World Journal of Advanced Pharmaceutical and Life Sciences

Journal homepage: https://zealjournals.com/wjapls/

ISSN: 2799-0222 (Online)

(RESEARCH ARTICLE)

Check for updates

Monosodium glutamate disrupts zebrafish sleep-like state likely through activation of peripheral nervous system glutamate receptors

Mohammad Kutub Ali * and Derron Ricardo Taite

Department of Basic Medical Science, Faculty of Medical Sciences, University of West Indies, Mona campus, Kingston, Jamaica, West Indies.

World Journal of Advanced Pharmaceutical and Life Sciences, 2024, 07(01), 034–043

Publication history: Received on 12 July 2024; revised on 28 August 2024; accepted on 31 August 2024

Article DOI: https://doi.org/10.53346/wjapls.2024.7.1.0034

Abstract

Monosodium glutamate (MSG), a widely used flavor enhancer, has raised concerns about its effects on behavior and sleep. This study investigates how MSG influences zebrafish sleep-like state (SLS) and interacts with other substances affecting neurobehavioral responses. Adult zebrafish were exposed to MSG at concentrations of 50, 100, and 150 mg/L, and their swimming speeds and SLS durations were recorded over a 14-hour night period. The results indicated that higher MSG concentrations increased swimming speeds and decreased SLS duration, suggesting heightened excitability and reduced sleep recovery.

The study further evaluated how MSG impacts anesthesia sensitivity induced by 2-phenoxyethanol (POE) compared to alcohol and nicotine. Zebrafish were pre-treated with 50 mg/L MSG, 0.5% alcohol, or 0.5 mg/L nicotine before being anesthetized with POE. Nicotine decreased anesthesia sensitivity in a concentration-dependent manner by enhancing neurotransmitter release. In contrast, MSG and alcohol had minimal effects on anesthesia. The limited impact of MSG on anesthesia sensitivity suggests that MSG primarily affects peripheral nervous system functions rather than central nervous system functions, likely due to its poor ability to cross the blood-brain barrier.

Overall, the findings demonstrate that MSG disrupts zebrafish behavior through peripheral mechanisms, affecting both swimming activity and sleep patterns. Nicotine, on the other hand, influences behavior through central nervous system interactions. These results highlight the need for further research to better understand how dietary additives like MSG impact neurobehavioral functions in aquatic models, offering insights into their broader implications for sleep and behavior.

Keywords: Monosodium glutamate; 2-Phenoxyethanol; Alcohol; Nicotine; Zebrafish; Sleep

1. Introduction

Monosodium glutamate (MSG), derived from L-glutamic acid—a naturally occurring amino acid in foods like meat, fish, cheese, and vegetables [1] has been a widely used flavor enhancer since its discovery. Identified as the fifth basic taste, umami, MSG enhances food flavor, with its effects varying by concentration and food matrix [2]. Its flavor-enhancing properties are further amplified by interactions with other umami compounds such as inosine monophosphate (IMP) and guanosine monophosphate (GMP), potentially allowing for reduced sodium chloride while maintaining taste [3].

Despite its widespread use, concerns about MSG's safety, especially with chronic exposure, remain. Glutamate, MSG's primary component, functions as a neurotransmitter involved in energy production and protein metabolism. Imbalances in glutamate levels are linked to brain damage and neurodegenerative disorders [4]. While most dietary

* Corresponding author: Mohammad Kutub Ali

glutamate is metabolized in the intestines, leading to minimal systemic absorption [5], the European Food Safety Authority (EFSA) has set an acceptable daily intake (ADI) for MSG at 30 mg/kg/day, though some studies suggest certain populations may exceed this threshold [6].

Preclinical studies have reported potential adverse effects of MSG, such as heart tissue damage, liver toxicity, diabetes, and obesity, at doses ranging from 0.04 g/kg to 8 g/kg [7]. However, these studies often use high doses or non-oral administration methods, which limits their relevance to typical human consumption [8]. MSG may also impact the central nervous system, potentially increasing aggressiveness and reducing locomotor activity due to overactivation of glutamate pathways [9].

High levels of glutamate, such as those from high doses of MSG, can disrupt blood-brain barrier (BBB) integrity, increasing permeability and contributing to neurotoxicity and inflammation [10]. Excessive glutamate is also associated with increased seizure susceptibility, with some preclinical studies suggesting MSG might lower the seizure threshold and worsen epilepsy symptoms [11]. Nonetheless, human studies on MSG's impact on BBB permeability and seizure activity are limited, necessitating further research to understand the real-world implications [12-13].

Additionally, high glutamate levels can disrupt sleep patterns [14-15], though human research on MSG's direct effects on sleep remains limited and inconclusive [16]. MSG's impact extends beyond BBB permeability; it also affects peripheral glutamate receptors, which are crucial for regulating pain, inflammation, and gastrointestinal functions [17-18]

These peripheral glutamate receptors also influence sleep regulation. NMDA receptors in peripheral tissues can impact sleep indirectly by affecting central pain processing, with chronic pain linked to disrupted sleep and insomnia [19-21]. AMPA receptors influence the sleep-wake cycle through interactions with pain pathways and inflammatory responses [22]. Peripheral glutamate receptors also modulate sleep by affecting inflammation and neurotransmitter release [23-25]. Understanding these interactions could lead to better management strategies for sleep disturbances related to pain and inflammation.

Recent advancements in zebrafish sleep research have enhanced the precision of sleep measurements through refined behavioral assays. These improvements have also shed light on how environmental factors, such as light exposure and water quality, affect sleep patterns in zebrafish. Additionally, emerging research highlights the influence of social interactions and group dynamics on sleep, offering valuable insights into the social determinants of sleep behavior [26]. In this context, our study aims to investigate the effects of monosodium glutamate (MSG) on zebrafish sleep. Specifically, we seek to elucidate how MSG impacts sleep patterns in zebrafish and explore the underlying mechanisms by which MSG might influence sleep. This research could provide a deeper understanding of MSG's effects on sleep and contribute to broader knowledge about sleep regulation in aquatic models.

2. Materials and Methods

- **Materials:** Adult zebrafish (Danio rerio), averaging 3 cm in length and weighing about 700 mg, were sourced from a local aquarium store. Prior to introducing the fish, 3 g of artificial instant ocean salts were added per liter of deionized distilled water for conditioning. This preparation was done on a large scale (200 L) and allowed it to stabilize over three days. Nicotine was obtained from Sigma-Aldrich (CAS Number: 54-11-5), and alcohol from Merck (Product Number: 1009711000). MSG from Fisher Scientific (CAS Number: 6106-04-3), 2-phenoxyethanol 99% v/v from Sigma Aldrich (CAS Number:122-99-6)
- **Zebrafish Maintenance and Selection:** Healthy adult zebrafish were selected using specific criteria and kept in large tanks measuring 45×60×25 cm. They were housed in conditioned fresh fish water with a conductivity of about 1,500 µS/cm and a pH of 7. The temperature was maintained at 28°C, with continuous aeration provided by an air pump to ensure proper oxygen levels. Water quality was managed through daily two-hour filtration and the addition of aquarium disinfectant. Additionally, 30% of the tank water was replaced daily with Millipore-filtered water containing the appropriate amount of instant ocean salt. The fish were fed three times daily (at 10 am, 2 pm, and 6 pm) with bloodworms, enough to be consumed within five minutes. After ten days of maintenance, zebrafish with similar morphology and length, having equal caudal fin length and exhibiting normal swimming and feeding behaviors, were chosen and housed in pairs separately in 1.5 L Yobiisolut mini aquarium tanks for further study.
- **Sleep Recording:** For sleep recording, zebrafish were placed individually or in pairs into a novel tank (Wide 50 cm × thickness 6.5 cm × height 30 cm) with a water-to-air interface surface of 325 cm^2. Daytime recordings were conducted using a CCD camera positioned in front of the tank, connected to a desktop computer, and

monitored with iSpy software at 4 frames per second for 10 hours (from 9 AM to 7 PM). Fish were fed twice daily, at 10 AM and 5 PM, with procedures in place to minimize stress during recording. At 6 PM, the tank was cleaned to remove uneaten food, and 80% of the water was replaced with fresh water. Oxygen saturation was maintained at 8.0 mg/L with an air stone connected to an air pump, and the water temperature was kept at 28°C.

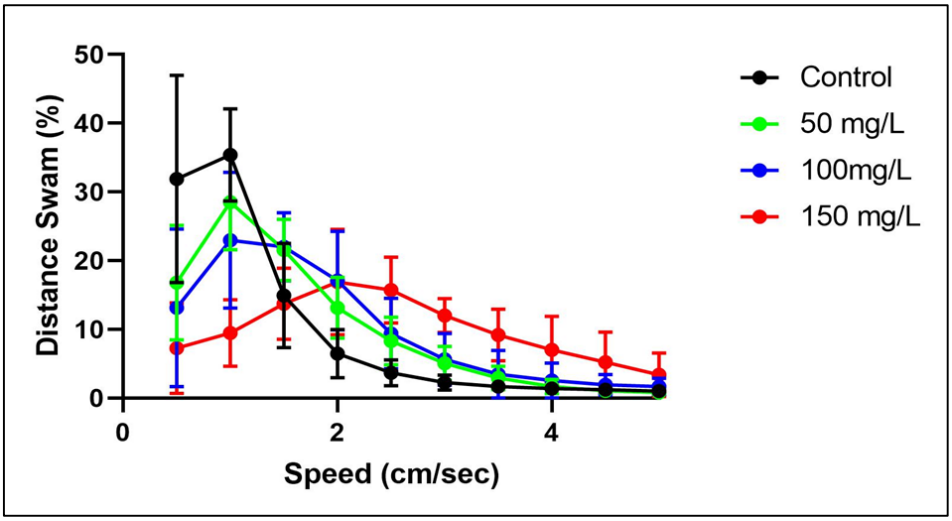
At 7 PM, a black night-light was turned on, and the daytime white light was turned off using an automatic timer. Night-time recordings, also at 10 frames per second, continued for 14 hours, including 2 hours of gradual dimming before the full activation of the night-light and 2 hours of gradual brightening after the night-light was turned off. The recordings were conducted in a closed room to prevent external disturbances. All fish were acclimated for 3 days under similar conditions to reduce anxiety related to environmental changes. The experiments, conducted four times with different sets of fish, tested conditions with 50, 100, or 150 mg/L MSG.

Video analysis was performed using NIH ImageJ software with the wrMTrck plugin. Total Sleep-Like State (SLS) duration was calculated by summing periods when fish swam uninterrupted above 6 second at a maximum speed of 0.3 cm/seconds. Statistical significance was assessed using Single Factor ANOVA in Microsoft Excel.

- **Preparation of 2-Phenoxyethanol (POE) Treatment Solutions**: To prepare a 5% POE stock solution, a precise volume of 99% was pipetted into 500 mL of distilled water. This stock solution served as the basis for generating all subsequent treatment solutions. Various treatment solutions with concentrations of 0.0450%, 0.0375%, 0.0370%, 0.0360%, 0.0350%, 0.0340%, 0.0330%, 0.0325%, and 0.0300% POE were prepared by diluting the 5% POE stock solution with 400 mL of distilled water. These concentrations were carefully chosen to cover a range of POE levels to assess their effects on zebrafish behavior.
- **Phenoxyethanol Assay**: In the phenoxyethanol assay, six healthy adult zebrafish were exposed to 400 mL of POE solutions with concentrations ranging from 0.0450% to 0.0300% for a period of 45 minutes. A key aspect of this assay involved assessing the tail response reflex, which was tested by gently pricking the tails of the fish with a pin at regular intervals. The absence of this reflex indicated that the fish were fully anesthetized. This procedure was repeated across different POE concentrations to determine the optimal levels for anesthesia. A graph was plotted to visualize the relationship between POE concentration, and the time taken for the loss of the tail response reflex, thereby identifying the most effective concentrations for subsequent experiments.
- **Drug Assay:** In the drug assay, six healthy adult zebrafish were pre-treated with various substances to evaluate their effects on anesthesia induced by POE. The pre-treatment involved three different substances: 150 mg/L monosodium glutamate (MSG) for 6 hours, 0.5% alcohol for 30 minutes, and 0.5 -1 mg/L nicotine for 30 minutes. After pre-treatment, the zebrafish were anesthetized using 400 mL solutions of 0.0350% POE. During the anesthesia process, the zebrafish behavior was monitored and recorded using iSpy software. The primary metric for assessing the level of anesthesia was the loss of the tail response reflex. This reflex was tested by gently pricking the tails of the fish at regular intervals. The disappearance of this reflex indicated the induction of anesthesia. By analyzing the response times and the effectiveness of anesthesia under different pre-treatment conditions, the study aimed to understand how MSG, alcohol, and nicotine influence the anesthetic efficacy of POE.

3. Results and Discussion

Figure 1 presents data on the percentage of distance traveled at various swimming speeds during nighttime recordings for two zebrafish in each tank across four experimental replicates. Control zebrafish exhibited a steady, slow swimming pace throughout the night. In contrast, higher concentrations of MSG in the water resulted in a greater percentage of distance traveled at higher speeds. MSG contains 12.28 mg of sodium per 100 mg, while sodium chloride (NaCl) contains 39.34 mg of sodium per 100 mg [27]. Adding 150 mg of MSG introduces approximately 18.42 mg of sodium, which minimally affects water conductivity, keeping it around 1,500 µS/cm. In comparison, adding 49 mg of NaCl, which provides a similar amount of sodium, raises conductivity to 1,540 µS/cm. This supports earlier findings that MSG dissociates less than NaCl, leading to lower conductivity [28]. Zebrafish can tolerate water conductivity up to 1,700 µS/cm [29], indicating that the observed changes in swimming speed are likely due to MSG's role as an excitatory neurotransmitter, which may disrupt relaxation and potentially increase aggression among the fish.

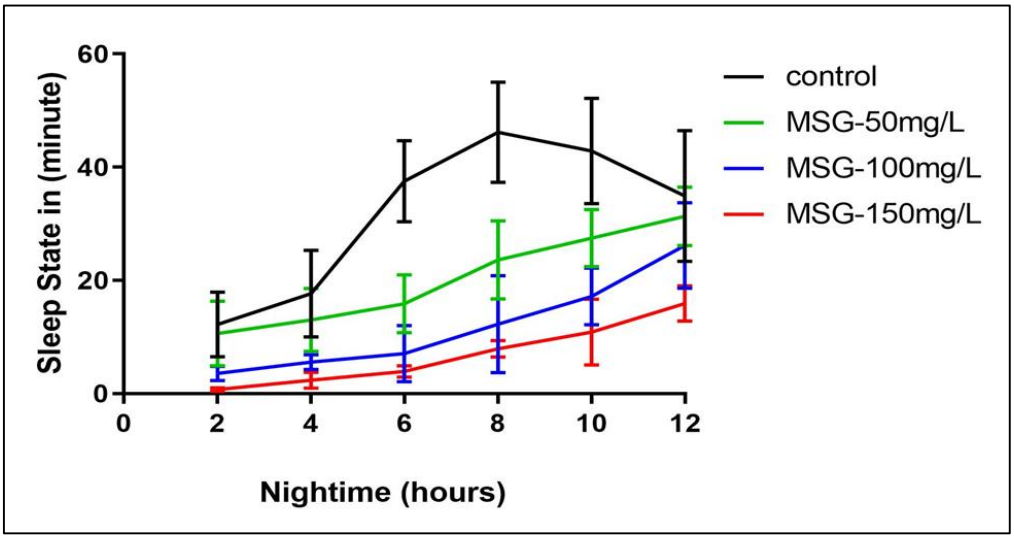


Each curve represents the mean ± SD from four independent experiments, each involving a pair of zebrafish.

Figure 1 Impact of Elevated MSG Concentrations on Nighttime Swimming Activity in Zebrafish

Zebrafish sleep is characterized by three key features: a quiescent state regulated by a circadian rhythm, reduced sensory responsiveness, and homeostatic regulation. Larval zebrafish display a diurnal pattern with peak activity during the light phase and periods of inactivity at night. Adult zebrafish sleep is defined by at least 6 seconds of inactivity. Both larval and adult zebrafish exhibit "sleep rebound," a compensatory increase in sleep following deprivation [30].

Figure 2 shows the total time spent in SLS each hour of the night. Control fish spent the most time in the SLS, with duration increasing until around 8 hours into the night before declining. Conversely, MSG-treated fish showed a concentration-dependent decrease in the time spent in the SLS. At 150 mg/L MSG, the duration of the SLS was the lowest, though it still increased throughout the night.



Each curve represents the mean ± SD from four independent experiments, each involving a pair of zebrafish.

Figure 2 Effect of Increasing MSG Concentrations on Nighttime duration of Sleep-like State in Zebrafish

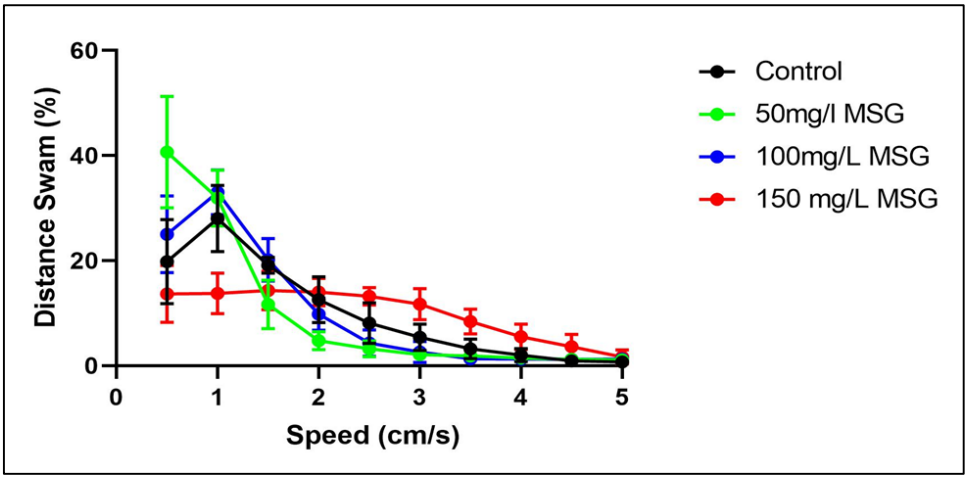
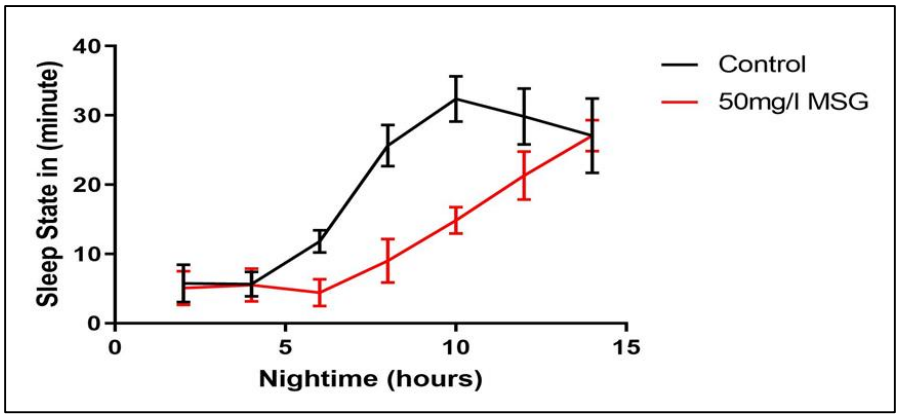


Figure 3 Sleep Rebound Activity in Zebrafish Pretreated Overnight with Various MSG Concentrations

Sleep was monitored throughout the entire daylight period following replacement of the treatment water with fresh fish water. Each curve represents the mean ± SD from four independent experiments, each involving a pair of zebrafish.

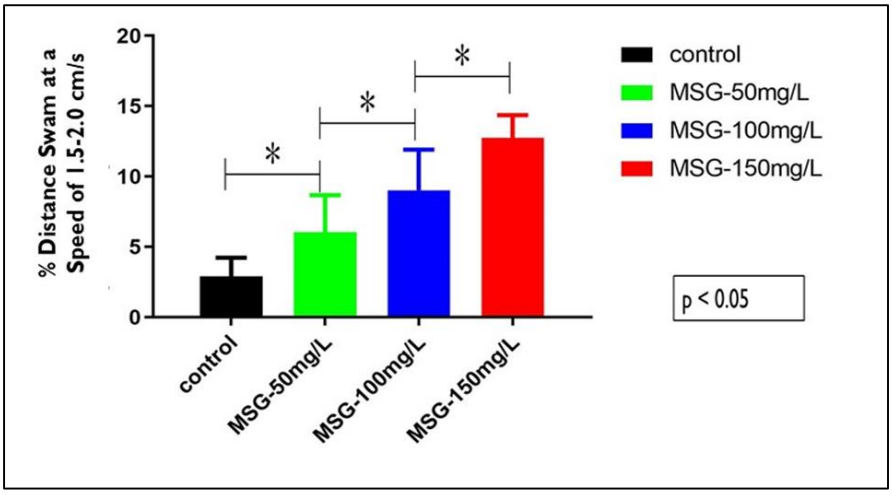
Figure 3 depicts the percentage distance traveled at each swimming speed during the light phase after replacing MSG-containing water with fresh water. Control fish exhibited minimal sleep rebound, while those previously exposed to 50 mg/L MSG showed the highest sleep rebound, followed by fish exposed to 100 mg/L MSG. Fish exposed to 150 mg/L MSG displayed the least sleep rebound. This suggests that prolonged MSG exposure may slow metabolism in a concentration-dependent manner [31], potentially inhibiting full recovery and sleep rebound even after MSG is removed from the water.

Zebrafish typically maintain certain distances from each other during sleep [26]. The excitatory effects of MSG might disrupt this spacing, leading to increased aggression and chase behavior. As a result, the duration of the SLS is expected to be lower. Figure 4 compares the duration of the SLS between zebrafish treated with 50 mg/L MSG and control fish. Control fish showed an increase in SLS duration after 4 hours of nighttime, whereas in MSG-treated fish, this increase was delayed until after 6 hours. Throughout the night, the SLS in MSG-treated fish remained consistently lower than in controls, except during the transition between night and day, when it approached control levels. This convergence is attributed to the reduced SLS in control fish after 10 hours of nighttime, a trend not observed in MSG-treated fish. These results suggest that MSG disrupts the relaxation state in zebrafish. Figure 5 illustrates the percentage of distance traveled at higher speeds by zebrafish treated with 50-150 mg/L MSG during the 7–10-hour nighttime period. Control fish displayed the longest duration of SLS but covered the least distance at higher speeds during this time. In contrast, the data reveals a significant increase in the percentage of distance traveled at higher speeds with rising MSG concentrations in the water. These results further confirm that MSG disrupts the SLS in a concentration-dependent manner.



Each curve represents the mean ± SD from four independent experiments, each involving single zebrafish.

Figure 4 Effect of Increasing MSG Concentrations on Nighttime duration of Sleep-like State in Zebrafish

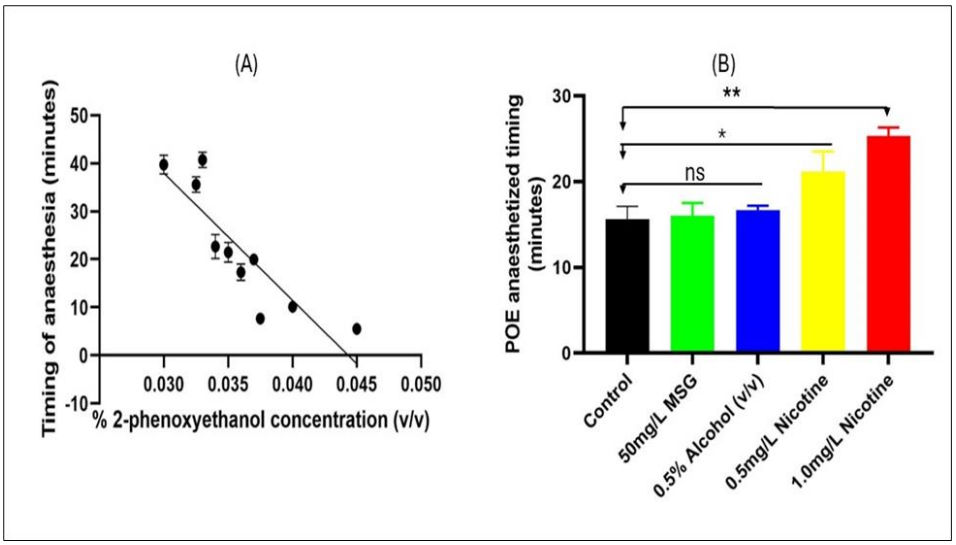


Each curve represents the mean ± SD from four independent experiments, with each experiment involving a single zebrafish.

Figure 5 Impact of Elevated MSG Concentrations on High-Speed Swimming Activity in Zebrafish During Progressive Nighttime Hours Between 7 to 10 hours

A major controversy surrounding MSG is that the blood-brain barrier effectively restricts glutamate from passing into the brain. As a result, brain glutamate levels only rise when blood glutamate concentrations are artificially elevated through non-physiological methods. This suggests that dietary MSG does not increase brain glutamate levels and, therefore, does not disrupt brain function [32].

POE, a commonly used anesthetic in adult zebrafish research [33], is known to reversibly inhibit N-methyl-D-aspartate (NMDA) receptors [34]. Since glutamate activates NMDA receptors [35-36], substances affecting NMDA receptor activity are of interest. Alcohol is known to inhibit NMDA receptor activity [37-38], whereas nicotine is known to enhance NMDA receptor activity [39-40]. Figure 6 (A) presents a time calibration plot showing the relationship between the increasing concentration of POE and the time required for loss of opercular beat and startle reflex. As the concentration of POE increased, there was a corresponding decrease in the time to achieve anesthesia. However, sensitivity to POE varied significantly among individual fish. At a concentration of 0.035%, the average anesthesia time was approximately 20 minutes, providing sufficient time to assess anesthesia without posing a high risk of fish mortality, which was a concern at higher concentrations. Additionally, fish at this concentration were relatively immobile, facilitating the detection of tail reflex. Conversely, at lower concentrations, fish remained more mobile and disoriented, complicating the detection of anesthesia and increasing the risk of injury during testing.



(A) Calibration curve for 2-Phenoxyethanol anesthesia. (B) Impact of MSG, alcohol, and nicotine on the anesthesia of zebrafish with 0.035% 2-Phenoxyethanol. Each point represents the mean ± SD from three independent experiments, each involving six zebrafish

Figure 6 Effects of MSG, Alcohol, and Nicotine on 2-Phenoxyethanol Anesthesia in Zebrafish.

Four groups of healthy fish, each consisting of six individuals, were treated as follows: Group 1 was exposed to 0.5% alcohol for 30 minutes; Group 2 and Group 3 were treated with 0.5 mg/L and 1.0 mg/L nicotine, respectively, for 30 minutes; and Group 4 was treated with 50 mg/L MSG for 6 hours. After these pretreatments, all fish were subjected to 0.035% POE for 20 minutes. The average percentage of fish showing anesthesia, based on three repeated experiments with six fish each, is shown in Figure 6(B). The data reveal that alcohol and MSG had an insignificant impact on fish anesthesia. In contrast, nicotine reduced anesthesia in a concentration-dependent manner.

Both POE and alcohol are known to inhibit NMDA receptors [34, 37-38], suggesting that their combined effect could lead to increased anesthesia. However, the lack of increased anesthesia in alcohol-pretreated fish may be attributed to the slow action of alcohol on the central nervous system (CNS) [41] and its first-pass metabolism [42].

Nicotine, on the other hand, binds to nicotinic acetylcholine receptors (nAChRs) in both the peripheral nervous system (PNS) and CNS [43]. Although POE does not affect these receptors, nicotine's rapid action in the CNS may lead to increased release of neurotransmitters such as glutamate, dopamine, serotonin, norepinephrine, and γ-aminobutyric acid when nAChRs are stimulated [44]. This increase in glutamate could provide competitive protection against the NMDA receptor inhibition caused by POE [34].

The lack of protection against POE in fish treated with 50 mg/L MSG suggests that MSG's effect on CNS glutamate levels is minimal, likely due to its poor ability to cross the blood-brain barrier [32]. Therefore, the concentration-dependent effects of MSG on zebrafish SLS might be mediated more by the PNS than the CNS. Zebrafish SLS relies on maintaining balance and spatial distance, and MSG might disrupt this balance through activation of PNS glutamate receptors [21, 23-25]. This disruption could contribute to restless behavior, as both CNS and PNS excitability are involved in restless legs syndrome [45]. Further research is needed to clarify the exact mechanisms involved.

4. Conclusion

This study demonstrates that MSG, nicotine, and alcohol differentially affect zebrafish behavior and physiological responses. Higher MSG concentrations increased the percentage of distance traveled at higher swimming speeds and decreased time spent in sleep-like states (SLS), indicating heightened excitability and reduced recovery. Nicotine reduced anesthesia sensitivity in a concentration-dependent manner by enhancing neurotransmitter release, offering competitive protection against NMDA receptor inhibition by POE. In contrast, alcohol and MSG had minimal impact on anesthesia, with MSG's effects likely limited by its poor ability to cross the blood-brain barrier. These findings suggest that MSG primarily disrupts zebrafish behavior through peripheral mechanisms, while nicotine's effects are mediated through central nervous system interactions. Overall, the results highlight the need for further research to better understand the underlying mechanisms and implications for neurobehavioral studies in aquatic models.

Compliance with ethical standards

Acknowledgments

We gratefully acknowledge the financial support from the University of the West Indies, Mona Campus, Kingston, Jamaica. We also extend our thanks to Hasina Nicholson for presenting the findings at the Ministry of Health Conference in Kingston, Jamaica

Disclosure of conflict of interest

The authors declare that there is no conflict of interest.

Statement of ethical approval

Ethical approval was granted by the Mona Campus Research Ethics Committee (Ref: AN4, 19/20).

Authors' Contribution

- **Mohammad Kutub Ali:** Research supervisor and coordinator, data analyser and manuscript drafting.
- **Derron Ricardo Taite:** 2-phenoxyethanol anaesthesia data collection.

References

[1] Stanska K, & Krzeski A (2016). The umami taste: from discovery to clinical use. *Otolaryngologia Polska*, 70(4), 10–15. doi:1199991 [pii] 10.5604/00306657.1199991

[2] Kurihara K (2015). Umami the Fifth Basic Taste: History of Studies on Receptor Mechanisms and Role as a Food Flavor. *BioMed Research International*, 189402. doi:10.1155/2015/189402

[3] Jinap S, Hajeb P, Karim R, Norliana S, Yibadatihan S, & Abdul-Kadir R (2016). Reduction of sodium content in spicy soups using monosodium glutamate. *Food & Nutrition Research,* 60, 30463. doi: 10.3402/fnr.v60.30463

[4] Meldrum BS (2000). Glutamate as a neurotransmitter in the brain: review of physiology and pathology. *The Journal of Nutrition*, 130(4S Suppl), 1007S–1015S. doi:10.1093/jn/130.4.1007S

[5] Blachier F, Boutry C, Bos C, & Tome D (2009). Metabolism and functions of L-glutamate in the epithelial cells of the small and large intestines. *The American Journal of Clinical Nutrition,* 90(3), 814S–821S. doi:10.3945/ajcn.2009.27462S

[6] EFSA Panel on Food Additives and Nutrient Sources added to Food. (2017). Re-evaluation of glutamic acid (E 620), sodium glutamate (E 621), potassium glutamate (E 622), calcium glutamate (E 623), ammonium glutamate (E 624) and magnesium glutamate (E 625) as food additives. *EFSA Journal,* 15(7). doi:10.2903/j.efsa.2017.4910

[7] Eweka A, Igbigbi P, & Ucheya R (2011). Histochemical studies of the effects of monosodium glutamate on the liver of adult wistar rats. Annals of Medical and Health Science Research, 1(1), 21–29.

[8] Shi Z, Luscombe-Marsh ND, Wittert GA, Yuan B, Dai Y, Pan X, & Taylor AW (2010). Monosodium glutamate is not associated with obesity or a greater prevalence of weight gain over 5 years: findings from the Jiangsu Nutrition Study of Chinese adults. British Journal of Nutrition, 104(3), 457–463. doi:S0007114510000760 [pii] 10.1017/S0007114510000760

[9] Swamy AH, Patel NL, Gadad PC, Koti BC, Patel UM, Thippeswamy AH, & Manjula DV (2014). Neuroprotective activity of Pongamia pinnata in monosodium glutamate-induced neurotoxicity in rats. Indian Journal of Pharmaceutical Sciences, 75(6), 657–663.

[10] Gruenbaum BF, Zlotnik A, Fleidervish I, Frenkel A, Boyko Mn (2022). Glutamate Neurotoxicity and Destruction of the Blood-Brain Barrier: Key Pathways for the Development of Neuropsychiatric Consequences of TBI and Their Potential Treatment Strategies. Int J Mol Sci. 23(17):9628. doi: 10.3390/ijms23179628.

[11] Singh M, Panda SP (2023). The Role of monosodium glutamate (MSG) in Epilepsy and other Neurodegenerative Diseases: Phytochemical-based Therapeutic Approaches and Mechanisms. Curr Pharm Biotechnol. doi: 10.2174/1389201024666230726161314.

[12] Sweeney MD, Zhao Z, Montagne A, Nelson AR, Zlokovic BV (2019). Blood-Brain Barrier: From Physiology to Disease and Back. Physiol Rev. 99(1):21-78. doi: 10.1152/physrev.00050.2017.

[13] Singh M, Panda SP (2023). The Role of monosodium glutamate (MSG) in Epilepsy and other Neurodegenerative Diseases: Phytochemical-based Therapeutic Approaches and Mechanisms. Curr Pharm Biotechnol. doi: 10.2174/1389201024666230726161314.

[14] Schneider L (2020). Neurobiology and Neuroprotective Benefits of Sleep. Continuum (Minneap Minn). 2020 26(4):848-870. doi: 10.1212/CON.0000000000000878.

[15] orenic SA, Klingaman EA, Wickwire EM, Gaston FE, Chen H, Wijtenburg SA, Rowland LM (2020). Sleep quality is related to brain glutamate and symptom severity in schizophrenia. J Psychiatr Res. 120:14-20. doi: 10.1016/j.jpsychires.2019.10.006.

[16] Mustafa, Z., Ashraf, S., Tauheed, S. F., & Ali, S. (2017). Monosodium glutamate, commercial production, positive and negative effects on human body and remedies - A review. International Journal of Scientific Research in Science and Technology, 3, 425-435. https://api.semanticscholar.org/CorpusID:103123922

[17] Du J, Li XH, Li YJ (2016). Glutamate in peripheral organs: Biology and pharmacology. Eur J Pharmacol. 784:42-8. doi: 10.1016/j.ejphar.2016.05.009.

[18] Chung MK, Ro JY (2020). Peripheral glutamate receptor and transient receptor potential channel mechanisms of craniofacial muscle pain. Mol Pain. 16:1744806920914204. doi: 10.1177/1744806920914204.

[19] Cairns BE, Svensson P, Wang K, Hupfeld S, Graven-Nielsen T, Sessle BJ, Berde CB, Arendt-Nielsen L (2003). Activation of peripheral NMDA receptors contributes to human pain and rat afferent discharges evoked by injection of glutamate into the masseter muscle. J Neurophysiol. 90(4):2098-105. doi: 10.1152/jn.00353.2003.

[20] O'Brien, M., & Cairns, B. E. (2016). Monosodium glutamate alters the response properties of rat trigeminovascular neurons through activation of peripheral NMDA receptors. Neuroscience, 334, 236-244. https://doi.org/10.1016/j.neuroscience.2016.08.007

[21] Benbow T, Teja F, Sheikhi A, Exposto FG, Svensson P, Cairns BE (2022). Peripheral N-methyl-D-aspartate receptor activation contributes to monosodium glutamate-induced headache but not nausea behaviours in rats. Sci Rep. 12(1):13894. doi: 10.1038/s41598-022-18290-w.

[22] Yang H, Xie J, Mu W, Ruan X, Zhang J, Yao L, Diao Z, Wu M, Li Y, Ren W, Han J (2020). Tea polyphenols protect learning and memory in sleep-deprived mice by promoting AMPA receptor internalization. Neuroreport. 31(12):857-864. doi: 10.1097/WNR.0000000000001462.

[23] Chun YH, Frank D, Lee JS, Zhang Y, Auh QS, Ro JY (2008). Peripheral AMPA receptors contribute to muscle nociception and c-fos activation. Neurosci Res. 62(2):97-104. doi: 10.1016/j.neures.2008.06.007.

[24] Gangadharan V, Wang R, Ulzhöfer B, et al. (2011). Peripheral calcium-permeable AMPA receptors regulate chronic inflammatory pain in mice. J Clin Invest. 121(4):1608-23. doi: 10.1172/JCI44911.

[25] Chen TJ, Kukley M (2020). Glutamate receptors and glutamatergic signalling in the peripheral nerves. Neural Regen Res. 15(3):438-447. doi: 10.4103/1673-5374.266047.

[26] Ali, M. K., & Nicholson, H. L. (2018). Increasing zebrafish (Danio rerio) numbers in a limited tank space reduces night-time fish sleep-like state and induces aggressive behaviour. World J Depress Anxiety, 1(1);1-6. https://api.semanticscholar.org/CorpusID:211006951

[27] Maluly HDB, Arisseto-Bragotto AP, Reyes FGR (2017). Monosodium glutamate as a tool to reduce sodium in foodstuffs: Technological and safety aspects. Food Sci Nutr. 5(6):1039-1048. doi: 10.1002/fsn3.499.

[28] Sinmyo, R., Keppler, H (2017). Electrical conductivity of NaCl-bearing aqueous fluids to 600 °C and 1 GPa. Contrib Mineral Petrol 172, 4.https://doi.org/10.1007/s00410-016-1323-z

[29] Aleström P, D'Angelo L, Midtlyng PJ, Schorderet DF, Schulte-Merker S, Sohm F, Warner S (2020). Zebrafish: Housing and husbandry recommendations. Lab Anim. 54(3):213-224. doi: 10.1177/0023677219869037.

[30] Chiu CN, Prober DA (2013). Regulation of zebrafish sleep and arousal states: current and prospective approaches. Front Neural Circuits. 9;7:58. doi: 10.3389/fncir.2013.00058.

[31] Zanfirescu A, Ungurianu A, Tsatsakis AM, et al. (2019). A review of the alleged health hazards of monosodium glutamate. *Compr Rev Food Sci Food Saf.* 18(4):1111-1134. doi: 10.1111/1541-4337.12448.

[32] Fernstrom JD (2018). Monosodium Glutamate in the Diet Does Not Raise Brain Glutamate Concentrations or Disrupt Brain Functions. *Ann Nutr Metab.* 73 Suppl 5:43-52. doi: 10.1159/000494782.

[33] Owen JP, Kelsh RN (2021). A suitable anaesthetic protocol for metamorphic zebrafish. *PLoS One.* 16(3):e0246504. doi: 10.1371/journal.pone.0246504.

[34] Musshoff U, Madeja M, Binding N, Witting U, Speckmann EJ (1999). Effects of 2-phenoxyethanol on N-methyl-D-aspartate (NMDA) receptor-mediated ion currents. *Arch Toxicol.* 73(1):55-9. doi: 10.1007/s002040050586.

[35] Yu A, Lau AY (2018). Glutamate and Glycine Binding to the NMDA Receptor. *Structure.* 26(7):1035-1043.e2. doi: 10.1016/j.str.2018.05.004.

[36] Costa BM, Kwapisz LC, Mehrkens B, Bledsoe DN, Vacca BN, Johnston TV, Razzaq R, Manickam D, Klein BG (2021). A glutamate concentration-biased allosteric modulator potentiates NMDA-induced ion influx in neurons. *Pharmacol Res Perspect.* 9(5):e00859. doi: 10.1002/prp2.859.

[37] Peoples RW, Ren H (2022). Effects of ethanol on GluN1/GluN2A and GluN1/GluN2B NMDA receptor-ion channel gating kinetics. *Alcohol Clin Exp Res.* 46(12):2203-2213. doi: 10.1111/acer.14965.

[38] Lai CC, Hsu JW, Cheng YS, Lin HH (2019). Involvement of metabotropic glutamate receptor 5 in ethanol regulation of NMDA receptor activity in rat substantia gelatinosa neurons. *Life Sci.* 233:116729. doi: 10.1016/j.lfs.2019.116729.

[39] Duan JJ, Lozada AF, Gou CY, Xu J, Chen Y, Berg DK (2015). Nicotine recruits glutamate receptors to postsynaptic sites. Mol Cell Neurosci. 68:340-9. doi: 10.1016/j.mcn.2015.09.002.

[40] Aramakis VB, Metherate R (1998). Nicotine selectively enhances NMDA receptor-mediated synaptic transmission during postnatal development in sensory neocortex. J Neurosci. 18(20):8485-95. doi: 10.1523/JNEUROSCI.18-20-08485.1998.

[41] Sadighi A, Leggio L, Akhlaghi F (2021). Development of a Physiologically Based Pharmacokinetic Model for Prediction of Ethanol Concentration-Time Profile in Different Organs. Alcohol Alcohol. 56(4):401-414. doi: 10.1093/alcalc/agaa129.

[42] Chi YC, Lee SL, Lai CL, Lee YP, Lee SP, Chiang CP, Yin SJ (2016). Ethanol oxidation and the inhibition by drugs in human liver, stomach and small intestine: Quantitative assessment with numerical organ modeling of alcohol dehydrogenase isozymes. Chem Biol Interact. 258:134-41. doi: 10.1016/j.cbi.2016.08.014.

[43] Gotti C, Zoli M, Clementi F (2006): Brain nicotinic acetylcholine receptors: native subtypes and their relevance. Trends Pharmacol Sci. 27:482-91. 10.1016/j.tips.2006.07.004

[44] Guo L, Mao J, Zhang Q, Fan W, Wang D, Li Z, Huang J, Xie J (2023). Pharmacokinetic and pharmacodynamic studies of nicotine in rat brain: a simultaneous investigation of nicotine metabolites and the release of neurotransmitters in vivo. Front Chem. 11:1275478. doi: 10.3389/fchem.2023.1275478.

[45] Lanza G, Bachmann CG, Ghorayeb I, Wang Y, Ferri R, Paulus W (2017). Central and peripheral nervous system excitability in restless legs syndrome. Sleep Med. 31:49-60. doi: 10.1016/j.sleep.2016.05.010.