A STUDIES ON MONOSODIUM L-GLUTAMATE TOXICITY IN ANIMAL MODELS - A REVIEW

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ABSTRACT
This review assesses many of the health implications and toxicity associated with monosodium glutamate (MSG) in animal models. MSG is the sodium salt of the amino acid responsible for their savoury taste of the food. The prevalence of this salt as a food additive in Asian, American and European cuisine and other diets. Debate over the healthiness of MSG and its effects on human obesity and other health problems has led to a negative public opinion. This literature review assesses that the consumption of MSG brings to pertain weight gain, obesity, locomotor and learning deficit, behavior and memory changes in rats. Increased obesity and body mass in rats is also associated with diabetes mellitus, hyperinsulinemia, atherosclerosis, cardiovascular disorder and fatty liver. The studies further suggest that MSG is neurotoxic in rats when injected neonatally. This review asserts the neonatal consumption of MSG leads to scarring of the neurons in the hippocampus and inhibited glutamate synthesis, resulting in impaired spatial memory and learning. Most of the studies provide evidence that ingestion of MSG is associated with neuro-endocrine disorders, metabolic dysfunction, oxidative stress and learning and memory deficit based on the clinical studies.

Keywords: Neurotoxicity, oxidative stress, learning and memory changes, MSG.

INTRODUCTION
Monosodium L-glutamate (MSG) is the sodium salt of the amino acid, Glutamic acid (Figure 1). Glutamate is one of the most abundant amino acids that make up proteins, found in protein rich foods such as milk, meat, fish, cheese, tomato products, soy sauces, and in many animal tissues and is responsible for their savoury taste. It is produced commercially by the fermentation of molasses and fermented proteins (soy sauce and hydrolyzed vegetable protein). Glutamate is also produced in the body and plays an important role in human metabolism.

Fig. 1: Chemical structure of MSG

MSG consumption has increased throughout the world in recent years as flavoring in cooking to increase palatability and food selection in a meal. It is used to provide in the food as meaty, savoury, or brothy taste by stimulating the glutamate receptors on the tongue. There are glutamate receptors in other parts of the body, especially the brain, where glutamate is acting as a neurotransmitter. This receptors induces more salivation, create greater stimulation of the olfactory and limbic system of the brain and promotes immune function. Almost all dietary glutamate, both in free form and as protein constituent, is metabolized in the intestinal mucosa. Dietary glutamate is a major energy source and an important substrate for the synthesis of glutathione and other amino acids in the gut. The average intake of glutamate as protein constituent (10g) and in its free form has been estimated approximately 1
g/day⁸. With respect to added glutamate mostly
in the form of MSG, the average intake ranges
0.3 to 0.5 g/day in European countries and 1.2
to 1.7 g/day in Asian countries⁹. These levels of
glutamate in the food are considered as safe⁸.⁹.
The aim of the present study was to review the
toxicity effect of MSG on neuro-endocrine,
metabolic and behavioral abnormalities in
pregnant and neonatal animals based on the
clinical studies.

OXIDATIVE STRESS
The uses of MSG as a safe food additive has been
questioned, due to a number of reports about its
toxic effects in humans as manifested by the
‘Chinese Restaurant Syndrome’ and the
production of lesions in the hypothalamus of
newborn mice and monkeys¹⁰. Many studies
show that MSG at dose levels above 4 mg/g b.w.
induced hyperlipidemia and hyperglycemia¹¹-¹³
and oxidative stress in the red blood cells¹⁴.
Elevated levels of glucose can result in
peroxidation of membrane lipids and red blood
cells, probably due to enolization of oxygen and yielding
α-keto aldehydes and free radical intermediates¹⁵.
The other research study showed that MSG (2
mg/g) administered in mice for 5 consecutive
days produced severe obesity, urinary glucose,
hyperglycemia, hyperinsulinemia, and a
decrease in both glucose tolerance and insulin
sensitivity. In these animals, a severe
hypertrophy of pancreatic islets due to the
proliferation of β cells was observed, indicating
that MSG mice could be used as the animal
model of human type 2 diabetes mellitus¹⁶ and
insulin resistance¹⁷. In addition, MSG-induced
obese animals, when administered MSG (2
mg/g) for 5 days, were a useful model of non-
alcoholic fatty liver disease (NAFLD)/non-
alcoholicsteatohepatitis (NASH) in humans¹⁸.
This is because these MSG mice showed the
development of marked centriflobular fatty
change with fibrosis progressing to hepatic
neoplasm in the liver. A single subcutaneously
administered an increasing dose of 4mg/g MSG
induced immediate severe obesity in mice¹⁹-²¹.
Nagata et al¹⁶ reported that MSG administered
at 2 mg/g subcutaneously for 5 consecutive
days to mice induced severe body weight, body
length, obesity, diabetes mellitus, and liver
lesions resembling NAFLD/NASH and several
kinds of dysfunction of lipid metabolism²²,²³.
The growth rate of all the mice was suppressed
and 10% of the animals in the 4 mg/g x5 course
MSG administered groups mice died¹⁶. The
cause of growth suppression resulting from MSG
treatment is thought to be brought about by the
impaired production of growth hormone
releasing factor, which accompanies the
necrosis of nerve cells in the arcuate nucleus²⁴,-²⁵.
It was estimated that the 4 mg/g x5 course of
treatment with MSG would be the maximum
possible dose because of its high toxicity²⁶.

NEUROTOXICITY STUDIES
Glutamate, an important excitatory amino acid,
is also a neurotransmitter distributed
ubiquitously in the mammalian brain²⁶-²⁷.
Glutamate is present in high levels in the brain
and select groups of neurons. The endogenous
L-glutamate, as the derived L-glutamate
of exogenous precursors, is liberated in a Ca²⁺-
dependent way after a depolarizing stimulus in
the CNS²⁸,²⁹. This glutamate may play a key role
in the induction of neuronal cell death occurring
in several neurological disorders including
Alzheimer’s disease³⁰-³², Huntington disease³⁰,³³
and Parkinson’s disease³⁰,³³. Glutamate elicits
neurotoxic effects via distinct receptor and non-
receptor-mediated mechanisms³⁴-³⁹. Glutamate
receptors play broad roles in neural plasticity,
nervous development and neurodegeneration,
while N-methyl-D-aspartate (NMDA) receptor
activity mediates the expression of
neuropeptides³⁹,⁴⁰.
MSG could penetrate the placental barrier and
distribute to embryonic tissues, particularly
 gaining high levels in brain tissue of fetal mice
after maternal administration⁴¹-⁴⁴. Glutamate
can produce obvious behavioral changes and
neuronal apoptosis⁴¹-⁴⁴. It was also reported that
neonatal administration of large doses of MSG
can cause neuronal necrosis in some brain
regions⁴⁵-⁴⁷. High doses of MSG in neonates
results in selective brain lesions accompanied
by endocrine, metabolic and behavioral
disturbances in adulthood⁴⁸-⁵⁰. Neonatal
exposure to excessive MSG may lead to
excitotoxicity and neuronal cell death during
development⁴¹,⁴²,⁴⁶,⁵²,⁵³.
That exogenous glutamate could be neurotoxic
was first proposed by Lucas and Newhouse⁵⁴
who described neuronal degeneration in the
inner layer of the retina following subcutaneous
administration of MSG to the neonatal mouse.
Subsequently, Olney⁴⁸ observed necrosis in the
hypothalamic (arcuate nucleus) neurons of
neonatal mice given MSG systemically. Other
investigators have also found
neurodegenerative changes after MSG
administration in various rodent species, usually
when the compound was administered
subcutaneously or by forced gavage.
It appears, however, that the neurotoxic
potential of exogenous glutamate in vivo is
critically dependent upon its route of
administration. Neuronal lesions have never been observed after ad libitum consumption of very high MSG doses. Thus, the studies observed hypothalamic lesions in weanling mice, probably the most sensitive species, following ad libitum MSG administration in the diet or drinking water at doses as high as 45.5 g/kg or 20.9 g/kg, respectively (These doses are 10-20 times higher than those required to induce neurodegenerative changes following parenteral or forced oral administration). This difference is probably related to differences in the pharmacokinetics of MSG depending on its route of administration which, in turn, determine its effects on extracellular brain glutamate concentrations. When glutamate is consumed orally, its effects on the brain are buffered by metabolism in the gastrointestinal tract, extrusion from the brain by active blood-brain transport systems, and local mechanisms mediating its uptake and metabolism in brain, these cause brain extracellular glutamate concentrations to remain relatively stable (The mechanism of MSG’s neurotoxic effects has been attributed to a prolonged increase in extracellular glutamate concentrations54).

EXCITOTOXICITY STUDIES
Glutamate interacts with two main subtypes of membrane receptors, ionotropic and metabotropic, coupled to ion channels and G proteins, respectively. The ionotropic receptors are further subdivided, based on selective agonists, into N-methyl-D-aspartate (NMDA), kainate, and a-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) subtypes27, 29, 55, 56. Interactions of glutamate with its ionotropic, mainly NMDA, receptors can lead to neurotoxic changes in some experimental situations by allowing excessive amounts of calcium to enter the neuron28. The metabotropic receptors are present in the presynaptic membrane and do not form ion channels; they are associated with G proteins and respond to the stimulus of second intracellular messengers56, 58.

Early studies in the 70’s, demonstrated that the administration of high concentrations of glutamate and other excitatory amino acids to the nervous system, produced degeneration and neuronal death in certain cerebral regions and that these effects are related to the excitotoxicity or neuronal damage due to excessive neuronal excitation through a specific on-activation of their ionotropic receptors27, 55.

Neuronal circuits’ construction is a dynamic process where both glutamate (Glu) and GABA (gamma-aminobutyric acid) mediated neurotransmissions have been largely implicated59-61. Moreover all neuronal networks in the vertebrate brain consist of excitatory principal neurons (glutamatergic) and inhibitory interneurons (GABAeric), which interact in active way establishing a functional balance to avoid any disease62. For this reason, changes in neuronal excitability during early development stages may modify chemically coded neural networks with possible pathological consequences63-66.

The neurotoxicity that is induced by an on-activation of these glutamatergic receptors has been associated with diverse neurodegenerative diseases64 as well as the excitotoxicity by nutritious ingestion of glutamate in the form of monosodic salts when consumed in high concentrations28, 65. It has also been demonstrated that the administration of MSG to immature animals induces destruction in certain regions of the brain that lack a blood-brain barrier, such as the arcuatus nucleus of the hypothalamus that is involved in the regulation of neuroendocrine functions27, 28, 64. However, these demonstrations have ignored the effects of the systemic administration of MSG that can develop high concentrations in organs such as liver and kidney; even when the presence of glutamatergic receptors has been demonstrated outside the CNS27, 28, 64, 66. These sub-types of receptors have been observed as the NMDA-R1, GluR 2/3 and mGluR 2/3 in liver, kidney, lungs, spleen and testicles27, 28, 64, 66. Additionally, Glutamate receptor over activation could lead to neuronal death in several brain regions, such as cerebral cortex, and hippocampus, between others67, 68. This kind of death is called excitotoxicity and is triggered by the Ca2+ influx mediated mainly by ionotropic glutamate receptors (iGlu-R) activation69, and it appears to be involved in several brain disorders, such as ischemic-hypoxic injury59, epileptic seizures70, and some chronic neurodegenerative diseases71, 72.

The susceptibility to excitotoxicity seems to be organism, age, sex, brain region, and neuronal type dependent73, 74. Therefore high Glu concentrations administered to male neonatal rats induce more extensive neuronal damage than in female or adult animals73. The results showed that four subcutaneous administration of Glu (4 mg/kg) induce neuronal death, which appears to be mediated through the activation of intracellular signaling p38 pathway and associated with changes in expression level of the three kinds of iGlu-R (NMDA, AMPA and Kainate-receptors)75-78.
NEUROTOXICITY IN PREGNANT AND FETAL RATS
MSG is given subcutaneously to pregnant rats caused acute necrosis of the acetylcholinesterase-positive neurons in the area postrema. The same effect has been observed in the area postrema of foetal rats. The process of neuronal cell death and the elimination of debris by microglia cells proved to be similar in pregnant animals and in their foetuses. However, embryonal neurons were more sensitive to glutamate as judged by the rapidity of the process and the dose-response relationship. These observations raise the possibility of transplacental poisoning in human foetuses after the consumption of glutamate-rich food by the mother.

NEUROTOXICITY IN NEONATES
MSG treatment of neonatal rodents results in a syndrome characterized by damage of the CNS, neuro-endocrine and behavioral abnormalities, arrested skeletal growth, hypophagia, and obesity. Obesity, associated with hypophagia and decreased body weight, is a specific feature of this syndrome. Recently, a decreased volume, density, and number of VMH neurons in neonatally MSG-treated rats have been documented.

The neonatal administration of large doses of MSG to rodents causes neuronal necrosis in some brain regions, along with behavioral and metabolic abnormalities. A dose of 4 mg/g body weight of glutamate (Glu), as MSG, induces excitotoxicity when administered to young rats. Evidence from various sources has indicated that both N-methyl-d-aspartate (NMDA) and non-NMDA receptors are expressed in the embryonic and developing rat neocortex. Several studies have demonstrated that glutamate receptors play an important role during development, both in shaping the neuronal circuitry, and in regulating synaptic plasticity in the central nervous system. On the other hand, expression of both AMPA receptors and kainate receptors has been strongly correlated with neuronal differentiation, maturation, and laminar formation. Abnormal changes in axodendritic synapses, with an increase in the width of the post-synaptic thickening, have reported recently to be induced by the local application of Glutamate to the rat neocortex. This strongly suggests that intense activation of Glutamate receptors by high-level exposure to exogenous Glutamate at an early age could modify the number of cortical neurons and their dendritic connectivity.

LOCOMOTOR AND LEARNING DEFICITS
The administration of large doses of MSG (4 mg/g, s.c. in the neonatal stage) to rodents causes neuronal necrosis of the hypothalamus along with behavioural abnormalities such as lethargy, changes in locomotor activity and learning deficits. The effects of neurotoxic higher doses of MSG (4 mg/g) on the locomotor activity of rats are variable. Thus, hyperactivity and hypoactivity have been reported in MSG-treated rats. Reasons for this inconsistency may include differences in the apparatus employed, route of administration and age of animals treated and/or length of time for which observations were made.

High doses of MSG to neonates have been reported to result in long-lasting deficits in the learning ability. Repeated treatment with 5 mg/g MSG in the neonatal stage induced deficits in discrimination learning in a T-maze experiment for food reinforcement. It has been postulated that Glutamate (N-methyl-d-aspartate (NMDA) receptors play an important role in many neurological functions, including long-term potentiation, learning and memory. Areas of the brain that are involved in learning and memory, such as the hippocampus and cortex, have a high concentration of NMDA receptors and NMDA receptor antagonists block the acquisition of behavioral tasks. It is well known that neonatal treatment with MSG destroys 80–90% of the arcuate nuclei neurons.

ENDOCRINE MALFUNCTION
The subcutaneous administration of large doses of MSG (4 mg/g, in the neonatal stage) results in severe adrenohypophysial endocrine malfunction as a result of hypothalamic neurotoxic lesioning. The hypothalamic lesion induces well characterized endocrinological alterations such as blunted growth, hypogonadism, hypothyroidism and obesity. Imunocytochemical studies showed that the large doses of MSG produce cytotoxicity in the mediobasal hypothalamus and a marked decrease in TH and GHRF, but not LHRH IR. These findings offer a reasonable explanation for the phenomenon that adult rats treated with MSG during the neonatal period reveal suppressed GH secretion and retardation of body growth.
HYPOTHALAMIC OBESITY

MSG-induced obese rat is a model associated with insulin resistance and dyslipidemia that may occur without the presence of hypertension or type 2 diabetes, depending on the age at which the animals are studied. The administration of MSG to newborn rats results in distinctive lesions in hypothalamic arcuate nucleus (ARC) neurons. The neuronal loss impairs insulin and leptin signaling and impacts energy balance as well as pituitary and adrenal activity. In contrast to other models of obesity, MSG-treated rats are characterized by increased plasma levels of corticosterone as well as increased lipogenesis and reduced lipolysis in the adipose tissue, despite their normophagia. An understanding of the alterations associated with MSG-induced obesity is of great relevance because the ARC is among the principal sites that regulate energy homeostasis. Although the endocrine, metabolic, and autonomic aspects of MSG-induced obesity have been extensively studied, the association between MSG and the development of vascular alterations is less understood.

MSG administration induces hyperphagia and increases the energy intake. Treatment might also induce hepatic metabolic shifting, which result in further injury. MSG at high concentrations (4 mg/g body weight or 4 g/kg) has been used experimentally to induce a variety of toxic effects, including hypothalamic lesions and obesity in neonatal animals. The development of hypothalamic obesity has been ascribed mainly to hyperphagia, but in addition to effect on food intake, hypothalamic lesion must also result in increased FCR, which is observed even when high blood glutamate levels are reached. The MSG-induced alterations are age dependent and were observed with subcutaneous injection in neonatal 1 and 5 days of age.

RETINAL DYSFUNCTION

Neonatal treatment with monosodium glutamate (MSG) causes neuronal cell death in specific central nervous system (CNS) regions such as the arcuate nucleus, the area postrema and the retina. Lucas and Newhouse noticed that severe retinal lesions could be produced in suckling mice by a single injection of MSG. Studies confirming their findings using neonatal rodents followed shortly, with others being reported from time to time. These studies concerned themselves not only with the confirmation of MSG induced retinal lesions, but with the formulation and testing of hypotheses to explain the phenomenon.

REFERENCES

3. IFIC. Review on Monosodium Glutamate: Examining the Myths 1994


57. Chol DW. Glutamate neurotoxicity in cortical cell culture is calcium dependent, Neurosct Lett, 1985; 58: 293-297


du Bois TM and Huang XF. Early brain development disruption from NMDA receptor hypofunction: relevance to schizophrenia. Brain Res Rev. 2007; 53: 260–270.


102. Collingridge GL and Bliss TV. NMDA receptors - their role in long-term potentiation, Trends Neurosci. 1987; 10: 288-293.
106. Beas-Zárate C, Flores-Soto ME and Armendariz-Borunda J. NMDAR-2C and 2D subunits gene expression is induced in brain by neonatal exposure


113. Jennes L, Stumpf WE, Bissette G and Nemeroff CB. Monosodium glutamate lesions in rat hypothalamus studied by immunohistochemistry for gonadotropin releasing hormone, neuropeptide Y, tyrosine hydroxylase, and glutamic acid decarboxylase and by autoradiography for (3H) estradiol, Brain Res. 1984; 308: 245 253.


