Toxicol* 2004; 42(2): 313-9.

Diniz YS, Faine LA, Galhardi CM, *et al*. Monosodium glutamate in standard and high fiber diets: metabolic syndrome and oxidative stress in rats. *Nutrition* 2005; 21(6): 749-55.

Eid T, Thomas MJ, Spencer DD, *et al*. Loss of glutamine synthetase in the human epileptogenic hippocampus: possible mechanism for raised extracellular glutamate in mesial temporal lobe epilepsy. *Lancet* 2004; 363(9402): 28-37.

Engel J, Williamson PD, Wieser HG. Mesial temporal lobe epilepsy. In: Engel J, Pedley TA, eds. *Epilepsy: a comprehensive textbook*. Philadelphia: Lippincott-Raven, 1997.

Eweka AO, Om'Iniabohs FAE. Histological studies of the effects of monosodium glutamate on the liver of wistar rats. *Int J Gastroenterol* 2008; 6(2): 1528-8323.

Fernandez-Tresguerres Hernández JA. Effect of monosodium glutamate given orally on appetite control (a new theory for the obesity epidemic). *An R AcadNac Med (Madr)* 2005; 122(2): 341-55.

Fernstrom JD. Monosodium glutamate in the diet does not raise brain glutamate concentrations or disrupt brain functions. *Ann Nutr Metab* 2018; 73(Suppl. 5): 43-52.

Gill SS, Mueller RW, McGuire PF, Pulido OM. Potential target sites in peripheral tissues for excitatory neurotransmission and excitotoxicity. *Toxicol Pathol* 2000; 28(2): 277-84.

González-Burgos I, López-Vázquez MA, Beas-Zárate C. Density, but not shape, of hippocampal dendritic spines varies after a seizure-inducing acute dose of monosodium glutamate in rats. *Neurosci Lett* 2004; 363(1): 22-4.

Hermanussen M, Garcia AP, Sunder M, Voigt M, Salazar V, Tresguerres JA. Obesity, voracity and short stature: the impact of glutamate on the regulation of appetite. *Eur J ClinNutr* 2006; 60(1): 25-31.

Hernandez-Ojeda M, Ureña-Guerrero ME, Gutierrez-Barajas PE, Cardenas-Castillo JA, Camins A, Beas-Zarate C. KB-R7943 reduces 4-aminopyridine-induced epileptiform activity in adult rats after neuronal damage induced by neonatal monosodium glutamate treatment. *J Biomed Sci* 2017; 24(1): 27.

Hwang LL, Wang CH, Li TL, *et al*. Sex differences in high-fat diet-induced obesity, metabolic alterations and learning and synaptic plasticity deficits in mice. *Obesity (Silver Spring)* 2010; 18(3): 463-9.

Ikeda K. New seasonings. *Chem Senses* 2002; 27(9): 847-9.

Kondoh T, Mori M, Ono T, Torii K. Mechanisms of umami taste preference and aversion in rats. *J Nutr* 2000; 130(4S Suppl): 966S-70S.

Kondoh T, Torii K. Brain activation by umami substances *via* gustatory and visceral signalling pathways and physiological significance. *Biol Pharm Bull* 2008; 31(10): 1827-32.

Masic U, Yeomans MR. Monosodium glutamate delivered in a protein-rich soup improves subsequent energy compensation. *J Nutr Sci* 2014; 3: e15.

Matyskova R, Maletinska L, Maixnerova J, Pirnik Z, Kiss A, Zelezna B. Comparison of the obesity phenotypes related to monosodium glutamate effect on arcuate nucleus and/or the high fat diet feeding in C57BL/6 and NMRI mice. *Physiol Res* 2008; 57(5): 727-34.

Mori N, Wada JA, Kumashiro H. Bidirectional transfer between kindling induced either by L-glutamate or L-aspartate and electrical stimulation in rats. *Brain Res* 1989; 498(1): 163-6.

Narayanan SN, Kumar RS, Paval J, Nayak S. Effect of ascorbic acid on the monosodium glutamate-induced neurobehavioral changes in periadolescent rats. *Bratisl Lek Listy* 2010; 111(5): 247-52.

Patel M, Day BJ, Crapo JD, Fridovich I, McNamara JO. Requirement for superoxide in excitotoxic cell death. *Neuron* 1996; 16(2): 345-55.

Paxinos G, Watson C. *The rat brain in stereotaxic coordinates, fourth edition*. San Diego, California: Academic press, 1998.

Racine RJ. Modification of seizure activity by electrical stimulation: II. Motor seizure. *Electroencephalogr Clin Neurophysiol* 1972; 32(3): 281-94.

Schousboe. A.Transport and metabolism of glutamate and GABA in neurons and glial cells. *Int Rev Neurobiol* 1981; 22: 1-45.

Li X, Staszewski L, Xu H, Durick K, Zoller M, Adler E. Human receptors for sweet and umami taste. *Proc Natl Acad Sci USA* 2002; 99(7): 4692-6.

Yu T, Zhao Y, Shi W, Ma R, Yu L. Effects of maternal oral administration of monosodium glutamate at a late stage of pregnancy on developing mouse fetal brain. *Brain Res* 1997; 747(2): 195-206.

Zlotnik A, Gruenbaum BF, Mohar B, *et al*. The effects of estrogen and progesterone on blood glutamate levels: evidence from changes of blood glutamate levels during the menstrual cycle in women. *Biol Reprod* 2011; 84(3): 581-6.

Author Offprint

TEST YOURSELF

(1) Does exogenous MSG have an effect on kindling-induced epileptogenesis?

(2) Did oral MSG change threshold values during the kindling process?

(3) What are the possible mechanisms involved in the effects of MSG?

Note: Reading the manuscript provides an answer to all questions. Correct answers may be accessed on the website, www.epilepticdisorders.com, under the section "The EpiCentre".

Author Offprint