



EFFECTS OF MONOSODIUM GLUTAMATE ON ANXIETY AND WORKING MEMORY OF MICE

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ABSTRACT

Monosodium glutamate (MSG) is a food additive commonly consumed as a flavour enhancer. The effects of sub chronic oral low dose administration of monosodium glutamate (MSG) on neurobehaviour were assessed using the elevated plus maze and Y-maze in mice. Twenty (20) albino mice weighing between 27-39 g were used for the study and assigned into four (4) groups A, B, C, D. Group A served as the control group while B, C, D received MSG dissolved in water at 0.5, 1.0, and 1.5 mg/kg dose for 14 days. After which the animals were exposed to the elevated plus maze and Y-maze. The results of the working memory and anxiety were expressed as mean \pm SEM. Mice administered 1.5 mg/kg dose of MSG had a significant decrease in the time spent in the open arm of the elevated plus maze ($p \leq 0.05$) compared with control group. Mice administered 1.5 mg/kg dose of MSG had a significant impairment of memory. The results demonstrated significant ($p \leq 0.05$) impairment in spatial memory and decrease in locomotor activities during 5 minutes exploration of the Y-Maze at 1.5 mg/kg dose of MSG. The findings suggest that monosodium glutamate poses a deleterious effect on memory.

Keywords: anxiety, memory, mice, monosodium glutamate

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INTRODUCTION

Glutamic acid was discovered and identified in 1866 by the German chemist Karl Heinrich Ritthausen, who treated wheat gluten with sulphuric acid [1]. Kikunae Ikeda of Tokyo Imperial University isolated glutamic acid as a taste substance in 1908 from the seaweed *Laminaria japonica* (Kombu) by aqueous extraction and crystallization, calling its taste umami [2]. Monosodium glutamate (MSG, also known as sodium glutamate; IUPAC name- Sodium 2-aminopentanedioate) is the sodium salt of glutamic acid, one of the most abundant naturally occurring non-essential amino acids [3, 4].

Glutamate is the excitatory neurotransmitter in the mammalian central nervous system (CNS) playing an important role in both physiological and pathological processes [3]. Glutamate receptors include three families of ionotropic receptors (NMDA-N-methyl-D-aspartate, AMPA- α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid and kainate) and three groups of metabotropic receptors (mGluR) [3]. They are dispersed throughout the central nervous system including amygdala, hippocampus and hypothalamus where they regulate many vital metabolic and autonomic functions [1, 5].

MSG is used as an agent which in high doses causes neuronal necrosis in hypothalamic arcuate nuclei in neonatal rats [6]. However, MSG effects are more extensive and not limited to hypothalamic area. Study confirmed that MSG (0.5 mg/g, subcutaneously, on postnatal days 1, 3, 5 and 7) led to prefrontal cerebral cortex changes, including fewer neurons,

shorter and less ramified dendritic processes and loss of cortical cell number from postnatal day 8-14 compared to control rats [7].

Studies have shown that monosodium glutamate (MSG) causes reduction in growth hormones and also how they affect platelet count, and other hemoglobin parameters as well as its effect on reproduction but little work have been done on its neurobehavioral effects, many studies done on MSG was administered at high doses. This research will focus on its neurobehavioral effect at low doses [3, 5, 8].

Since monosodium glutamate is widely used to enhance taste and flavor of food, previous study by Kraeuter *et al.* [2] revealed that MSG was neurotoxic and produced seizures, memory, deficits, anxiety and other neurological disorders in rodents. The doses used in the study were too high and far above the possible amount consumed by humans. Although the effects of MSG have been extensively studied for over a century, it will still continue to be evaluated for safety profiles in light of current scientific knowledge and methods of testing. This research is aimed at investigating the effect of MSG on anxiety and working memory.

MATERIALS AND METHODS

Twenty (20) male mice weighing 27 – 39 g was gotten from the animal house in the Department of Physiology, Faculty of Basic Health Sciences, Bingham University, Karu, Nigeria and was housed there throughout the period of the research.

The mice were acclimatized for two (2) weeks and were fed with feed and water. Animals were trained on the elevated plus maze and Y-maze for a week. Administration of monosodium glutamate (ajinomoto) began and lasted for 14 days. The animals were made to run the elevated plus maze and Y-maze test

GROUP 1: Served as the control group and they were given animal feed and distilled water for 14 days.

GROUP 2: Were administered 0.5 mg/kg body weight of MSG orally for 14 days

GROUP 3: Were administered 1.0 mg/kg body weight of MSG orally for 14 days

GROUP 4: Were administered 1.5 mg/kg body weight of MSG orally for 14 days [5]

The mice were carried into the test room in their cages and were handled by the base of their tails at all times. The mice were placed in the central square of the plus maze facing an open arm where they were allowed to explore the apparatus for 5 minutes and the animals behaviour on the maze were recorded. After five (5) minutes, the mice were removed by the base of their tails and returned to their home cage. The maze was cleaned with diluted methylated spirit and permitted to dry between tests to remove any odour residue of the previous animal.

Behavioural studies methods (Adopted from [5])

Y-maze: The mice were placed at the end of one arm and allowed to move freely through the maze for a duration of five (5) minutes, the entries into each arm are recorded. After each trial, the Y-maze was cleaned, disinfected and dried prior to placing the next mouse. The following readings taken includes, number of alternations, number of entries and percentage alternations.

Open arm entries: frequency with which the animal entered the open arms was recorded

Closed arm entries; frequency with which the animal entered the closed arms was recorded. All four of the mouse paws were required to be in the arms to be counted as an entry

Open arm duration: length of the time the animals spent in the open arms

Closed arm duration: length of time the animal spent in the closed arms [2]. The percentage of the number of entries into the open and closed arms based on the total number arms entries was calculated for each animal. The percentage of time spent in the open and closed arms were calculated over the 5 minutes test [2].

Statistical analysis

All behavioral data were analyzed using one-way analysis of variance (ANOVA) followed by post hoc test (Students Newman Keul’s) carried out to determine the source of a significant effect. Results were expressed as Mean ± SEM, p ≤ 0.05 was taken as accepted level of significant difference from control.

Ethical clearance

Ethical clearance was obtained from the ethical committee on animals in Bingham university, Karu and was given reference number BHU121

RESULTS

Twenty (20) mice were used for this experiment for both the elevated plus maze and Y-maze test. The test was carried out twice for each animal for both EPM and Y-maze and the results are as follows;

Table 1: Body weight of mice before administration of MSG

Control Group (A) (g)	Group B (g)	Group C (g)	Group D (g)
28.0 ± 0.5	33.8 ± 1.3	33.4 ± 0.9	36.3 ± 1.9
33.2 ± 1.3	30.0 ± 1.5	29.7 ± 1.4	36.8 ± 2.3
27.0 ± 0.9	33.9 ± 1.6	32.1 ± 0.8	35.0 ± 1.5
30.9 ± 1.2	33.0 ± 0.8	36.0 ± 1.0	36.4 ± 1.8
35.1 ± 1.8	32.2 ± 1.7	39.4 ± 2.1	36.4 ± 1.7

Table 2: Body weight of mice after administration of MSG

Control Group (A)	Group B	Group C	Group D
30.0 ± 1.8	34.0 ± 2.0	33.4 ± 1.3	36.3 ± 2.4
33.2 ± 1.3	30.0 ± 0.9	31.1 ± 2.2	36.8 ± 1.9
33.0 ± 0.8	33.9 ± 1.7	32.1 ± 1.8	35.0 ± 0.5
33.1 ± 1.2	33.0 ± 1.4	36.0 ± 2.5	37.8 ± 2.6
37.1 ± 1.6	31.8 ± 0.7	39.4 ± 1.8	36.8 ± 1.6

Effect of monosodium glutamate on percentage time spent in the open or closed arms

Figure 1 shows the effect of monosodium glutamate on the percentage time spent in the open and closed arms following 5 minutes of exploration in the elevated plus

maze. There was a significant (p ≤ 0.05) reduction in time spent in the open arm at 1.5 mg/kg dose of MSG compared to the control group. There was also an increase in time spent in the closed arm with increasing doses of MSG compared to control. Comparing

percentage time spent in the open arm to the closed arm, the animals spent more time in the closed arm than open

arm however neither effect was statistically significant.

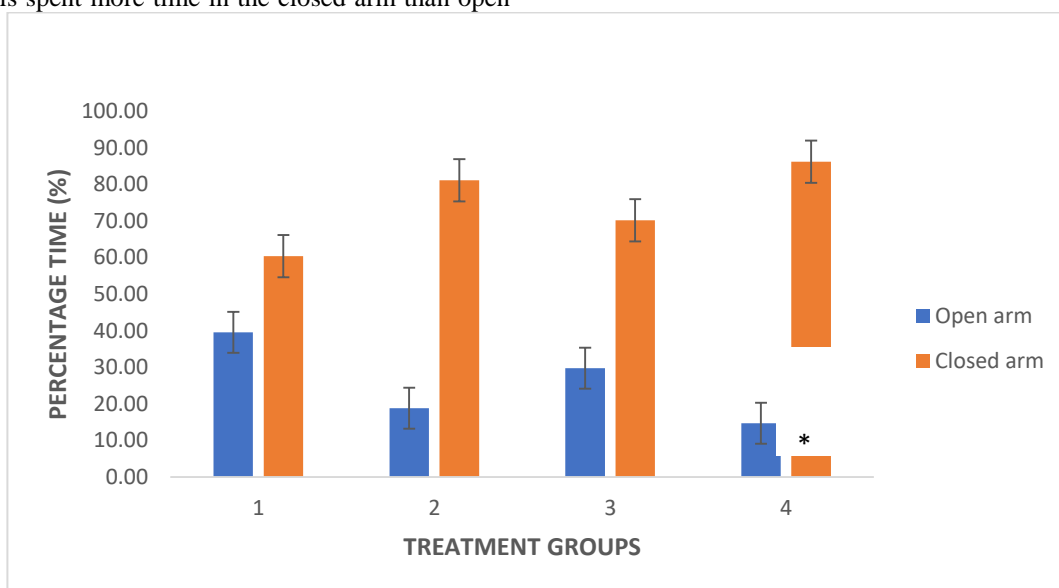


Figure 1: Effect of MSG (0.5, 1.0, 1.5 mg/kg) on time spent in open and closed arms during 5 minutes of EPM exploration. Each bar represents Mean \pm SEM, * $p \leq 0.05$ compared to control. 1 is control group, 2, 3, and 4 received MSG at 0.5, 1.0, 1.5 respectively.

Effect of monosodium glutamate on number of open or closed arm entries

Figure 2 shows the effect of monosodium glutamate on the number of entries into the open and closed arm following 5 minutes of exploration in the elevated plus maze there was a significant ($p \leq 0.05$) reduction in number of entries into the open arm at 1.5 mg/kg

compared to control, while at the other two doses there's slight increase compared to control. There was also a non-significant ($p \geq 0.05$) increase in number of entries into close arm at 0.5 mg/kg compared to control and other doses. Comparing the number of entries into the closed arm to open arm entries revealed that the animals spent more time in the closed arm than in the open arm.

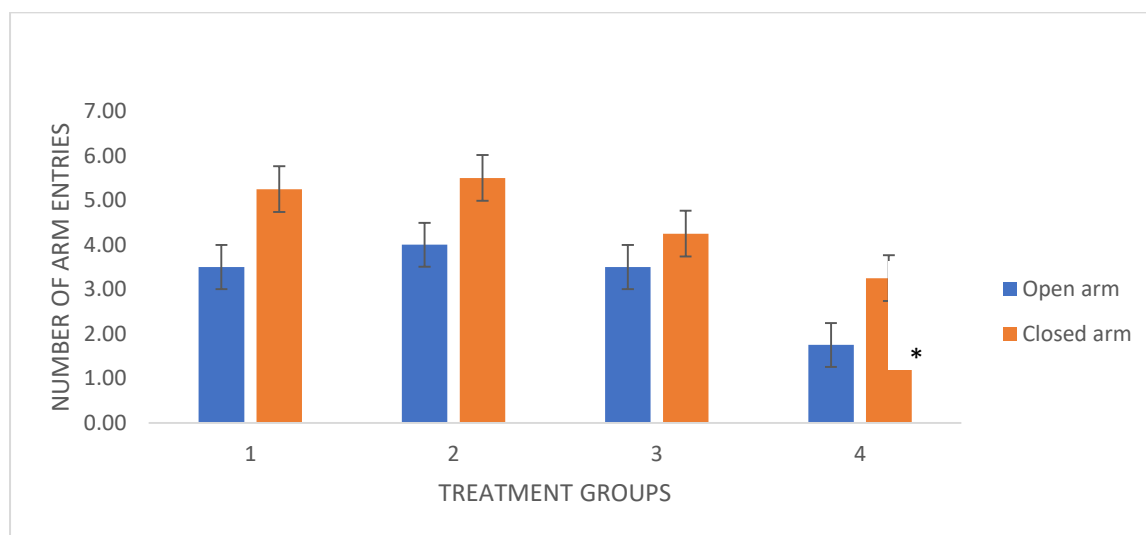


Figure 2: Effect of MSG (0.5, 1.0, 1.5 mg/kg) on number of entries in open and closed arms during 5 minutes of EPM exploration. Each bar represents Mean \pm SEM, * $p \leq 0.05$ compared to control. 1 is control group, 2, 3, and 4 received MSG at 0.5, 1.0, 1.5 mg/kg respectively.

Effect of monosodium glutamate on total number of arm entries

Figure 3 shows effects of MSG on total number of arm entries following 5 minutes of exploration in elevated plus maze there was a decrease in the number of arm

entries at 1.0 mg/kg (8.00) and 1.5 mg/kg (6.00) there was a slight increase in the group treated with 0.5 mg/kg compared to control (9.00) ($p \leq 0.05$).

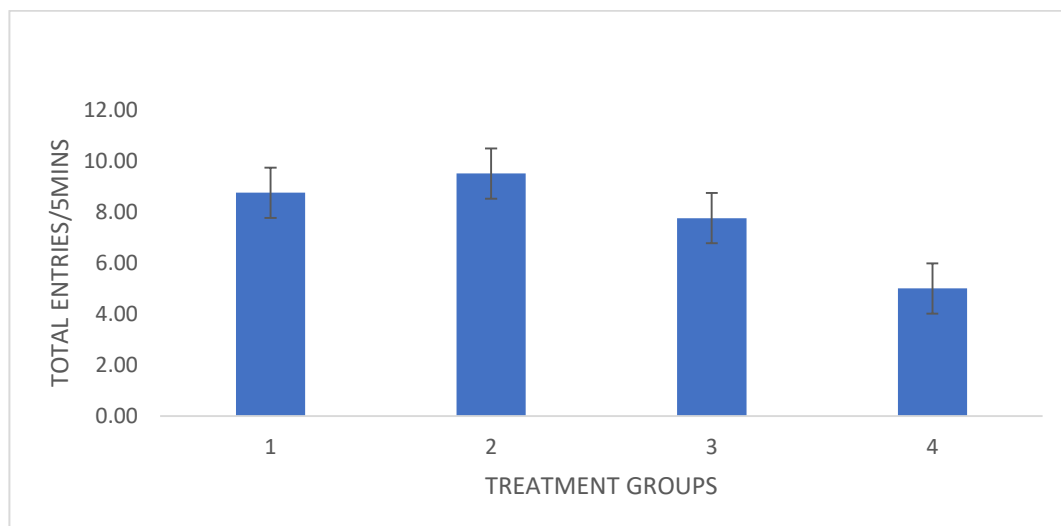


Figure 3: Effect of MSG (0.5, 1.0, 1.5 mg/kg) on total number of arm entries during 5 minutes of EPM exploration. Each bar represents Mean ± SEM, * $p \leq 0.05$ compared to control. 1 is control group, 2, 3, and 4 received MSG at 0.5, 1.0, 1.5 mg/kg respectively. (9.50 ± 1.44), (7.75 ± 1.80), (5.00 ± 1.47).

Effect of monosodium glutamate on Y-maze locomotor activity

Figure 4 shows the effect of MSG on locomotor activity following 5 minutes of exposure in the Y-maze. There

was an increase in locomotor activity with increasing doses of MSG compared to control ($p \leq 0.05$).

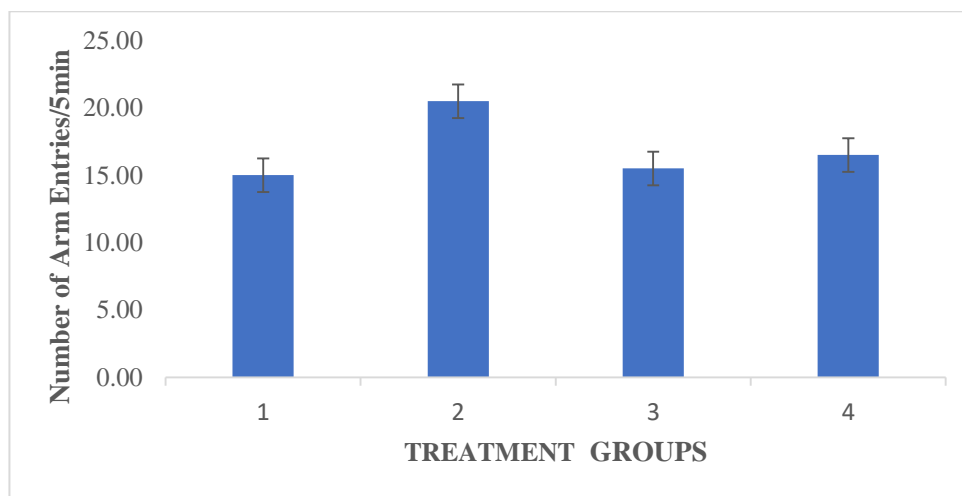


Figure 4: Effect of MSG (0.5, 1.0, 1.5 mg/kg) on spatial memory during 5 minutes of exploration in the Y-maze. Each bar represents Mean ± SEM, * $p \leq 0.05$ compared to control. 1 is control group, 2, 3, and 4 received MSG at 0.5,1.0,1.5 mg/kg respectively.

Effect of monosodium glutamate on Y-maze on spatial memory

Figure 5 shows the effect of MSG on spatial working memory following five (5) minutes of exposure in the Y maze. There was a dose dependent reduction in spatial

working memory at 1.5 mg/kg dose ($p \leq 0.05$) (41.37 ± 0.71) compared to control (42.54±0.5). A dose dependent reduction in spatial working memory was also observed at 0.5 mg/kg and 1.0 mg/kg although it was not statistically significant ($p \geq 0.05$).

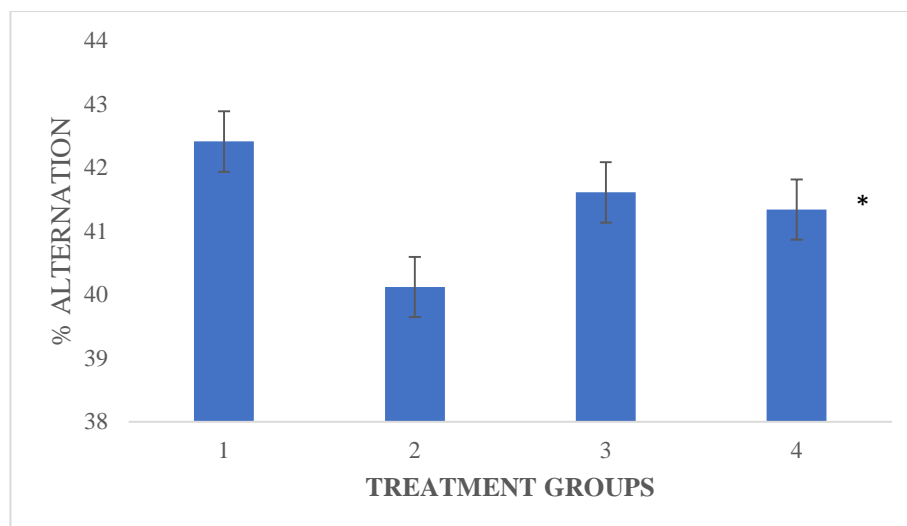


Figure 5: effect of MSG (0.5, 1.0, 1.5 mg/kg) on spatial memory during 5 minutes of exploration in the Y-maze. Each bar represents Mean ± SEM, * $p \leq 0.05$ compared to control. 1 is control group, 2, 3, and 4 received MSG at 0.5, 1.0, 1.5 mg/kg respectively. (40.12 ± 1.5), (41.61 ± 1.9), (41.37 ± 0.7).

DISCUSSION

This study revealed that acute and repeated administration of monosodium glutamate (MSG) was associated with; no significant effect on body weight of mice, Impairment of spatial recognition and spatial working-memory in mice at 1.5 mg/kg body weight dose of MSG, and also reduction of locomotor activity following 1.5 mg/kg dose administration of MSG in mice. In this study, administration of monosodium glutamate (MSG) did not significantly ($p \geq 0.05$) alter body weight compared to control. This corroborates with an earlier study in which similar result were reported [5].

The result also shows that after 5 minutes of exploration in the elevated plus maze following 1.5 mg/kg administration of MSG, there was a significant ($p \leq 0.05$) reduction in time spent in the open arm of the maze (1.75±0.85) compared to the control (3.50±0.29). This result corroborates the outcome of a study conducted by Kraeuter *et al.* [2, 11], which reported a reduction in time spent in open arm of elevated plus maze at 1.5 mg/kg dose of MSG. Glutamate-GABA (Gamma amino butyric acid) imbalance has been implicated in anxiety disorders and the response elicited following oral administration of MSG could mean that MSG resulted in an increase in brain glutamate level enough to alter the glutamate GABA balance [2, 5, 11]. However, a decrease in locomotor activities was also observed in group two (0.5 mg/kg dose of MSG) and group three (1.0 mg/kg dose of MSG) though with no significant ($p \geq 0.05$) changes.

The results also demonstrated significant decrease ($p \leq 0.05$) in spatial memory during 5 minutes exploration of the Y-Maze at 1.5 mg/kg dose of MSG. This finding contradicts the non-significant changes in spatial memory at low dose of MSG (0.5-1.5 mg/kg) of MSG reported by Onalapo *et al.* [5]. The possible mechanism of impaired spatial memory following 1.5

mg/kg dose of MSG is as a result of interference with glutamate synthesis in the hippocampus [4, 5, 9]. Also, the impairment of spatial memory at 1.5 mg/kg dose of MSG could be secondary to a combined effect of slight elevation of glutamine. There was an impairment of spatial memory in group two (0.5 mg/kg dose of MSG) and group three (1.0 mg/kg dose of MSG), though with no statistically significant ($p \geq 0.05$) changes.

CONCLUSION

Monosodium glutamate (MSG) is well known as food additives and is widely used across the globe to enhance the taste and flavor of a variety of food preparations including many processed food and snacks. Our study revealed that consumption of MSG low dose decreases locomotor activities and impairs spatial memory. MSG was found to possess a deleterious effect in the body of mice.

RECOMMENDATION

There should be awareness towards educating people on the negative effect MSG pose to health. Further studies should be done for a longer duration of time and at molecular level to determine the effect of consumption of monosodium glutamate.

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