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Research Article

Dose-Dependent Neurotoxic Effects of Monosodium Glutamate and its Potential Genetic Transmission in Mice

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Abstract: This study aimed to preliminarily investigated the neurotoxic effects of monosodium glutamate on mice and their pups, and the potential possibility of a genetic mechanism influencing the pups. In this study, mice were given different doses of Monosodium Glutamate (MSG) at doses of 2.0 or 4.0 g/kg body weight for 10 continuous days, and its impacts on both the mice and their pups were evaluated by means of appearance assessments, morphological examination and animal behavior experiments. The results showed that high doses of MSG induced clear, dose-dependent toxic symptoms in both mice and their pups. Increasing MSG dosage resulted in greater brain injury in both mice and their pups, along with more pronounced deficits in Y-maze and water maze learning and memory, heightened central nervous system excitability, the inhibition of the exploratory and curious behaviors of the pups, and the more serious the damage to the high-altitude coordinated motor performance. These findings provide a foundation for studying the dose-dependent damage to the central nervous system in mice caused by exogenous glutamate and its potential genetic effects on pups.

Keywords: Monosodium Glutamate (MSG), Mice, Neurotoxicity, Genetic Mechanism

Introduction

Monosodium Glutamate (MSG) is a sodium salt of glutamic acid, commonly used as a flavor enhancer in food processing. It decomposes into glutamate and sodium ions in the body, playing a role in physiological functions (Sharma, 2015). While glutamate is an important energy source, excessive intake can be harmful. Since 1969, when Olney (1969) first reported that administering 2 mg/g of MSG to neonatal mice caused acute neurological damage and led to obesity and hyperleptinemia in adulthood, the safety of MSG consumption has remained an enduring topic of investigation.

Monosodium Glutamate (MSG) is widely used as a food additive to enhance flavor, particularly in Asian countries (Zhao *et al*., 2021), and there is no clear limit on the usage amount (Fernstrom, 2018; Jin *et al*., 2018; Jubaidi *et al*., 2019). Therefore, MSG is sometimes overused. Although the intake of MSG has been increasing in recent years, and numerous studies indicate that excessive intake can elevate the risk of conditions such as diabetes, obesity and Alzheimer's disease (Ko *et al*., 2022). As the primary excitatory amino acid, glutamate is extensively present in the mammalian brain's central nervous system, participating not only in neural signal transmission (Nishizawa, 2001), but also in autonomic functions and energy metabolism. When too much MSG is ingested, the cumulative concentration of glutamate in the brain rises dramatically and severe neuroexcitotoxicity occurs, resulting in neuronal damage or death. It has been reported that MSG can lead to chemical brain damage, a decline in cognitive function, an increase in neurodegeneration and psychiatric symptoms (such as depression, anxiety, sleep disorders and irritability) (Franco *et al*., 2017; Madhavadas *et al*., 2016; Sasaki-Hamada *et al*., 2015). Multiple studies have also supported the finding that, due to the incomplete development of the blood-brain barrier in newborn animals, administering MSG orally or via subcutaneous injection can lead to neuronal necrosis in specific areas of the central nervous system (Hernandez-Ojeda *et al*., 2017; Liao *et al*., 2020; Rivera-Carvantes *et al*., 2017). This indicates that excessive MSG intake may also cause neurotoxicity in newborn mice (Madhavadas *et al*., 2017). However, while the neurotoxic effects of MSG on the

adult CNS are well-documented, there is limited research on how MSG affects brain functions such as learning and memory in adult mice, particularly in relation to any potential genetic mechanisms that may influence pups' health. This gap in the literature forms the basis of the current study, which aims to explore these understudied areas in depth.

Therefore, this paper investigates the neurotoxic effects of MSG on mice and their pups, and and examines whether genetic mechanisms influence the pups, through observations of external signs, morphological examinations, and animal behavioral experiments. It also examines the effects of different doses of MSG on the physiology and health of adult mice and their pups, provides data support for studying the mechanism through which glutamate exerts neurotoxic effects on the body, and serves as a warning against misuse and overdosage in humans.

Materials and Methods

Instruments

Automatic activity program controller: ZIL-2, Institute of Materia Medica, Chinese Academy of Medical Sciences; Cavity plate tester: XDB-2, Institute of Materia Medica, Chinese Academy of Medical Sciences; Water Labyrinth Program Automatic Controller: SMG-2, Institute of Materia Medica, Chinese Academy of Medical Sciences; Y Labyrinth stimulator: MG-3, Zhangjiagang Biomedical Instrument Factory, Jiangsu Province; Shanghai Pan electronic balance: JA12002 model, Shanghai Tianping Instrument Factory; Paraffin slicer: Leica Germany; OLYMPUS Microscope: OLYMPUS Corporation, Japan.

Reagent

Monosodium L-glutamate: Shanghai Boao Biotechnology Co., Ltd; Pentobarbital sodium: Fluka Corporation; All the other reagents were domestic analytical pure.

Animal

The experimental animals were 100 KM mice, SPF grade, female/male, 6-8 weeks, weighing 18-22 g. They were purchased from Changsha Tianqin Biotechnology Co, Ltd, License No.: SCXK (Xiang) 2022-0011. They were placed in plexiglass mouse cages for rearing. Food was standard rat feed and drinking water is clean tap water. Feeding and drinking were ad libitum. Bedding was changed every 3 days. The ambient temperature was maintained at 23±1℃, 12 h lighting, 12 h of darkness. The experimental protocols were reviewed and approved by Hainan Technology and Business College and complied with the relevant ethical guidelines for animal experimentation.

Methods

Sixty healthy Kunming mice were used. They were randomly allocated to control and experimental groups, with equal numbers of males and females. The experimental group was subsequently separated into two different dosage subgroups. Based on previous experimental data, 2.0 and 4.0 g/kg were established as doses inducing pathological alterations. Therefore, this study selected 2.0 and 4.0 g/kg for further investigation (Olney, 1969; Tabassum *et al*., 2020). The mice in these two dose groups were given intragastric administrations of MSG (2.0 or 4.0 g/kg body weight) continuously for 10 days. Meanwhile, the mice in the control group were given intragastric administrations of the same volume of normal saline continuously for 10 days. In this experiment, we implemented a single-blind design to ensure that the experimenters responsible for behavioral observations and data recording were unaware of the group assignments of the mice throughout the experiment. The experimenters were responsible only for standardized behavioral observations and data collection, with all group assignments concealed through coding. Data analysis was conducted independently to avoid any potential bias. In addition, in this study, to prevent stress responses that might be induced by the gavage procedure, we ensured that all mice underwent the same gavage procedure, maintaining calm and consistency throughout the process. Furthermore, environmental conditions such as temperature, lighting, and noise levels were strictly controlled to avoid external factors from interfering with the experimental results. The following observations and experiments were carried out.

In addition, 30 healthy KM mice were randomly paired with half of the male and half of the female mice were reared in the same cage after administration of the drug in the above manner. After the females became pregnant, they were reared in separate cages, and the resulting pups were subjected to the following observations and experiments.

Appearance and Signs Observation

The responses of the animals' nervous system, respiratory system, digestive system, genitourinary system and changes in skin and fur color were observed and recorded before and after the administration of the drug to the mice twice a day until the end of the experiment. The reactions of the nervous system, respiratory system, digestive system and genitourinary system as well as changes of eyes, skin and hair color were observed and recorded every day from birth to 90 days of age.

Morphological Examination

Histopathology: Every two days following drug administration, two mice were selected and anesthetized

via intraperitoneal injection of sodium pentobarbital (60 mg/kg), and the mice were executed with 10% formalin solution by cardiac perfusion. Decapitated brain, fixed in formalin solution at the same concentration for a week. Paraffin-embedded, serial sections of whole brain coronal plane of 10 μm thickness were made, and 1 slice was taken every 100 μm. HE staining, light microscopy. After 90 days since the administration of the medicine, four mice from each group were taken for histopathologic inspection as above. One week after the administration of the drug, four mice from each group were taken at 90 days after birth for histopathological analysis, and the method was the same as above.

Behavioral Experiment

Learning and Memory Ability

Y-maze

It was used for evaluation of Learning and Memory Capacity. MSG was formally tested at the end of gavage in a dimly lit and quiet environment on days 1, 2, 3, 4, 5, 6, 36, and 90 after adulthood and on days 41, 42, 43, 44, 45, 46, and 76 after their pups. The mice were put into a maze first and acclimatized for 20 s prior to testing. Randomly turn on a Y maze device arm light, at this time the arm was not energized as a safety zone, the other two arms without lights and the junction area were energized by heat (60~80°C) and become a non-safety zone. At the same time, the mice were transferred to the non-safety zone, the mice escaped to the safety zone after thermal stimulation and stayed for 5 s as the correct response, otherwise it was the wrong response. The training was repeated for each mouse until it learned (≥18 correct responses in 20 consecutive training sessions was considered to meet the learning criterion) (Timothy *et al*., 2019). Finally, it was analyzed the Training-Session Count and intra-experimental errors between the groups whether the learning and memory abilities of adult and pup mice were diminished. The Y-maze needed to be re-cleaned and washed after each mouse's training was completed to exclude subsequent experimental mice from being affected by odor (Liang *et al*., 2021).

Morris Water Maze

It was employed to assess the ability of mice to learn and remember their sense of spatial location and orientation. The particular experimental operations were as described below: Pour clear water was put into the pool to a depth of 30 cm (with dimensions of 0.8×0.21×0.42 m^3), and ink was added to muddy the water so as not to allow the mice to see the underwater platform. The safety platform was located in the middle of the quadrant remote from the entry point. The platform was fixed with a distance of one centimeter to the water surface. The site of the safety platform was kept the same throughout the experiments, with the water temperature kept at 23±2°C. The point where the mouse entered was marked. The conditions and environment were kept constant for each experiment, and the training was conducted four times a day with 15-20 min between training sessions, with four starting positions randomly placed in the east, west, south, and north. The time for training once was specified to be 90 s. Mice that located the platform within the allotted time remained on it for 15 s to complete the training. Those failing to find it were placed on the platform for the same duration once the time expired (Yong *et al*., 2010). Such training for 5 days, followed by testing on the 6^{th} day, during which the safety platform was removed. Each mouse test once, and computer software automatically recorded and analyzed the latency of the mouse's first platform crossing (Ridolo *et al*., 2014).

Autonomous Activity Experiment

It was evaluated central nervous system excitability by autonomous activity experiments. After 10 consecutive days of MSG gavage in adult mice, they were put in a Mouse ZIL-2 Program Controlled Autonomous Activity Device. The test was started after 5 min of Acclimatization. Then the amount of activity of mice was keep a record during a period of 5 min the mice were carried out the training three times daily and the average number was obtained. The operation of pups was the same as that of adult mice on days 30, 40, 60, and 90.

Exploring Curious Behaviors

Cavity plate experiment: The mouse hole plate tester was 50 cm × 50 cm in size, with 16 holes of 3 cm in diameter neatly arranged on the plate. The animals were placed on the hole board, and the computer automatically recorded the number of times the mouse probed the holes within 3 min.

Coordinated Motor Skills at Altitude

Rope climbing experiment: The rope was 2 meters long and 1 meter above the ground. Mice were placed on the rope and their behavior was observed. Those who could climb the rope freely were labeled as "able to climb the rope"; those who could only grasp the rope but could not climb on the rope or could not hold the rope and fell to the ground were labeled as "unable to climb the rope or fell to the ground". The results were statistically analyzed using the chi-square test.

Statistical Processing

The experiment was carried out at least three independent replications. Excel 2019 software was applied to analyze and process the data. The experimental data were subjected to statistical analysis using SPSS 26.0. The t-test was employed to compare the statistical differences between the two groups of data, and one-way

analysis of variance (ANOVA) was used to compare the statistical differences among multiple groups of data. The data were expressed as mean ± Standard Deviation (SD). Statistical significance was indicated by *P<0.05, **P<0.01, and ***P<0.001, indicating statistical disparities, remarkable statistical disparities, and extremely remarkable statistical disparities among the data respectively.

Results

The Impact of MSG on the Appearance and Physical Signs of Mice

MSG was administered to mice via oral gavage (2.0 g/kg body weight) for 10 days. Most of the mice showed increased activity and aggressiveness (especially males) starting on the 4th day of administration; a small proportion showed poorer, fluffy, and lusterless fur.

MSG was administered to mice via oral gavage (4.0 g/kg body weight) for 10 days consecutively. Starting from the 4th day of administration, there was a marked decrease in activity, dull, lackluster fur, diminished responsiveness to mechanical stimuli, slow action, slow reaction, dull and depressed; unsteady gait, ataxia, head bowed down; occasional full-body convulsions or spasms, tonicity; sedentary immobility; respiratory distress, wheezing, open-mouthed respiration; loss of appetite, abdominal distension was found after dissection; urine became dark (yellowish - green), incontinence; the fur appeared discolored, fluffy, and lackluster, dirty and unclean, easy to shed; weight loss was obvious, emaciation, and some of them show eye abnormalities.

After 10 days of consecutive MSG (2.0 or 4.0 g/kg body weight) gavage in mice, half of which were male and half of which were randomly paired and housed in the same cage, most of the resulting litters (pups 1) showed increased activity and aggressiveness (especially in males), and some showed eye abnormalities and red eyelids.

Morphological Examination

Pathohistological analysis of MSG gavage treatment induced brain tissue damage in mature mice and pups. To examine how MSG treatment affects the hippocampal tissue of adult mouse brains, mice were continuously gavaged with various doses of MSG for 10 days in a row. The histopathological results from Fig. 1 and Fig. 2 indicate that MSG induces both neuronal cell death and cellular proliferation in the hippocampal region of mice. As the treatment duration increased, apparent signs of neuronal degeneration were observed, including sparse neuronal arrangement, irregular cell morphology, and vacuolar degeneration, suggesting that MSG causes widespread neuronal death and disrupts brain structures associated with cognition. Meanwhile, in the dentate gyrus region, the cellular layer was markedly thickened and more densely arranged, showing characteristics of reactive proliferation, aligning with the central nervous system's compensatory repair response to injury. Moreover, under the same treatment duration, both changes were more pronounced in the high-dose groups.

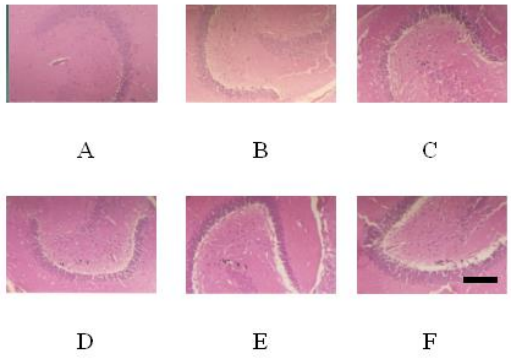

Fig. 1: The impact of MSG on the histopathology of the hippocampal region

A. Control
B. MSG (2.0 g/kg) (HE × 200) (2 days)
C. MSG (2.0 g/kg) (HE × 200) (4 days)
D. MSG (2.0 g/kg) (HE × 200) (6 days)
E. MSG (2.0 g/kg) (HE × 200) (8 days)

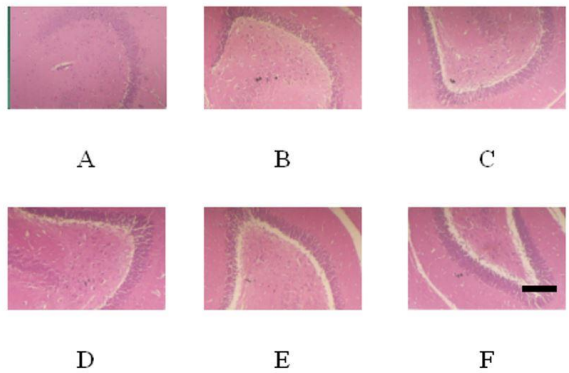

Fig. 2: The impact of MSG on the histopathology of the hippocampal region

A. Control
B. MSG (4.0 g/kg) (HE × 200) (2 days)
C. MSG (4.0 g/kg) (HE × 200) (4 days)
D. MSG (4.0 g/kg) (HE × 200) (6 days)
E. MSG (4.0 g/kg) (HE × 200) (8 days)
F. MSG (4.0 g/kg) (HE × 200) (10 days)

After 10 days of MSG gavage and a 90-day recovery period, histopathological examination showed that, compared to the control group (Fig. 3A), all dosage groups exhibited varying degrees of damage, with more severe damage observed at higher doses. At a dose of

2.0 g/kg, the hippocampal region of the adult mice displayed degenerative characteristics such as sparse neuronal arrangement, loose structure, pale or fragmented chromatin, and vacuolar degeneration, suggesting neuronal death. In addition, the dentate gyrus cell layer was markedly thickened and more densely arranged, with increased yellowish regions, indicating significant cellular proliferation, in line with the central nervous system's compensatory repair process (Fig. 3B-C). At a dose of 4.0 g/kg, the adult mice's hippocampus exhibited more pronounced neuronal death and dentate gyrus cell proliferation (Fig. 3D), with obvious edema or degeneration observed (Fig. 3E-F). To further investigate the effects of MSG gavage on pups, F1 pups were gavaged after 90 days of mating. Histopathological analysis of the brains of F1 pups showde that at a dose of 2.0 g/kg MSG, hippocampal neurons in the same litter also exhibited neuronal death and dentate gyrus cell proliferation (Fig. 3G); at a dose of 4.0 g/kg MSG, neuronal death and dentate gyrus cell proliferation were more pronounced (Fig. 3H). In summary, these results suggest that MSG administration may cause hippocampal neuronal damage in both adult mice and their pups, and this effect is dose-dependent.

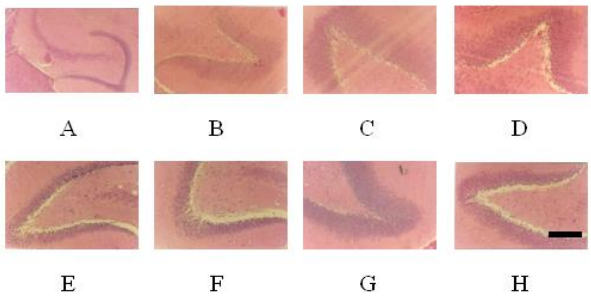

Fig. 3: Morphological effects of intragastric administration of MSG on the hippocampus in the adult mice

A. Control (HE × 100)
B. MSG (2.0 g/kg) (HE × 200)
C. MSG (2.0 g/kg) (HE × 200)
D. MSG (4.0 g/kg) (HE × 200)
E. MSG (4.0 g/kg) (HE × 200)
F. MSG (4.0 g/kg) (HE × 200)
G. MSG (2.0 g/kg) (HE × 200), F1
H. MSG (4.0 g/kg) (HE × 200), F1

Impact of MSG Exposure on Mouse Behavioral Learning and Memory Ability

As shown in Fig. 4, adult mice gavaged with 10 days of MSG (2.0 g/kg body weight) showed some impairment of Y-maze learning and memory abilities, but no significant damage to their pups. However, adult mice that were gavaged with MSG (at a dose of 4.0 g/kg of body weight) continuously for 10 days and pups demonstrated significant differences in disruption of the Y-maze learning and memory skills. These results indicate that higher doses of MSG lead to more severe cognitive impairments in both adults and pups.

As shown in Fig. 5, in the water maze experiment, normal mice had shorter swim-out times and fewer entries into the blind end (i.e., the number of errors) as the number of training sessions increased. Gavage with MSG for 10 days significantly impaired the mice's ability to discriminate spatial orientation in the water maze. This impairment was reflected by increased escape latency and more errors. The corresponding pups also showed increased escape latency and increased number of errors compared with the control group. The results indicate that MSG impairs spatial orientation and memory in mice and their pups.

Excitability

As shown in Fig. 6, after 10 days of MSG gavage in mice, the number of voluntary activities in mice in the groups increased, and the number of voluntary activities in mice of the groups was more and more significant with the increase of the dose, which suggests that MSG has an over excitatory effect on the CNS of adult mice. The same situation also existed in the corresponding pups. This result indicates that MSG triggers dose-dependent excitatory responses in the central nervous system of both adult mice and their pups, with these effects being more pronounced in the pups.

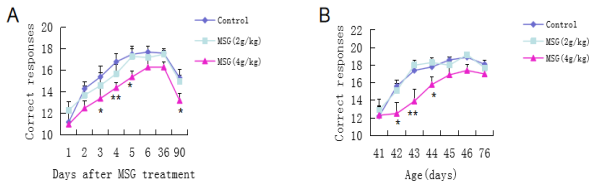

Fig. 4: Y-maze evaluation of cognitive function in mice (A) and pups (B) that were gavaged with MSG continuously for 10 days

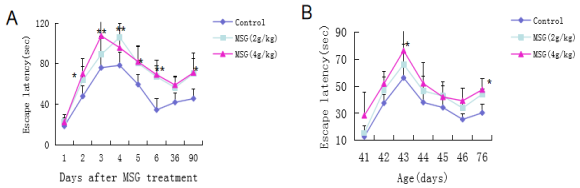

Fig. 5: Water maze evaluation of cognitive function in mice (A) and pups (B) that were gavaged with MSG continuously for 10 days

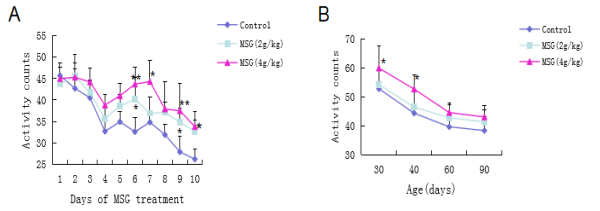

Fig. 6: The spontaneous activities of mice (A) and pups (B) that were gavaged with MSG continuously for 10 days

Exploring Curious Behavior

As shown in Fig. 7, MSG significantly reduced the number of probing holes in adult mice, and the influence of different dose sizes did not show a significant difference, but there was still significant variability in their corresponding pups, with a greater effect at higher doses. This also suggests that MSG can inhibit the exploratory curiosity behavior of mice and their pups, with these effects being more pronounced in the pups and exhibiting a dose-dependent relationship.

Coordinated Motor Skills at Altitude

As shown in Tab. 1 and Tab. 2, in the rope-climbing experiment, most of the control mice could climb the rope freely. After the mice were continuously gavaged with MSG (at a dose of 2.0 or 4.0 g/kg of body weight), the number of those that "could not climb the rope or fell to the ground" increased significantly. Some could only grasp the rope but were unable to crawl on it, while others could not hold the rope firmly and thus fell to the ground. The results were consistent with those of the pups. The results showed that continuous gavage of MSG caused a significant dose-dependent impairment of the coordinated aerial locomotor activity in mice and their pups.

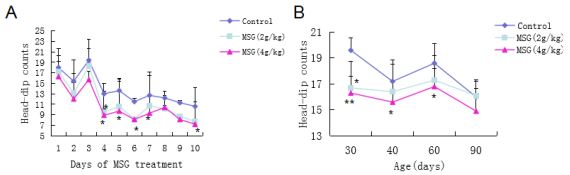

Fig. 7: The exploratory and curious behaviors of mice (A) and pups (B) that were gavaged with MSG continuously for 10 days

Table 1: The impact of MSG on the high-altitude coordinated motor ability of mice P<0.01, P<0.001, vs. control group

	Animal number	20 days post-dose		30 days post-dose		40 days post-dose	
		Can't climb	P	Can't climb	P	Can't climb	P
Control	10	1		0		0	
MSG (2.0 g/kg)	10	4	<0.001	3	<0.01	4	<0.001
MSG (4.0 g/kg)	10	6	<0.001	4	<0.001	5	<0.001

Table 2: Effects of intragastric administration of MSG on the cooperation

	Animal number	20 days old		30 days old		40 days old	
		Can't climb	P	Can't climb	P	Can't climb	P
Control	10	1		0		0	
MSG (2.0 g/kg)	10	2	<0.05	1	<0.05	1	<0.05
MSG (4.0 g/kg)	10	3	<0.05	3	<0.05	3	<0.05

Discussion

Glutamate serves as the primary excitatory neurotransmitter in the brain, present in the human and mammalian central nervous system, functioning as an excitatory neuromodulator with potential neurotoxic effects (Mahmoud *et al*., 2019). The exact mechanism underlying its neurotoxic effects has not been fully elucidated. By the end of the last century, scientists studying this neurotransmitter had found that the release of glutamate leading to excitotoxicity is an important pathological basis for classical neurological injuries and diseases such as traumatic brain injury and cerebral ischemia.

In the experiment, MSG was found to cause increased activity, dull hair color, short body stature, fat and developmental delay. The enhanced activity may result from glutamate being the primary excitatory neurotransmitter in the mammalian brain, where it is essential for central nervous system communication, but excessive levels may lead to overstimulation (Yan, 2019). The lack of a shiny coat, short and fat body and developmental delay may be associated with endocrine deficiency syndrome induced by MSG (Otter *et al.*, 2016; Pedroso *et al*., 2019). Zinc, an important trace element in mammalian, is vital for proper growth and development. High intake of MSG can convert zinc in the body into zinc glutamate, which is then excreted, leading to zinc deficiency. As a result, symptoms such as dry skin and hair may occur. This phenomenon may be characterized by the destruction of endocrine-related neurons by MSG, resulting in hypothalamic neuropediatric hypofunction (Marta *et al*.,

2019; Mazzone and Nistri, 2019). However, it is important to note that stress induced by the gavage procedure or environmental factors may have influenced the observed effects. Gavage itself may cause stress, potentially impacting the overall health and behavior of the animals. Additionally, environmental factors such as temperature, lighting, and noise levels may also affect the results. While every effort was made to control for these potential confounding factors, their influence cannot be completely ruled out.

In this experiment, histopathological examinations revealed that MSG induced a dose-dependent degree of necrosis or hyperplasia in the hippocampal regions of both adult mice and their pups. Studies have shown that damage to hippocampal neuronal cells significantly affects the spatial memory, learning abilities, and other cognitive functions of mice, leading to a decline in overall cognitive abilities (Wang *et al*., 2025). These findings suggest that MSG may negatively affect spatial orientation, learning ability, exploratory behavior, and emotional regulation in both adult mice and their pups by inducing neuronal damage in the hippocampal region. Furthermore, although previous studies have indicated that MSG can cause varying degrees of damage to different brain regions in mice, and that MSG does correlates with a reduction in the number of normal hippocampal cells (Bai *et al*., 2000), research on the effects of MSG on pups remains limited. This study partially fills this gap and provides preliminary evidence that MSG not only damages the hippocampal neurons in adult mice but also causes damage to the hippocampal neurons in their pups.

The results of this study indicate that varying doses of MSG lead to different levels of decline in learning and memory performance, CNS, exploratory curiosity, and coordination abilities in adult mice and their pups. Existing studies suggest that a low dose of MSG (0.375 g/kg) does not significantly affect the learning and memory abilities of adult mice (Wang *et al*., 2012). However, after 5-week-old male Wistar rats were continuously gavaged with 2 g/kg of MSG, it inhibited Na^+-K^+-ATPase activity in the hippocampus and cortex, leading to long-term learning and memory dysfunction (Liao *et al*., 2020). Moreover, with increasing MSG dosage, the CNS becomes excessively excited, causing significant damage to exploratory curiosity behavior and coordination ability (Abu-Taweel *et al*., 2014; Ahanger *et al*., 2021; Kouzuki *et al*., 2019). In combination with histopathological findings, this study further confirms that MSG has a dose-dependent effect, damaging the hippocampal regions and CNS in mice, which in turn affects behaviors and abilities (such as learning and memory, exploratory curiosity, and coordination). Based on this, the experiment also suggests that the damage and effects caused by MSG in mice are similarly manifested in their pups. Interestingly, pups appear more sensitive to MSG, and their behavioral changes, such as exploratory curiosity, are more pronounced compared to the adults. Moreover, the damage is irreversible. However, the specific mechanisms of action still require further genetic or molecular analysis for validation, which will be the focus of subsequent investigations.

As a flavoring agent, MSG has long been commonly added to everyday foods to improve culinary flavor, as well as to stimulate appetite and aid digestion, and is now considered safe for healthy populations in the quantities and methods of use specified by the Joint FAO/WHO Expert Organization on Food Additives in 1973, with an "acceptable daily intake" for MSG ranging from 0 mg to 120 mg. However, at the 19th session of the FAO/WHO Food Additives Regulatory Committee in 1987, it was officially announced that the relevant limits had been abolished and it was agreed that MSG is a safe food flavor enhancer for use. However, in 1987, the 19th FAO/WHO Committee on Food Additives Regulations meeting formally announced that the regulations on MSG consumption limits were abolished, and it was agreed that MSG is a food flavor enhancer and is safe for use. However, it has been shown that long-term intake of MSG, even at a daily intake as low as 0.3-1.0 g, can result in a series of abnormal reactions, and the risk of abnormal reactions in humans increases after 5 years of continuous intake (Shi *et al*., 2012). In this paper, mice given 2 g/kg of MSG for 10 consecutive days were shown to cause significant dose-dependent damaging effects on the CNS of mice. If calculated according to the equivalent dose coefficient conversion method in "Pharmacological Experiment Methodology" and the equivalent dose ratio calculated based on the conversion of body surface area between humans and animals, the dose for mice = 9.1 × the clinical dose for humans (Xu *et al*., 2022). When the MSG intake reaches 220 mg/kg, the clinical dose for humans may exhibit toxic effects. However, it is important to note that this is only theoretical data, and such extrapolation is speculative and should be interpreted with caution. Nevertheless, long-term intake of large amounts of exogenous MSG does increase the concentration of glutamate in the human body, and the accumulation of large amounts of glutamate not only has neurotoxic effects but also poses risks of reproductive toxicity (Wang *et al*., 2022), metabolic syndrome, and renal damage. This is not only relevant for the normal population, but also if MSG is abused or overdosed, especially for special groups such as the elderly, infants and children, as well as certain diseases, more experimental studies are still needed in order to formulate appropriate management policies and dosage guidelines.

Conclusion

This study demonstrated that MSG can induce significant neuronal damage in the hippocampal region

of adult mice, thereby impairing their learning, memory, spatial orientation, and overall cognitive functions. In addition, MSG can cause overexcitation of the CNS, suppress exploratory and curiosity-driven behaviors, and markedly reduce high-altitude coordinated motor abilities. Most of these adverse effects exhibited a clear dose-dependent pattern. Notably, these neurotoxic effects were also observed in pups, with certain impairments being even more pronounced compared to adult mice. This indicates that, beyond its direct harm to adult individuals, MSG may exert sustained effects on offspring through genetic or developmental pathways. The findings suggest that long-term or high-dose intake of MSG may lead to irreversible damage to the CNS and pose potential risks to offspring health, providing important experimental evidence for further elucidating the neurotoxic mechanisms of MSG and for establishing scientifically sound dietary safety standards.

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Authors Contributions

Yanan Zou: Designed the research plan, supervised experiments, and revised the manuscript.

Qiong Liang: Writing - original draft, Software.

Lu Liu: Methodology, Data curation.

Dingguo Li: Data curation, Resources.

Ting Wang: Writing - review & editing, Grammar revision.

Mingzhu Pan: Editing, Grammar revision. Tongxiang Zong: Methodology, data curation.

Yuxia Zhang: Data curation, resources.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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