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Original Research Article

Chronic Effects of Monosodium Glutamate on Anxiety and Motor Function in Swiss Albino Mice: Evaluation Using Hole Board and Rear Cylinder Tests

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ABSTRACT

Background: Monosodium glutamate (MSG) is a widely used flavour enhancer. While generally considered safe, concerns persist regarding the neurological consequences of long-term exposure, as excessive glutamate activity may lead to excitotoxicity and behavioural disturbances. This study investigated the chronic effects of MSG on anxiety-like behaviour and motor function in Swiss albino mice.

Methods: Forty adult male mice were randomly assigned to four groups (n=10) and administered either distilled water (control) or MSG (40, 60, or 80 mg/kg) via intraperitoneal injection daily for three months. Behavioural changes were assessed using the Hole Board Test to measure anxiety (head-dipping frequency) and the Rear Cylinder Test to evaluate locomotor activity (rearing movements). Statistical significance was determined at $p < 0.05$.

Results: Results demonstrated a significant, dose-dependent reduction in head-dipping frequency in MSG-treated groups compared to controls, indicating anxiogenic effects, with the most pronounced outcomes at 60 mg/kg and 80 mg/kg doses. Conversely, there were no statistically significant differences in rearing movements across any groups, indicating preserved motor coordination.

Conclusion: In conclusion, chronic MSG administration induces dose-dependent anxiety-like behavioural patterns, likely mediated by altered glutamatergic neurotransmission, without significant motor impairment. These findings highlight the potential for long-term MSG exposure to impact emotional regulation pathways and warrant further histopathological and neurochemical investigation to elucidate the underlying mechanisms.

Keywords: Monosodium Glutamate, Anxiety, Hole Board Test, Cylinder Test, Motor Function, Behavioural Neuroscience, Swiss Albino Mice.

INTRODUCTION

As a sodium salt of glutamic acid, monosodium glutamate (MSG) serves as a widespread food additive designed to improve palatability and flavor by delivering a distinct umami taste. It is regularly integrated into packaged goods, restaurant dishes, and processed food products. Driven by accelerating urbanization and the expansive availability of processed options, dietary exposure to MSG has grown considerably across numerous populations globally. The average daily intake of MSG in humans is estimated to range between 0.3 g and 1 g, depending on dietary patterns and regional food habits.[1]

Glutamate acts as the chief excitatory neurotransmitter within the mammalian central nervous system, where it is critical for neuronal plasticity, synaptic transmission, memory, and learning. Even so, a process called excitotoxicity can occur when glutamate activity becomes excessive, resulting in neuronal damage.Overactivation of glutamate receptors such as N-methyl-D-aspartate (NMDA)

receptors results in increased intracellular calcium influx, oxidative stress, mitochondrial dysfunction, and eventual neuronal death.[2]

Experimental studies have shown that high levels of glutamate exposure may contribute to several neurological and psychiatric disorders, including neurodegenerative diseases, epilepsy, depression, and anxiety disorders.[3] Animal studies have demonstrated that chronic exposure to MSG may induce behavioural alterations, neuronal degeneration, and oxidative stress in brain regions involved in emotional regulation and motor control.[4]

Globally, anxiety disorders stand as a prominent public health issue and rank among the most widespread psychiatric illnesses. To explore the neurobiological pathways that drive anxiety and to assess how environmental factors or pharmacological substances impact behavior, researchers frequently rely on behavioral animal models. Rodents are particularly useful experimental models because of their genetic similarity to humans and the well-characterised behavioural paradigms available for assessing emotional and motor functions.[5]

Among the various behavioural paradigms used to assess anxiety in rodents, the Hole Board Test is a commonly used unconditioned model that measures exploratory behaviour. Rodents naturally explore holes present in the apparatus by dipping their heads into them. Increased head-dipping behaviour is associated with reduced anxiety levels, whereas decreased head-dipping indicates increased anxiety-like behaviour.[6]

Motor coordination and locomotor activity are important components of neurobehavioral studies, as impairment in motor function may influence behavioural outcomes. The Rear Cylinder Test is widely used to assess locomotor activity and motor coordination in rodents by measuring spontaneous rearing behaviour. Reduced rearing activity may indicate impairment of motor function or neurological dysfunction.[7]

Even though numerous investigations have explored the toxicity profiles associated with MSG, the majority have concentrated on acute exposure. Consequently, data addressing the long-term behavioral implications of chronic MSG exposure are still scarce. To address this gap, the current research was designed to assess the long-term effects of chronic MSG intake on anxiety-related behavioral patterns and motor capabilities in Swiss albino mice, utilizing the specific paradigms of the Hole Board Test and the Rear Cylinder Test.

MATERIALS & METHODS

This study employed a prospective, interventional, experimental animal model to assess the long-term impact of monosodium glutamate (MSG) on anxiety-like behavior and motor coordination.

Animal Subjects

The study utilized forty adult male Swiss albino mice, with weights ranging between 20 and 30 g. These subjects were procured from a certified breeding facility and acclimated to standardized laboratory conditions, including a 12-hour light/dark cycle and controlled temperature (22 ± 2°C). All animals were provided with ad libitum access to water and a standard pellet diet throughout the experimental period.

Ethical Considerations

The experimental protocols were reviewed and authorized by the Institutional Animal Ethics Committee. Every procedure was conducted in strict adherence to the guidelines established by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Experimental Design and Administration Protocol

The study subjects were randomly allocated into four distinct cohorts (n = 10 per group): a control group and three experimental groups receiving MSG at dosages of 40 mg/kg, 60 mg/kg, and 80 mg/kg, respectively. MSG was administered via once-daily intraperitoneal (i.p.) injections for a continuous duration of three months. To maintain experimental integrity and minimise procedural bias, the control group received an equivalent volume of distilled water through the same route. This standardised administration protocol ensured that all animals, regardless of treatment, were subjected to identical handling and injection-related stressors throughout the chronic exposure period.

Behavioral Tests

Hole Board Test (Anxiety)

Anxiety-like behavior was assessed by observing exploratory activity in a hole-board apparatus. Each mouse was tracked for 5 minutes, and the frequency of head-dipping into the board's holes was recorded. A lower frequency of dipping is interpreted as an

anxiogenic response, whereas a higher frequency suggests anxiolytic effects.

Rear Cylinder Test (Motor Function)
Motor coordination was evaluated using a transparent cylinder where spontaneous rearing-defined as the animal elevating its forelimbs against the cylinder walls-was recorded over 3 minutes. A reduction in these rearing movements signifies locomotor impairment.

Statistical Analysis
Behavioural data are presented as medians with interquartile ranges (IQR). Longitudinal changes within groups were analysed using Wilcoxon signed-rank and Friedman tests. For between-group comparisons, the Kruskal–Wallis test was employed. Statistical significance for all assessments was established at $p < 0.05$.

RESULTS

Baseline Group Characteristics

Group	Hole Board Head Dips Median (IQR)	Cylinder Test Rearing Median (IQR)
Control	13 (10.8–15.3)	18 (15–20)
MSG 40 mg/kg	12 (10.8–13.3)	17 (15–19)
MSG 60 mg/kg	11 (10–13.3)	17 (14–19)
MSG 80 mg/kg	12.5 (11–14)	16 (14–18)

Table 1: **Baseline Group Characteristics**

Table 1 summarises the initial behavioural performance of the experimental cohorts before the commencement of MSG administration. Statistical evaluation confirmed that there were no significant inter-group disparities at the baseline, establishing a uniform starting point across all study arms.

Effect of MSG on Anxiety (Hole Board Test)

Group	Baseline Median (IQR)	3 Months Median (IQR)	p value
Control	13 (10.8–15.3)	10 (10–11)	0.03
MSG 40	12 (10.8–13.3)	9 (6.5–12)	0.04
MSG 60	11 (10–13.25)	6.5 (4.75–8.25)	0.01
MSG 80	12.5 (11–14)	6 (4–7)	0.01

Table 2: Baseline vs 3 months

The greatest reduction in head dipping was observed in the MSG 60 mg/kg group, indicating increased anxiety-like behaviour.

Effect of MSG on Motor Function (Rear Cylinder Test)
Motor function was evaluated using the Rear Cylinder Test by recording the number of spontaneous rearing movements performed by mice within a transparent cylinder for three minutes. Rearing behaviour reflects locomotor activity and exploratory motor function.

Baseline vs Three-Month Comparison (Table 3)
The baseline values of rearing counts were comparable among all experimental groups. Following three months of MSG administration, a slight reduction in rearing activity was observed in the MSG-treated groups; however, the changes were not statistically significant when compared with the control group.

Group	Baseline Median (IQR)	3-Month Median (IQR)	p value
Control	18 (15–20)	17 (15–19)	0.31
MSG 40 mg/kg	17 (15–19)	16 (14–18)	0.28
MSG 60 mg/kg	17 (14–19)	15 (13–17)	0.21
MSG 80 mg/kg	16 (14–18)	15 (13–16)	0.19

Table 3

Between-Group Comparison

Between-group analysis using the Kruskal–Wallis test revealed no statistically significant difference in rearing counts among the control and MSG-treated groups after three months of administration ($p > 0.05$).

Overall, the findings suggest that chronic administration of MSG at doses of 40 mg/kg, 60 mg/kg, and 80 mg/kg did not produce significant impairment in motor coordination or locomotor activity in mice as assessed by the Rear Cylinder Test.

Effect of MSG in the Cylinder Test in adult mice at various time intervals.

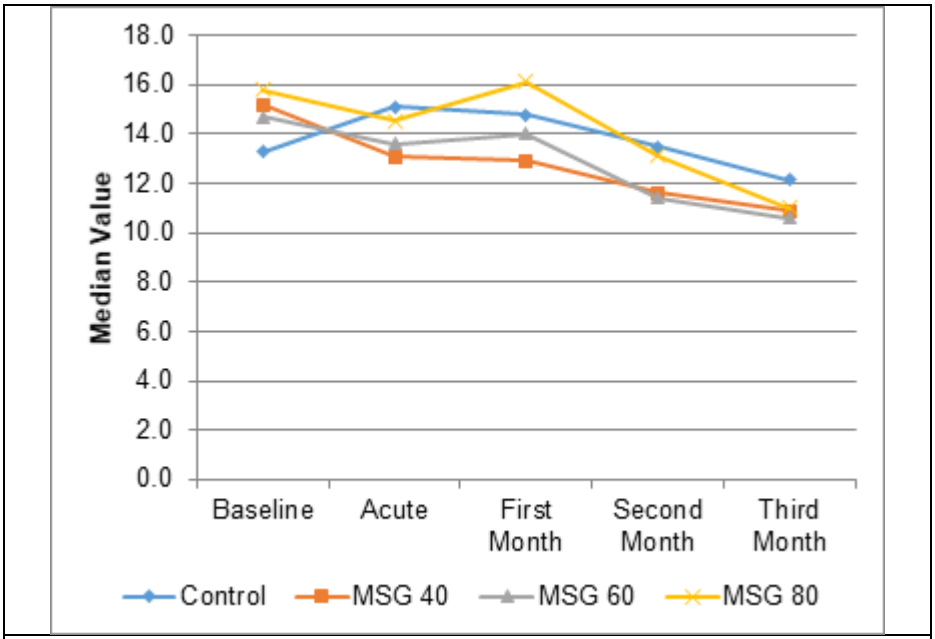

Image 1

DISCUSSION

The present study evaluated the chronic effects of monosodium glutamate (MSG) administration on anxiety-like behaviour and motor function in Swiss albino mice using the Hole Board Test and Rear Cylinder Test. The results demonstrated a progressive reduction in head-dipping behaviour in MSG-treated groups during the three-month experimental period, indicating increased anxiety-like behaviour. However, motor function assessed using the Rear Cylinder Test did not show statistically significant impairment across the experimental groups.

Glutamate is the principal excitatory neurotransmitter in the central nervous system and plays a vital role in synaptic transmission, neuronal plasticity, learning, and memory.[1] Under physiological conditions, glutamatergic neurotransmission is tightly regulated. However, excessive activation of glutamate receptors can lead to excitotoxic neuronal injury through increased calcium influx and oxidative stress.[2] Excitotoxicity has been implicated in several neurological and psychiatric disorders, including anxiety disorders and neurodegenerative diseases.[3]

In the present study, chronic MSG administration significantly reduced head-dipping behaviour in the Hole Board Test. Exploratory behaviour in rodents is considered an indicator of emotional status, and decreased head dipping is generally associated with increased anxiety-like behaviour.[6] These findings are consistent with previous studies reporting that prolonged MSG exposure can alter behavioural responses and induce neurochemical changes in brain regions associated with emotional regulation.[4] Experimental evidence suggests that increased extracellular glutamate levels may disrupt neurotransmitter balance in the hippocampus and amygdala, leading to heightened anxiety responses.[8]

The anxiogenic effect observed in the present study appeared to be dose-dependent, with the most pronounced behavioural changes observed in the 60 mg/kg and 80 mg/kg MSG groups. Previous animal studies have also reported dose-dependent behavioural alterations following MSG exposure. Chronic administration of MSG has been shown to increase oxidative stress and neuronal degeneration in various brain regions, including the hippocampus and cerebellum.[9] Such

neuronal changes may interfere with normal neurotransmitter signalling and contribute to behavioural disturbances.

Several studies have suggested that MSG may influence behavioural outcomes through mechanisms involving oxidative stress and inflammatory responses. Experimental studies have demonstrated that MSG administration can increase lipid peroxidation and reduce antioxidant enzyme activity in neural tissue.[10] These biochemical alterations may contribute to neuronal damage and functional impairment in neural circuits involved in emotional processing. Furthermore, alterations in glutamatergic neurotransmission have been associated with anxiety-like behaviours in experimental animals.[11]

Despite the significant changes observed in anxiety-like behaviour, motor function evaluated using the Rear Cylinder Test did not show statistically significant differences between groups. Rearing behaviour is widely used as an indicator of locomotor activity and neuromuscular coordination in rodents.[7] The absence of significant motor impairment suggests that chronic MSG exposure at the doses used in this study did not markedly affect motor pathways or cerebellar function. Similar findings have been reported in studies where moderate doses of MSG produced behavioural alterations without significant locomotor deficits.[12]

The preservation of motor function observed in the present study may indicate that the doses of MSG administered primarily affected neural circuits involved in emotional regulation rather than those responsible for motor coordination. Emotional behaviour is largely regulated by limbic system structures such as the amygdala, hippocampus, and prefrontal cortex, whereas locomotor activity depends mainly on cerebellar and basal ganglia circuits.[13] Disruption of neurotransmission within limbic pathways may therefore lead to anxiety-like behaviour without producing overt motor dysfunction.

Previous histopathological studies have also demonstrated that MSG exposure can induce neuronal degeneration and astrocytic activation in brain regions associated with behavioural regulation.[14] Astrocyte activation and increased expression of glial fibrillary acidic protein (GFAP) have been reported following glutamate-induced neurotoxicity.[15] Such changes may alter neuronal signalling and contribute to behavioural abnormalities.

Overall, the findings of the present study support existing experimental evidence suggesting that chronic MSG exposure may influence emotional behaviour through alterations in glutamatergic neurotransmission and oxidative stress mechanisms. However, the absence of significant motor impairment indicates that the behavioural changes observed are unlikely to be due to locomotor deficits. Further studies incorporating neurochemical analysis, oxidative stress markers, and histopathological evaluation of brain tissue are required to better understand the mechanisms underlying MSG-induced behavioural alterations.

CONCLUSION

Our findings indicate that long-term MSG exposure in Swiss albino mice results in marked anxiety-like behavioural patterns, evidenced by a dose-dependent decrease in head-dipping frequency during the Hole Board Test. Conversely, the Rear Cylinder Test showed no statistically significant motor deficits across all treatment groups. This suggests that chronic MSG intake primarily impacts emotional and anxiety-related pathways-likely mediated by glutamatergic transmission-rather than affecting motor or locomotor function. Future investigations focusing on neurochemical and histopathological assessments are recommended to further elucidate the precise underlying mechanisms of these MSG-induced behavioural shifts.

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