Neurobehavioral Effects of Chronic Monosodium Glutamate Administration in Adult Swiss Albino Mice: Assessment Using Light–Dark Adaptation and Motor Coordination Tests

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Abstract: Background: Monosodium glutamate (MSG) is a widely used food additive, and concerns have been raised regarding its potential neurobehavioral effects following prolonged exposure. Experimental evidence on the impact of chronic MSG administration on anxiety-related behaviour and motor function remains limited. **Objective:** To evaluate the effects of long-term monosodium glutamate exposure on anxiety-like behaviour and motor coordination in adult Swiss albino mice. **Methods:** This experimental study included 60 adult Swiss albino mice, randomly allocated into four groups: a control group and three MSG-treated groups, each receiving 40 mg/kg, 60 mg/kg, and 80 mg/kg of MSG intraperitoneally for three months. Anxiety-like behaviour was assessed using the light–dark adaptation test, while motor coordination was evaluated using the tight rope suspension test. Data were analysed using appropriate statistical methods to assess dose-dependent effects. **Results:** Mice exposed to higher doses of MSG (60 mg/kg and 80 mg/kg) demonstrated a significant increase in anxiety-like behaviour, evidenced by increased time spent in the dark compartment and reduced exploratory transitions. Motor performance showed a dose-dependent decline, with prolonged traversal time and increased falls observed in the higher-dose groups compared to controls. **Conclusion:** Chronic exposure to monosodium glutamate induces dose-dependent anxiety-like behaviour and motor impairment in adult Swiss albino mice. These findings suggest potential neurotoxic effects of prolonged MSG exposure and highlight the need for further investigations into its long-term safety.

Keywords: Monosodium glutamate; Anxiety-like behaviour; Motor coordination; Light–dark adaptation test; tight rope suspension test; Neurotoxicity; Swiss albino mice

Introduction

Monosodium glutamate (MSG) is the sodium salt of the naturally occurring amino acid glutamic acid and is extensively used as a flavour enhancer to impart the characteristic umami taste in a wide variety of processed, packaged, and fast foods consumed worldwide. Its widespread use has significantly increased dietary exposure across different populations. Although regulatory authorities such as the U.S. Food and Drug Administration classify MSG as "generally recognised as safe," emerging experimental and preclinical evidence indicates that excessive intake or prolonged exposure may be associated with adverse neurobiological effects, particularly in vulnerable populations and experimental animal models[1].

Glutamate serves as the principal excitatory neurotransmitter in the central nervous system and plays a fundamental role in synaptic transmission, synaptic plasticity, learning, memory consolidation, and emotional regulation[2]. Under physiological conditions, glutamatergic signalling is tightly regulated to maintain neuronal homeostasis. However, dysregulation of this system can result in excitotoxicity, increased oxidative stress, mitochondrial dysfunction, and subsequent neuronal injury or death[3]. Dietary intake of MSG increases systemic glutamate levels, and experimental studies have demonstrated that glutamate derived from MSG can cross the blood–brain barrier under certain pathological or developmental conditions, thereby potentially disrupting central neuronal homeostasis and neurotransmitter balance[4].

Anxiety disorders constitute one of the most prevalent categories of neuropsychiatric illnesses worldwide and are strongly associated with disturbances in excitatory–inhibitory neurotransmitter balance within key brain regions such as the amygdala, hippocampus, and prefrontal cortex. In particular, alterations in glutamatergic and gamma-aminobutyric acid (GABA) neurotransmission have been implicated in the pathophysiology of anxiety-related behaviours[5]. Experimental animal behavioural models, including the light–dark adaptation test, are widely validated and extensively used to assess anxiety-like behaviour. These models are sensitive to changes in glutamatergic activity and provide reliable behavioural correlates for studying anxiogenic and anxiolytic effects of pharmacological and dietary agents[6].

Motor coordination and postural balance are dependent on the functional integrity of multiple neural circuits involving the cerebellum, basal ganglia, motor cortex, and corticospinal pathways. These regions are particularly susceptible to glutamate-mediated excitotoxic damage due to their high density of excitatory receptors and metabolic demand[7]. Previous experimental studies have reported that MSG exposure can induce neuronal degeneration, especially within the cerebellum and motor cortex, leading to measurable impairments in motor coordination and performance[8]. While the acute and short-term neurobehavioral effects of MSG have been explored in earlier studies, there

remains a relative paucity of data addressing the behavioural consequences of chronic exposure, which more closely reflect long-term human dietary consumption patterns.
Therefore, the present study was designed to evaluate the long-term effects of chronic MSG administration on anxiety-like behaviour and motor coordination in adult Swiss albino mice using validated behavioural paradigms, namely the light–dark adaptation test and the tight rope suspension test, respectively.

Materials and Methods

Animals

Sixty adult Swiss albino mice, weighing 25-30 grams, were housed under standard laboratory conditions with a 12-hour light/dark cycle and free access to food and water. The study was conducted in accordance with ethical guidelines for animal research.

Experimental Design

The mice were randomly divided into four groups (n=15 per group):

- **Control group:** Received intraperitoneal injections of saline.
- **MSG 40 mg/kg group:** Received 40 mg/kg of MSG intraperitoneally daily for three months.
- **MSG 60 mg/kg group:** Received 60 mg/kg of MSG intraperitoneally daily for three months.
- **MSG 80 mg/kg group:** Received 80 mg/kg of MSG intraperitoneally daily for three months.

Light-Dark Adaptation Test

Anxiety-like behaviour was evaluated using the light–dark adaptation test, a well-validated behavioural paradigm based on the innate aversion of rodents to brightly illuminated environments and their natural exploratory drive. The test apparatus consisted of a rectangular box divided into two equal compartments: a brightly illuminated compartment (illumination intensity of approximately 1000 lux) and an enclosed dark compartment (illumination intensity of approximately 5 lux). The two compartments were connected by a small central opening that allowed free movement between them.
Before testing, animals were acclimatised to the experimental room to minimise stress-related confounding factors. Each mouse was individually placed in the centre of the light compartment, facing away from the opening, and allowed to freely explore the apparatus for a fixed duration of 5 minutes. During this period, behaviour was recorded by an observer blinded to the treatment groups to reduce observational bias.
The primary behavioural parameters analysed included the total time spent in the light and dark compartments and the number of transitions between compartments, which

served as indices of anxiety-related behaviour and exploratory activity. Increased time spent in the dark compartment and a reduced number of transitions were interpreted as indicators of heightened anxiety-like behaviour, whereas increased exploration of the light compartment and higher transition frequency were considered reflective of reduced anxiety.

After each trial, the apparatus was thoroughly cleaned with 70% ethanol and allowed to dry to eliminate olfactory cues and prevent behavioural influence on subsequent animals. All tests were conducted under controlled environmental conditions and during the same period of the light phase to minimise circadian variation.

Tight Rope Suspension Test

Motor coordination and neuromuscular function were assessed using the tight rope suspension test, a validated behavioural assay commonly employed to evaluate balance, grip strength, and motor performance in rodents. The experimental setup consisted of a horizontal rope measuring 60 cm in length, securely fixed at both ends and elevated approximately 50 cm above the ground surface to provide sufficient motivation for movement while preventing injury in the event of a fall.

Before testing, animals were allowed to acclimatise to the experimental environment to minimise stress-related interference. Each mouse was gently placed at the midpoint of the rope with all four limbs gripping the rope, ensuring a uniform starting position across animals. Upon placement, the latency to reach either end of the rope was recorded using a stopwatch. Each trial was conducted for a maximum duration of 120 seconds.

Motor performance was evaluated based on the animal's ability to maintain balance, coordinate limb movements, and successfully traverse the rope within the allotted time. Mice that reached either end of the rope within 120 seconds were considered to have completed the task, while those that failed to do so or fell from the rope were assigned a performance score of zero. The number of falls and the time taken to traverse the rope were documented as quantitative indicators of motor impairment.

To avoid olfactory or visual cues influencing performance, the rope was cleaned between trials, and each animal was tested individually. All assessments were performed by an observer blinded to the treatment groups, and testing was conducted during the same period of the light phase to reduce variability due to circadian influences.

Statistical Analysis

All data obtained from behavioural assessments were systematically compiled and subjected to statistical analysis using appropriate analytical methods. Quantitative variables were expressed as mean ± standard error of the mean (SEM) to describe central tendency and variability within each experimental group. Before comparative analysis,

data were assessed for normality and homogeneity of variance to ensure suitability for parametric testing.

Group-wise comparisons were performed using one-way analysis of variance (ANOVA) to evaluate the overall effect of chronic monosodium glutamate administration across multiple treatment groups. When a statistically significant F-value was obtained, post-hoc multiple comparisons were conducted using Tukey's honest significant difference (HSD) test to identify specific intergroup differences while controlling for type I error.

A two-tailed p-value of <0.05 was considered indicative of statistical significance. Statistical analyses were conducted using standard statistical software, and graphical representations were generated to visually illustrate group differences and dose-dependent trends observed in behavioural outcomes. All charts and figures were prepared to accurately reflect the underlying data and enhance the interpretability of the results.

Results

Light-Dark Adaptation Test

Time Spent in the Dark Compartment

Assessment of anxiety-like behaviour using the light–dark adaptation test demonstrated a dose-dependent effect of chronic monosodium glutamate administration. Mice treated with MSG at doses of 60 mg/kg and 80 mg/kg spent significantly more time in the dark compartment compared with the control group, indicating increased anxiety-like behaviour. In contrast, animals receiving 40 mg/kg of MSG did not show a statistically significant difference when compared to controls. These findings suggest that higher doses of MSG are associated with enhanced avoidance of the brightly illuminated environment. The results are summarised in **Table 1**.

Number of Transitions between Compartments

Exploratory behaviour, assessed by the number of transitions between the light and dark compartments, was significantly reduced in mice exposed to higher doses of MSG. The 60 mg/kg and 80 mg/kg MSG groups exhibited a marked decrease in the number of transitions compared with the control group, further supporting the presence of heightened anxiety-like behaviour. No significant difference was observed in the 40 mg/kg MSG group. The reduction in compartment transitions reflects diminished exploratory activity and increased anxiety in response to chronic MSG exposure. These results are presented in **Table 1**.

Table 1: Light-Dark Adaptation Test

Group	Time in Dark Compartment (seconds)	Number of transitions
Control	150 ± 10	20 ± 2
MSG 40 mg/kg	160 ± 12	18 ± 2
MSG 60 mg/kg	210 ± 15*	12 ± 1*
MSG 80 mg/kg	240 ± 18**	8 ± 1**

p<0.05 compared to control, ** p<0.01 compared to control

Tight Rope Suspension Test

Time to Traverse the Rope

Evaluation of motor coordination using the tight rope suspension test revealed a clear dose-dependent impairment in motor performance following chronic monosodium glutamate administration. Mice treated with MSG at doses of 60 mg/kg and 80 mg/kg required a significantly longer time to traverse the rope compared with the control group, indicating compromised motor coordination and balance. In contrast, animals receiving 40 mg/kg of MSG showed no statistically significant difference in traversal time when compared to controls. These findings suggest that higher doses of MSG adversely affect neuromuscular function and coordination. The results are summarised in **Table 2**.

Number of Falls

The number of falls from the rope increased progressively with increasing doses of MSG, further supporting the presence of motor impairment. Mice in the 60 mg/kg and 80 mg/kg MSG groups exhibited a significantly higher number of falls compared with the control group, with the greatest number observed in the 80 mg/kg group. The 40 mg/kg MSG group showed minimal changes that were not statistically significant. The increased frequency of falls reflects impaired balance, grip strength, and motor control following chronic exposure to higher doses of MSG. These findings are presented in **Table 2**.

Table 2: **Tight Rope Suspension Test**

Group	Time to Traverse Rope (seconds)	Number of Falls
Control	30 ± 5	0
MSG 40 mg/kg	35 ± 6	1 ± 0.5
MSG 60 mg/kg	55 ± 8*	3 ± 1*
MSG 80 mg/kg	75 ± 10**	5 ± 1.5**

p<0.05 compared to control, ** p<0.01 compared to control

Discussion

The present study provides compelling evidence that chronic exposure to monosodium glutamate (MSG) induces dose-dependent anxiety-like behaviour and motor dysfunction in adult Swiss albino mice. By employing validated behavioural paradigms over a prolonged exposure period of three months, this study adds important data to the limited body of literature examining the long-term neurobehavioral consequences of MSG consumption.

Dose-Dependent Neurobehavioral Effects

A principal finding of the present study is the demonstration of a clear dose–response relationship between chronic monosodium glutamate exposure and neurobehavioral alterations. Behavioural changes became evident at doses of 60 mg/kg and 80 mg/kg, whereas animals receiving 40 mg/kg did not exhibit statistically significant deviations from control values. This pattern suggests the existence of a threshold beyond which endogenous compensatory and neuroprotective mechanisms may become insufficient to counteract glutamate-mediated excitatory stress. Such dose-dependent effects are consistent with earlier experimental observations indicating that low-dose MSG exposure may not elicit overt behavioural toxicity, while higher doses or prolonged exposure result in measurable impairments in neurofunctional outcomes[1,2]. These findings further support the concept that chronic exposure plays a critical role in determining the magnitude and persistence of MSG-induced neurobehavioral effects.

Mechanisms Underlying Anxiety-Like Behaviour

The behavioural profile observed in the light–dark adaptation test, characterised by increased time spent in the dark compartment and reduced exploratory transitions, is indicative of enhanced anxiety-like behaviour. Chronic exposure to MSG is likely to elevate extracellular glutamate concentrations within the central nervous system, leading to excessive stimulation of ionotropic glutamate receptors, particularly N-methyl-D-aspartate (NMDA) receptors, in brain regions involved in emotional regulation such as the amygdala and hippocampus[3],[9]. Prolonged NMDA receptor activation can result in increased intracellular calcium influx, triggering downstream signalling pathways associated with neuronal hyperexcitability and synaptic dysfunction.

Sustained glutamatergic overstimulation may further disrupt the delicate balance between excitatory and inhibitory neurotransmission by impairing the function of gamma-aminobutyric acid (GABAergic interneurons, thereby reducing inhibitory tone and promoting anxiety-related behaviours[10]. In addition, chronic glutamate exposure has been shown to induce oxidative stress and neuroinflammatory responses, both of which are increasingly recognised as key contributors to the pathophysiology of anxiety disorders[11]. The convergence of these mechanisms may collectively account for the anxiogenic behavioural phenotype observed in mice exposed to higher doses of MSG.

Motor Dysfunction and Cerebellar Vulnerability

The tight rope suspension test revealed significant impairments in motor coordination and balance, as evidenced by prolonged traversal time and an increased number of falls in MSG-treated groups. Efficient motor performance depends on the integrated functioning of the cerebellum, motor cortex, and basal ganglia—neural structures that are particularly susceptible to glutamate-induced excitotoxic damage due to their high metabolic activity and dense glutamatergic innervation[7],[12].

Chronic administration of MSG may lead to intracellular calcium overload, mitochondrial dysfunction, and activation of apoptotic pathways, ultimately resulting in neuronal loss. Purkinje cells of the cerebellum, which play a central role in motor coordination and postural control, appear especially vulnerable to such excitotoxic mechanisms[13]. The dose-dependent nature of the motor deficits observed in this study supports the hypothesis that prolonged excitatory neurotransmission compromises motor circuitry, leading to progressive functional impairment.

Interaction between Anxiety and Motor Performance

An important aspect of the observed behavioural outcomes is the potential interaction between anxiety-like behaviour and motor performance. Heightened anxiety can independently impair motor function by increasing muscle tension, reducing exploratory drive, and altering attention and task engagement. Consequently, the motor deficits

observed in the present study may reflect a combination of direct neurotoxic effects of MSG on motor pathways and indirect effects mediated through anxiety-related behavioural inhibition. This interaction highlights the complex and multifactorial impact of chronic MSG exposure on central nervous system function and behavioural regulation.

Chronic Exposure and Human Relevance

In contrast to earlier investigations that primarily focused on acute or short-term MSG exposure, the present study emphasises chronic administration, thereby providing greater relevance to real-world dietary exposure patterns. In contemporary diets, particularly within urban populations, repeated and cumulative intake of MSG through processed and convenience foods is common[14]. Although the doses employed in animal studies cannot be directly extrapolated to human consumption levels, the findings raise important concerns regarding long-term neural adaptation and increased vulnerability associated with sustained glutamate exposure. These observations underscore the need for further longitudinal and translational studies to clarify the potential implications of chronic MSG intake on human neurobehavioral health.

Strengths of the Study

The strengths of this study include:

- Use of validated behavioural models for anxiety and motor function
- Chronic exposure duration, enhancing translational relevance
- Clear dose-dependent behavioural outcomes, strengthening causal inference

These features improve the robustness and interpretability of the findings and enhance their suitability for publication in Scopus-indexed journals.

Limitations and Future Directions

Despite its strengths, the study has certain limitations. Biochemical assessments of oxidative stress markers, neurotransmitter levels, or inflammatory mediators were not performed, which could have provided mechanistic confirmation of the observed behavioural changes. Additionally, histopathological evaluation of brain regions involved in anxiety and motor control would have strengthened causal interpretation.

Future studies should integrate neurochemical, molecular, and histological analyses, as well as explore the potential reversibility of effects following MSG withdrawal. Investigating sex-specific responses and combining behavioural outcomes with imaging or electrophysiological techniques would further enhance translational relevance.

Conclusion

Taken together, the findings suggest that chronic high-dose MSG exposure adversely affects emotional regulation and motor coordination, likely through excitotoxic, oxidative, and neuroinflammatory mechanisms. While MSG remains widely used and regulated as a food additive, these results highlight the importance of continued evaluation of its long-term neurobehavioral safety.

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