

Monosodium Glutamate Maintains Antioxidant Balance in the Neuro-Retinal Axis of Male Wistar Rats

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Abstract

Purpose Information on monosodium glutamate (MSG) safety in the eye-brain axis remains scarce. The objective of the study was to establish changes in calcium (Ca^{2+}) concentrations, catalase activity, Malondialdehyde (MDA) levels and identify any major histological changes associated with parenteral and feed supplementation of MSG in male Wistar rats. This is important to guide policy on human consumption of food additives.

Methods Thirty adult male Wistar rats were divided into 5 groups ($n = 6$) for a period of one month. Group I and IV were treated with MSG 6g/kg and 60 mg/kg subcutaneous, while groups II and III at 5% and 2% MSG feed supplement. Group V was the control group treated with normal saline (no MSG). The eye and brain were analyzed for Ca^{2+} , catalase, MDA levels and histological analysis was done using Hematoxylin and Eosin staining.

Results Calcium levels in the eye was reduced at high (6g/kg/day and 5% s.c) MSG treatments while in the brain these remained constant. Since Ca^{2+} plays a crucial role in catalase activity, levels of catalase were not affected in eye-brain axis. MDA levels were elevated at high MSG treatments and no structural changes in the retina of the eye and no damage were found in the brain.

Conclusion MSG maintained catalase levels, Ca^{2+} and no structural changes were observed in the eye and brain.

Introduction

Physiological function and relationships between the eye and the brain have led to the establishment of dominance in ocular function (Ooi & He, 2020). Sensory impulses are transmitted to the brain through the optic nerve, which is a white matter tract comprising of axons of retinal glial cells (Butt et al., 2004). In the brain, the occipital lobe which consists of Brodmann's area 17, 18, and 19, is responsible for the processing of visual information (Stanley & Elliott, 2008). Neuro-dysfunction has been associated with the accumulation of reactive oxygen species (ROS) which lead to lipid peroxidation of cellular membranes i.e. Malondialdehyde (MDA) levels (Gawel et al., 2004; Haider et al., 2011). Some studies have claimed ROS arise from food additives such as monosodium glutamate (MSG) however there is no consensus on the cause-effect of MSG in body tissues (Afolabi et al., 2020; "Corrigendum for 'A Review of the Alleged Health Hazards of Monosodium Glutamate,'" 2020; Rim, 2017; Sharma & Deshmukh, 2015b, 2015a; Zanfirescu et al., 2019). The method of administration and the used doses in most of these studies were not comparable with human MSG intake (Husarova & Ostatnikova, 2013).

High concentrations of calcium (Ca^{2+}) in the brain have been associated with an onset of oxidative stress and cell death (Fei et al., 2020), while in the eye, this has been associated with blindness-as is common in humans following prolonged use of artificial tears which contain high levels of Ca^{2+} (Bernauer, 2006). This is important since MSG has been associated with an increase in Ca^{2+} intake (Beyreuther et al., 2007; Kazmi et al., 2017), although information correlating MSG and Ca^{2+} levels

remains scarce. Furthermore, Ca²⁺ is a second messenger whose concentrations modulate the activity of catalase enzyme (Afiyanti & Chen, 2014). Catalase antioxidant activity helps protect tissues against ROS in disease conditions (MatÉs et al., 1999). In the brain, MSG has been associated with a decrease in catalase activity (Farombi & Onyema, 2006), although this varies with the route of administration.

MSG administration at high concentrations has been associated with toxicities such as atrial fibrillation (Burkhart, 2013), however, rats treated with MSG at 4.0 g/kg subcutaneous (s.c) produced no evidence of pain nor oxidative stress (Rosa et al., 2018), and this was contrary to previous findings of pain in masseter muscles with MSG at 150 mg/kg (Shimada et al., 2016). MSG feed as a feed supplement at 0.1, 0.2, 0.4 g/kg had an increase in MDA levels (Onaolapo et al., 2019) demonstrating interactions of MSG with biochemical parameters. Additionally, glutamate itself may affect neuronal control of bone mass, as monosodium glutamate-sensitive neurons can stimulate bone formation (Cowan et al., 2012). MSG administered at 5, 10, and 15 mg/kg of MSG respectively for 28 days in rodents was associated with no effects on biochemical parameters such as Alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, creatinine and urea (Kolawole, 2013). Evidence of 2% MSG toxicity to humans remains scarce on food intake, body weight, body mass, gastric evacuation, and organ mass, however, chronic MSG feed supplementation promotes glutamate synthesis in the body (Claire et al., 2011).

Policymakers have failed to find evidence relating to MSG toxicity as reported in many experimental studies (EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS) et al., 2017). In addition, neurotoxicity at 3.2 g/kg/day was absent and an acceptable daily intake of 30 mg/kg/day was established for human consumption. In lieu of the existing controversies and importance of MSG in the food industry, a need to re-evaluate the safety of MSG administered via parenteral and feed supplementation in the eye-brain axis created a rationale for the study. This was important to generate basic information that would guide policy on MSG consumption once findings are adopted by policymakers.

2 Methods

2.1 Study Design

This was an experimental study in which qualitative data was collected from Wistar rats, in which 30 adult, 7 weeks old male Wistar rats weighing between 110-130 g were used. The rats were randomly allocated to experimental groups using a random number generator in MS Excel while MSG used in the current study was donated by Ajinomoto Co. Inc, Japan through the Institute of Innovation. Rats were divided into 5 groups, each consisting of 6 rats. Group I rats were administered with MSG at 6 g/kg/day s/c. Group II and III rats received MSG at 5% and 2% in feed as a supplement, respectively. Group IV and V received MSG at 60 mg/kg/day s/c and normal saline (control), respectively. Rats were offered food and water ad libitum. The experiment was conducted for 30 days after which the rats were sacrificed.

2.2 Laboratory Analysis

Rats were euthanized using sodium pentobarbitone injected intraperitoneally (Close et al., 1997). The eye and brain were harvested from each rat and placed in sterile sample bottles. Samples were divided into duplicates for biochemical and histological analysis. Samples for biochemical analysis were subsequently homogenized in 50 mmol L⁻¹. Phosphate buffer (at pH = 7.0) was added at a ratio of 1 : 100 (v/v), and centrifuged at 3000 rpm for 5 min (Magalhães et al., 2016). The filtrate was collected into sterile eppendorf tubes which were stored in a refrigerator at -20°C for biochemical analysis. Samples for histological analysis were placed in 10% buffered formalin.

2.2.1 Determination of tissue calcium

Calcium concentrations in the experimental diets, and the tissues were determined using an atomic absorption spectroscopy (AAS) (Kopl, 2013). The AAS (Perkin-Elmer, model GBC932AA, USA) was set up according to manufacturer's recommendations and an equation from the standard curve (absorbance = 450 nm) was used to determine calcium concentrations from each sample.

2.2.2 Determination of catalase concentration in animal tissues

Catalase was determined as a major antioxidant according to methods by Johansson & Borg, (1988) for small animal samples. This was based on the principle that dichromate in acetic acid was reduced to chromic acetate when heated in the presence of hydrogen peroxide which formed per chromic acid as an unstable intermediate. The chromic acetate produced was measured calorimetrically at wavelength 570-610 nm. Since dichromate had no absorbance in this region the presence of the compound in the assay mixture did not interfere at all with the spectrometric determination of chromic acetate. The 0.2M hydrogen peroxide was diluted to different concentration of standards. Different concentrations of H₂O₂, ranging from 10 to 160 µM were pipetted in Eppendorf tubes (2.5ml) and 0.5 ml of dichromate/acetic (prepared by adding 5% solution of potassium dichromate (K₂Cr₂O₇) with glacial acetic acid (1:3) by volume) added to each. Addition of the reagent to H₂O₂ instantaneously produced an unstable blue precipitate of perchromic acid. On subsequent heating for 10 minutes in a boiling water bath, the color of the solution changed from yellow to stable green due to the formation of chromic acetate. After cooling at room temperature, the volume of solution was transferred into cuvettes and the optical density was then obtained at 580 nm using a spectrometer while using a blank solution. The catalase preparation was stopped at a particular time (1 minute) by addition of dichromate/acetic acid mixture and the remaining hydrogen peroxide determined by measuring chromic acetate calorimetrically after heating the reaction mixture. A standard curve at an absorbance of 580 nm i.e. absorbance = 0.0013 (concentration) + 0.0521, $R^2 = 0.9719$ which was in the form of $y = mx + c$ was obtained.

2.2.3 Determination of malondialdehyde in animal tissues

Determination of **malondialdehyde** (MDA) was done by the nonspecific Thiobarbituric acid reactive substances (TBARS) measurement as previously described (Kasozi et al., 2018). In particular the concentration was determined by using the equation;

Concentration = Absorbance/ (Molar extinction co-efficient x 1L) x 10⁶ nm where an extinction co-efficient of 156 was used.

2.2.4 Determination of histopathological changes in animal samples

Sections of smooth and skeletal muscle blocks of each rat were analyzed according to a systematic random embedding, random sectioning and sampling method as described in our previous study (Kasozi et al., 2018). Microscopic changes were assessed using a light microscopy and described descriptively.

2.3 Statistical analysis

Quantitative data was analyzed using Graph Pad Prism Version 6. Information was presented as mean ± SD and a Tukey's test was conducted to determine sources of variation at significance of 95%. Significant differences were reported when $P < 0.05$. Data from histological analysis was summarized and presented in paragraphs.

3. Results

3.1 MSG maintained levels of Ca²⁺ in eye and brain of male wistar rats

In the eye, monosodium glutamate (MSG) administered subcutaneous led to significantly ($P < 0.05$) higher Ca²⁺ levels in blood when compared to 5% MSG, while Ca²⁺ levels were highest in MSG administered at lower concentrations (Table 1) i.e. parenteral and feed supplement (Fig1 A). Furthermore, Ca²⁺ levels in the brain were not affected ($P > 0.05$) by the different concentrations and routes of administration used in the study (Fig2 B).

3.2 Catalase-Malondialdehyde status in the eye and brain under MSG

Catalase levels in the eye and brain were not affected under MSG administration (Fig2 A & B). Malondialdehyde (MDA) levels were highest in the eye at 6g/kg and 5% MSG ($P > 0.05$), and these were significantly different (Table 1) from the low MDA levels associated with low concentrations of MSG in the eye (Fig2 C & D). However, levels of MDA were higher in the brain than in the eye.

3.3 MSG doesn't change the structure in the eye and brain of male Wistar rats

No significant changes were seen in the retina and optic nerve of rats exposed to MSG in the study groups as shown below (Fig 3). In addition no significant changes in the Brain tissue of rats were seen following MSG exposure (Fig 4).

4. Discussion

The study showed that supplementation of foods with 5% MSG was associated with low ocular accumulation of Ca²⁺. These findings demonstrate the risk of MSG toxicities are lowest in feed

supplementation as compared to MSG administered parenterally. Since humans consume MSG as a feed supplement, findings in this study demonstrate the importance of evaluating toxicities in reflection of routes of administration used in humans (Husarova & Ostatnikova, 2013). These findings, though basic, provide an insight on the cellular changes in the eye since high Ca^{2+} levels in the eye increase the risk of blindness (Bernauer, 2006). In the brain, the study didn't find evidence of Ca^{2+} accumulation associated with MSG. Ca^{2+} homeostasis plays a crucial role in neurofunction (Zündorf & Reiser, 2011) demonstrating a low oxidative stress signature associated with MSG consumed at low concentrations in body tissues. This is important since excessive Ca^{2+} influx following glutamate receptor stimulation following MSG consumption would lead to accumulation of reactive oxygen species (ROS), which propagate oxidative stress and tissue damage (Hazzaa et al., 2020).

Catalase levels in the eye-brain axis were not affected by MSG concentrations used in the study. Since Ca^{2+} modulates the activity of catalase enzyme (Afiyanti & Chen, 2014), relatively stable levels of Ca^{2+} in the eye and brain stabilized the antioxidant marker in this study. Since catalase helps protect the eye and the brain against oxidative stress (MatÉs et al., 1999), findings in this study emphasize the need to consume MSG at concentrations recommended by policymakers on food additives i.e. the Joint European Committee on Food Additives (JECFA) and the European Food Safety Authority (EFSA) to ensure that toxicological monitoring studies can help influence policy in the right direction (EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS) et al., 2017). The study also showed that 6g/kg and 5% MSG administered s.c and as a food supplement had the potential to elevate lipid peroxidation in the eye and the brain. High concentrations above the JECFA and EFSA limits recommended for human consumption would lead to excessive accumulation of ROS. This subsequently would reduce the catalase levels in the brain and lead to neurodysfunction (Farombi & Onyema, 2006). Furthermore, the brain presented a higher risk to accumulate MDA than the eye demonstrating selective predisposition to oxidative stress in the eye-brain axis. Since the occipital lobe is charged with the processing of visual impulses in the brain (Butt et al., 2004; Ooi & He, 2020), findings in this study demonstrate a risk of ocular-neural damage once MSG is consumed at very high concentrations.

Concentrations of MSG used in this study didn't affect the retina nor the optic nerves in the eye. These findings are contrary to previous invitro embryonic study in which MSG damage in the retina was reported (Blanks et al., 1981), demonstrating key differences between invitro and invivo studies. Furthermore, a 3 month study following MSG administration in rodents was able to demonstrate retinal damage (Ohguro, 2002), however this translates into humans for 6.5 years (Sengupta, 2013) consuming MSG at concentrations at excessively high levels (10-20 g) not recommended by the JECFA and EFSA. In the brain, MSG affects neurons with little effect on glial cells at high concentrations (Xiong et al., 2009), however no significant changes were found in our study since concentrations used were relatively low and this was in agreement with previous study on MSG controversy in the brain (Xiong et al., 2009).

Conclusion

The study showed that consumption of MSG at concentrations below 5% as a feed supplement and administered at 6g/kg parenteral had no effects on Ca²⁺ levels, catalase while these concentrations increased MDA levels although not sufficient to cause structural changes in the eye and brain of male wistar rats. The study offers an updated perspective on the safety of MSG consumption at low concentrations promoted by International Committee on Food Additives.

Declarations

Authors' contributions

KIK conceptualized and designed the study. HN, HIN, KM, FS collected the data, LOO, RN, MAN, GESB conducted data interpretation. HN and KIK drafted the initial version of the manuscript, while all authors revised, approved the final version for publication and remain in agreement on all aspects of the work. The authors declare that all data were generated in-house and that no paper mill was used.

Availability of data and material

Data used in the study can be accessed at <https://figshare.com/s/22fcfd4929ff2d84df31>

Ethical approval

All authors contributed equally to the study.

This was acquired from the Kampala International University Western Campus Ethics and Scientific review board. Consent to participate was not applicable to this study.

Consent for publication

Not applicable.

Consent to participate

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Tables

Table 1. Adjusted P values on group comparisons on Eye and Brain Calcium, catalase and MDA levels in male wistar rats following administration of MSG.

Tukey's multiple comparisons test	Eye	Brain	Eye	Brain	Eye	Brain
	Calcium		Catalase		MDA	
6g/kg s.c. vs. 5% MSG in feed	0.0002	0.8100	0.9857	0.7666	0.5477	0.8496
6g/kg s.c. vs. 2% MSG in feed	0.0003	0.9988	0.8976	0.9989	0.0007	< 0.0001
6g/kg s.c. vs. 60 mg/kg/day MSG	< 0.0001	0.9482	0.9676	0.9471	0.0043	< 0.0001
6g/kg s.c. vs. Control	0.0013	0.9988	0.7100	> 0.9999	0.0043	< 0.0001
5% MSG in feed vs. 2% MSG in feed	< 0.0001	0.9065	0.9942	0.8709	0.0156	< 0.0001
5% MSG in feed vs. 60 mg/kg/day MSG	< 0.0001	0.3418	> 0.9999	0.2979	0.0695	< 0.0001
5% MSG in feed vs. Control	< 0.0001	0.9065	0.9279	0.7956	0.0695	< 0.0001
2% MSG in feed vs. 60 mg/kg/day MSG	0.6426	0.8348	0.9988	0.8353	0.9969	0.9689
2% MSG in feed vs. Control	0.9562	> 0.9999	0.9942	0.9998	0.9969	0.9694
60 mg/kg/day MSG vs. Control	0.2619	0.8348	0.9616	0.9021	> 0.9999	> 0.9999

Figures

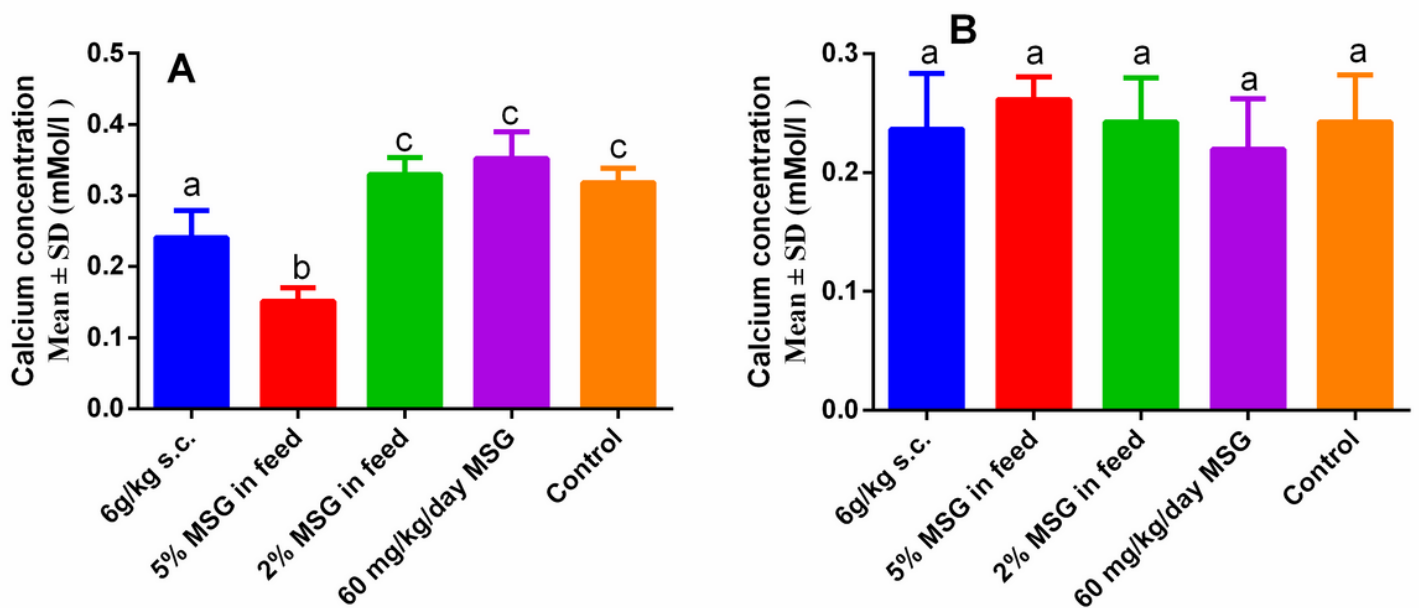


Figure 1

Calcium concentration variations in Wistar rats. A = Eye calcium levels, B = Brain calcium levels. Different superscripts = $P < 0.05$.

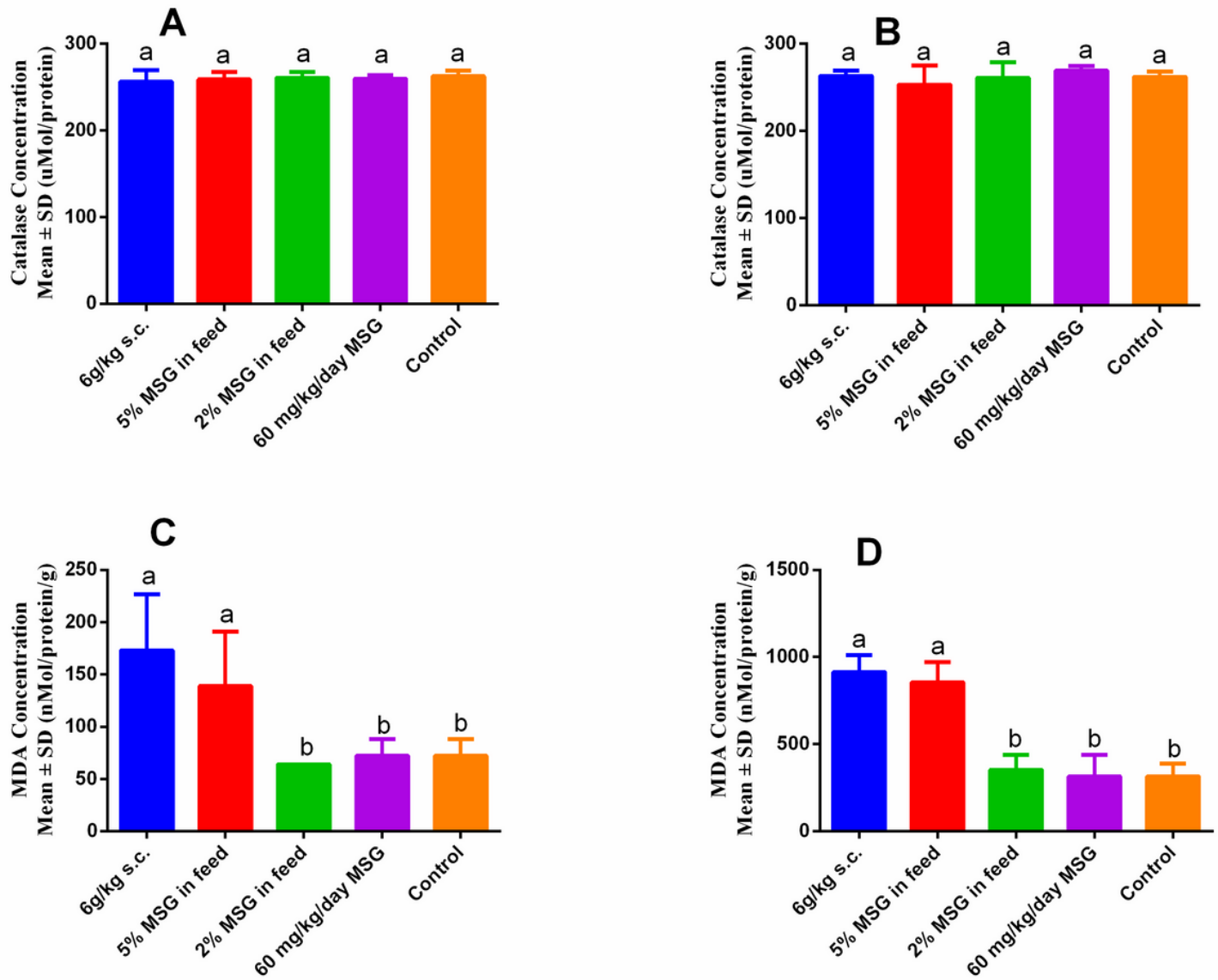


Figure 2

Antioxidant variations in Wistar rats. A = Eye catalase, B = Brain catalase, C = Eye MDA, D = Brain MDA, MDA = Malondialdehyde. Different superscripts = $P < 0.05$.

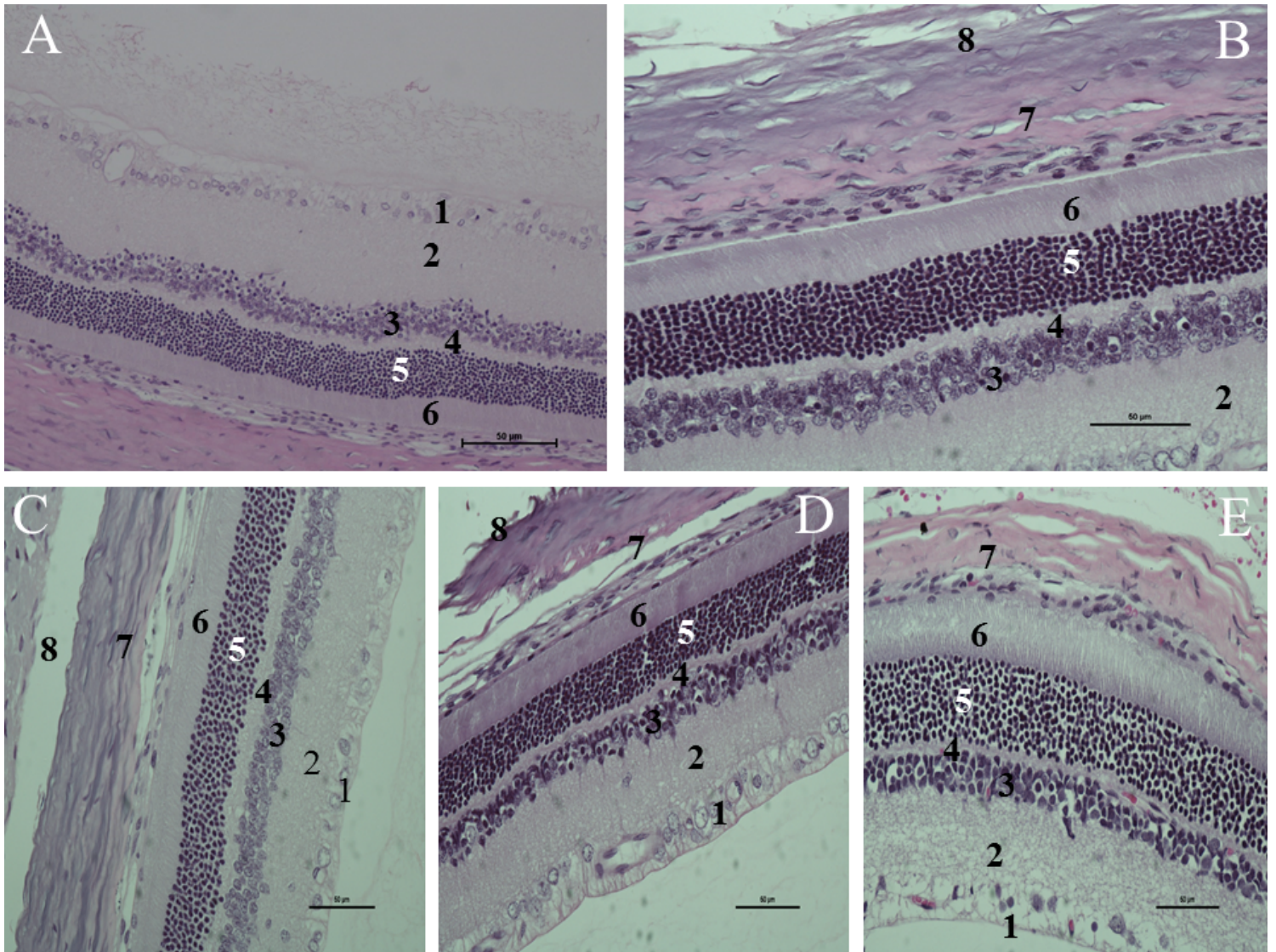


Figure 3

Changes in the Eye after MSG exposure in Rats. Group A = Rats treated with MSG at 6g/kg/day s.c; B = 5% MSG in feed; C = 2% MSG in feed; D = 60 mg/kg/day MSG s.c; E = No MSG. In the photomicrographs: 1 = Optic nerve fibers and ganglion cell layer; 2 = inner plexiform layer; 3 = inner nuclear layer; 4 = outer plexiform layer; 5 = Outer nuclear layer; 6 = Outer processes of rods and cones; 7 = choroid and 8 = Sclera.

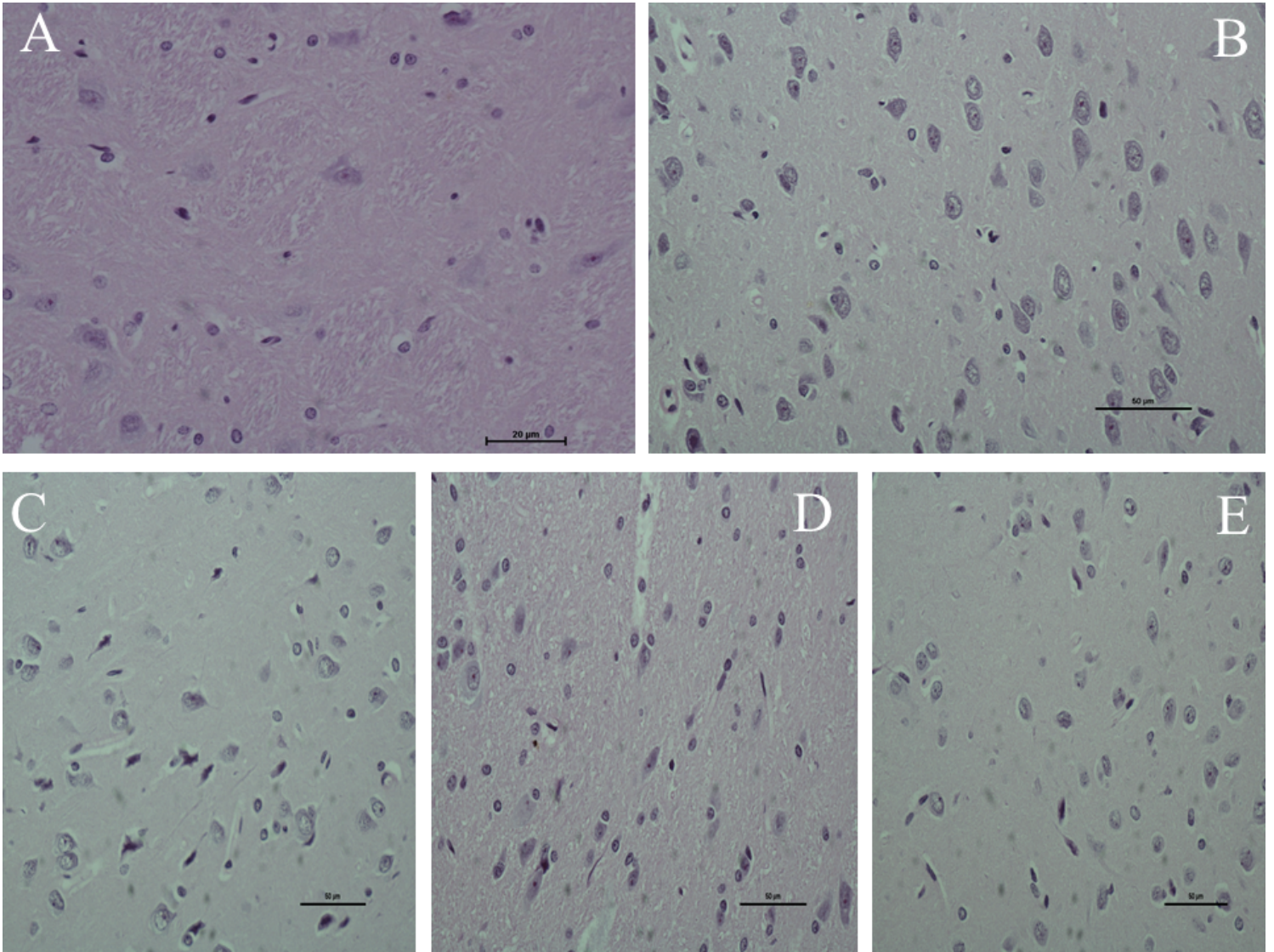


Figure 4

Brain changes after MSG exposure in Wistar Rats. Group A = Rats treated with MSG at 6g/kg/day s.c; B = 5% MSG in feed; C = 2% MSG in feed; D = 60 mg/kg/day MSG s.c; E = No MSG.