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Monosodyum Glutamatın Normal Sıçan Yaşlanmasında Korteks ve Hipokampüs Üzerine Etkilerinin Araştırılması

Investigation of the Effects of Monosodium Glutamate on Cortex and Hippocampus in Normal Aging Rats

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ÖZ

Amaç: Bu araştırma, Monosodyum Glutamat (MSG) uygulanan yaşlı sıçanların korteks ve hipokampüs dokularında dopamin taşıyıcısı (DAT), dopamin reseptörü 1 (D1) ve D2 ekspresyonlarını araştırmak amacıyla yapılmıştır.
Yöntem: Deney gruplarının beyin dokularında hipokampüs ve korteksteki nöronal dejenerasyon ve genişlemiş kan damarları histopatolojik analizle değerlendirildi. DAT, D1 ve D2'nin immünoreaktivitesi immünohistokimyasal analizle belirlendi.
Bulgular: Korteksteki nöronal dejenerasyon yaşlı sıçanlarda kontrol grubuna kıyasla anlamlı derecede daha yüksekti. Yaşlı sıçanların hipokampüsünde ve korteksinde DAT, D1 ve D2 reaktiviteleri kontrol grubuna kıyasla anlamlı derecede artmıştı.
Sonuç: Yaşlı sıçanlar ve kontrol grubu arasında hipokampüs ve korteksteki genişlemiş kan damarları açısından anlamlı bir fark bulunmadı. Çalışmamızda yaşlı sıçanların hipokampüs ve korteks dokularında DAT, D1 ve D2 ekspresyonlarının artması dopaminin önemini vurgulayabilir. Verilerimiz yaşa bağlı motor ve bilişsel işlevlerdeki bozulmaların anlaşılmasında dopaminerjik yolaklara katkı sağlayabilir.

Anahtar Kelimeler: Yaşlanan Beyin, D1, D2, Dopamin Taşıyıcısı, İmmünohistokimya.

ABSTRACT

Objective: This study aims to investigate the expressions of the dopamine transporter (DAT), dopamine receptor 1 (D1), and dopamine receptor 2 (D2) in the cortex and hippocampus tissues of monosodium glutamate (MSG)-administered aged rats.
Method: Neuronal degeneration and dilated blood vessels in the hippocampus and cortex of the experimental groups' brain tissues were evaluated by histopathological analysis. Immunoreactivity of DAT, D1, and D2 was determined by immunohistochemical analysis.

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Yazar Katkıları: A) Fikir/Kavram, B) Tasarım, C) Veri Toplama ve/veya İşleme, D) Analiz ve/veya Yorum, E) Literatür Taraması, F) Makale Yazımı, G) Eleştirel İnceleme

Results: Neuronal degeneration in the cortex was significantly higher in aged rats than in the control group. DAT, D1, and D2 reactivities in the hippocampus and cortex of aged rats were significantly increased compared to those in the control group.
Conclusion: No significant difference was found between the aged rats and the control group in terms of dilated blood vessels in the hippocampus and cortex. The increased DAT, D1, and D2 expressions in the hippocampus and cortex tissues of aged rats in our study may highlight the importance of dopamine. Our data may contribute to understanding age-related impairments in motor and cognitive functions through dopaminergic pathways.

***Key words**:* Aging Brain, D1, D2, Dopamine Transporter, Immunohistochemistry.

1. INTRODUCTION

Aging is a physiological process that results in a progressive loss of function characterized by molecular and anatomical changes (1). As with every tissue, significant changes are seen in the brain. Changes in the metabolism of the neurons in the aging brain can result in decreased numbers and synapses in areas such as neurons cerebral and cerebellar cortex, hippocampus, locus coeruleus, and substantia nigra. At the same time, the reduction in neurogenesis in the aging brain can lead to a reduction in tissue integrity, function, and regenerative response (2). As a result of this drop, it is known that cognitive processes such as processing speed, processing memory, and long-term memory are beginning to be disrupted (3). Cognitive changes that are failing can affect the daily function and quality of life of an elderly adult (4). Therefore, a better understanding of the basic mechanisms of aging may result in minimizing functional loss. In this respect, it is important to understand the changes in the aging brain at the molecular level.

Dopamine is one of the main neurotransmitters. It plays a role in providing motor coordination and cognitive functions. Dopamine shows its effects through five receptors divided into 2 families, such as D1 (D1 and D5) and D2 (D2, D3, and D4) dopamine receptors. All dopamine receptors are extensively expansive in the central nervous system (CNS) (5). The dopamine receptors associated with protein G (D1, D2, D3, D4, and D5), the catecholaminergic neurotransmitter dopamine, mediate all physiological functions such as voluntary movement, reward mechanisms, and neurohormonal regulation (6). The functions that the receptors are intermediating may vary by subunit. With receptor D1, memory, attention, impulse control, and voluntary movements are mainly performed. The D2 receptor plays a role in the planning of learning, attention, sleep, memory, and voluntary movements (7). These cognitive functions are known to begin to be lost in old age (8). In line with this information, the dopaminergic activity in aging has been highlighted in recent years, when it may have been degraded.

With dopaminergic activity, brain neuronal systems have a long-known role in the regulation of motor functions. New evidence suggests that dopamine plays a critical role in high-grade cognitive functions such as memory, learning, and the reward system (9). The decrease in cognitive function and the reduction in motor functions due to dopaminergic activity in old age support the view that age-dependent changes depend on dopamine loss (10). As well as reduced motor functions, age-dependent cognitive and emotional disorders reflect dopaminergic disorder (11).

In the study by Erixon-Lindroth et al. (2005), it was shown that there are net age-related losses in striatal dopamine transporter (DAT) binding. In the data, it has been shown that there is a significant deterioration in episodic memory (word and shape recall, face recognition) and executive functions (visual working memory, verbal fluency) with advancing age. It has been reported that these age-related cognitive deficits are mediated by reductions in DAT binding

(12). In addition, it can be said that changes in emotion, interest, and behavior with age may be due to dopaminergic activity. It is important to investigate these changes molecularly and to evaluate them in terms of different molecular exposures in order of gaining new perspectives.

Glutamate, the anion of glutamic acid, is the most abundant amino acid in the brain and functions as the main excitatory neurotransmitter in the central nervous system (CNS) (13). It is involved in cognitive functions such as learning and memory (14). In addition to its physiological functions, Glutamate, the sodium salt of glutamic acid, "Monosodium Glutamate" (MSG), is frequently used as a flavor enhancer in the food industry (15). However, studies conducted in recent years show that exposure to MSG may cause problems in many physiological functions, especially in the CNS (16). In addition, it has been reported that monosodium L-glutamate administration can induce dopamine uptake and release from the caudate nucleus (17). The data show that MSG exposure may cause changes in the CNS by affecting dopaminergic activity at the synaptic level. However, the role of the relationship between MSG exposure and the dopaminergic system in the aging process has not been investigated yet.

In summary, dopaminergic function and structural changes that occur in the brain with aging lead to a decrease in motor and cognitive skills. However, the relationship of this change with MSG has not been investigated molecularly. Accordingly, in this study, we investigated the effects of MSG administration on D1,D2, and DAT expressions in the hippocampus and cortex of aged rats.

2. MATERIALS AND METHODS

Animals

In the study conducted by Tunca et al. (2019), 15-16-month- aged rats were used (18). Twelve aged male Wistar albino rats of a minimum of 15 months old and 550 to 650 g body weight were used in this study. Rats were purchased from the laboratory animal house of [Blinded] University. They were acclimatized for 10 days before the beginning of the experiment (19). All rats were fed standard rat diet, allowed to drink water “ad libitum”, and kept under normal daylight/dark cycle and room temperature during the study.

Experimental Design

Rats were weighed and assigned to two experimental groups. It is planned to have 6 rats in each group. The application of MSG took 28 days. The 3R (Replacement, Reduction, and Refinement) has become a guiding principle for the ethical use of animals in research (20). MSG is used to induce oxidative damage to the brain tissue of rats after long-term consumption. All administrations were made by oral gavage with saline, MSG, and they were repeated for 28 days of the experiments. The MSG group was given a solution of 120 mg/kg for 28 days by the gavage method (21). In this study, the housing, handling conditions of the animals, MSG dosage and method of application were consistent with those previously described and aligned with the existing literature.

The research protocol was approved by the ethics committee of the Hatay Mustafa Kemal University Faculty of Veterinary Medicine (IRB Approval Number: 143582, Decision No: 2022/02-04), and it was carried out according to the Declaration of Helsinki’s guidelines.

MSG was obtained from Sigma Company, Germany.

Control group: Distilled water by the gavage method for 28 days

MSG group: 120 mg/kg/day MSG was administered by gavage (21).

Histological Analysis

Brain tissues removed from the experimental groups were fixed with 10% formaldehyde solution and then washed in tap water (1 night), and then water recovery was performed by keeping the tissues in increasing alcohol series (70%, 80%, 90%, and 100%). Tissues made transparent by soaking in xylol were embedded in paraffin in the appropriate orientation. 5 µm-thick sections were taken from brain tissue samples embedded in paraffin using a microtome. It was stained with Harris Hematoxylin and Eosin and examined histomorphologically under a light microscope (Olympus BX53). Neuron degeneration and dilated blood vessels in the hippocampus and prefrontal cortex were evaluated in the brain tissues examined in the frontal section plane of the experimental groups. The pyknotic nucleus, perineural space enlargement, and cell swelling or shrinkage were considered as neuron degeneration (22). Histopathological results in each category were scored as follows: 0 = absent, 1 = mild, 2 = moderate, and 3 = severe. A minimum of 20 slices per rat for 6 rats per experimental group were scored, and blind observers of the study groups performed all histological evaluations.

Immunohistochemical Analysis

Anti-D1 Dopamine receptor (ADR001, Alomone labs), anti-D2 Dopamine receptor (ADR-002, Alomone labs), and anti-Dopamine transporter (DAT) (AMT-003, Alomone labs) primary antibodies were used at a dilution ratio of 1:100, and their immunoreactivity was determined. The biotinylated goat anti-polyvalent secondary antibody (Thermo Fisher Scientific, UK, TP-125-BN) was used. Since it is a ready-made solution, there is no dilution ratio (23). In summary, after deparaffinization of sections on slides coated with 5 µm-thick poly-L-lysine, citrate buffer was used to open epitopes (pH: 6.0; Thermo Fischer Scientific, UK, AP-9003-500). The slides were then placed in a 3% hydrogen peroxide solution in methanol to inhibit endogenous peroxidase activity. Ultra V block solution (Thermo Fischer Scientific, UK, TA-125-UB) was applied to prevent non-specific staining. Then, the sections were incubated with the primary antibody to be examined at 4 oC overnight. Then, biotinylated goat anti-polyvalent secondary antibody (Thermo Fisher Scientific, UK, TP-125-BN) was incubated for 40 minutes in a 37 oC incubator. After washing several times with PBS, it was incubated with streptavidin peroxidase (Thermo Fisher Scientific, UK, TS-125-HR) for 30 minutes in a 37 oC incubator. The antibody complex was visualized by incubation with diaminobenzidine (DAB) chromogen (Thermo Fisher Scientific, UK, TA-125-HD). Sections were then counterstained with Gill III Hematoxylin (Merck, Germany, 1.05174.1000). It was dehydrated by passing through a series of increasing alcohol concentrations and covered with a sealant called Entellan. Sections were examined with an Olympus BX53 light microscope. TIFF images were imported into Image J Version 1.46 (National Institutes of Health, Bethesda, Maryland) software to quantify the immunohistochemical staining intensity for each protein. The mean intensity of the background was obtained by averaging the values of the negative control images treated with secondary antibody alone (24). The immuno staining intensity level

value was calculated by dividing the average signal intensity above the background for a minimum of 20 images per rat for 6 rats per experimental group.

Statistical Analysis

For histopathological and immunohistochemical analysis, an independent sample t-test was applied. Statistical analysis of the results was performed using GraphPad Prism 9 software, and the obtained results are presented as mean ± standard deviation (SD). $P<0.05$ was considered statistically significant.

3. RESULTS

Histological Findings

As shown in Figure 1 and Table 1, no significant difference was found between the groups in terms of dilated blood vessels in the hippocampus and cortex tissues. It was observed that neuron degeneration in cortical areas was higher in the MSG group (*$p<0.05$). Degenerating neurons are indicated with arrows in Figure 1.

Table 1. Damage Rates in Hippocampus and Cortex Tissues Across Experimental Groups.

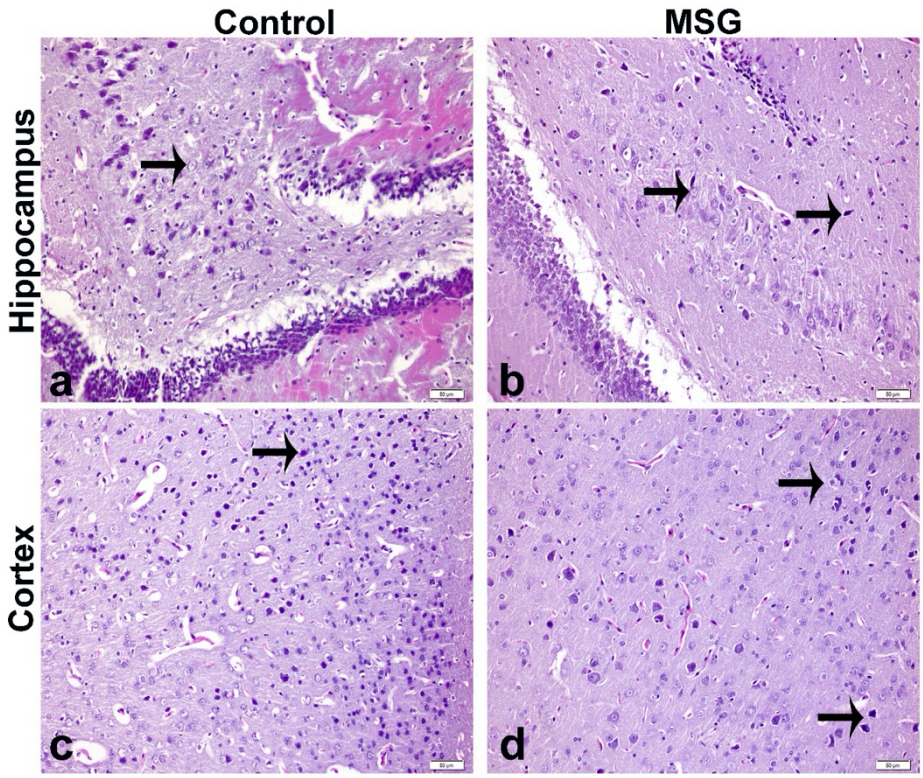
Groups	Hippocampus		Cortex	
	Dilated blood vessel	Neuron degeneration	Dilated blood vessel	Neuron degeneration
Control	1.66±0.81	0.83±0.75	0.66±0.81	0.83±0.75
MSG	1.83±0.75	1.83±0.75*	1.33±0.51	2.16±0.4*

Data shown in the table are expressed as mean±SD. An independent sample t-test was applied. In the hippocampus, *p=0,0442 and in the cortex, *p=0.0034 represent statistically different from the control group.

Immunohistochemical Findings

According to the findings, D1, D2, and DAT immunostaining intensity in the hippocampus and cortex areas of the MSG group was significantly increased compared to the control group (Figures 2, 3, and 4; *$p<0.05$). The entire area of the prefrontal cortex was scanned, and the cells stained with the Image J software program were measured. The Image J software evaluates the cells stained in the entire area in the sections from all groups. Intensity indicates the average percentage of the total area that is stained. Photographs that were consistent with the data obtained as a result of statistical analyses were placed on the panel. Similarly, CA1, CA2, and CA4 parts of the hippocampus were scanned and photographed, and cells stained with the Image J software program were measured to obtain quantitative data. Photographs that were consistent with the results of statistical analyses were placed on the panel.

Figure 1.



Hematoxylin-eosin staining images of the hippocampus and cortex in the brain tissues of the experimental groups. a: Hippocampus of the control group, b: Hippocampus belonging to the MSG group, c: The cortex of the control group, and d: The cortex belonging to the MSG group. Magnification 20x, scale bar: 50µm.

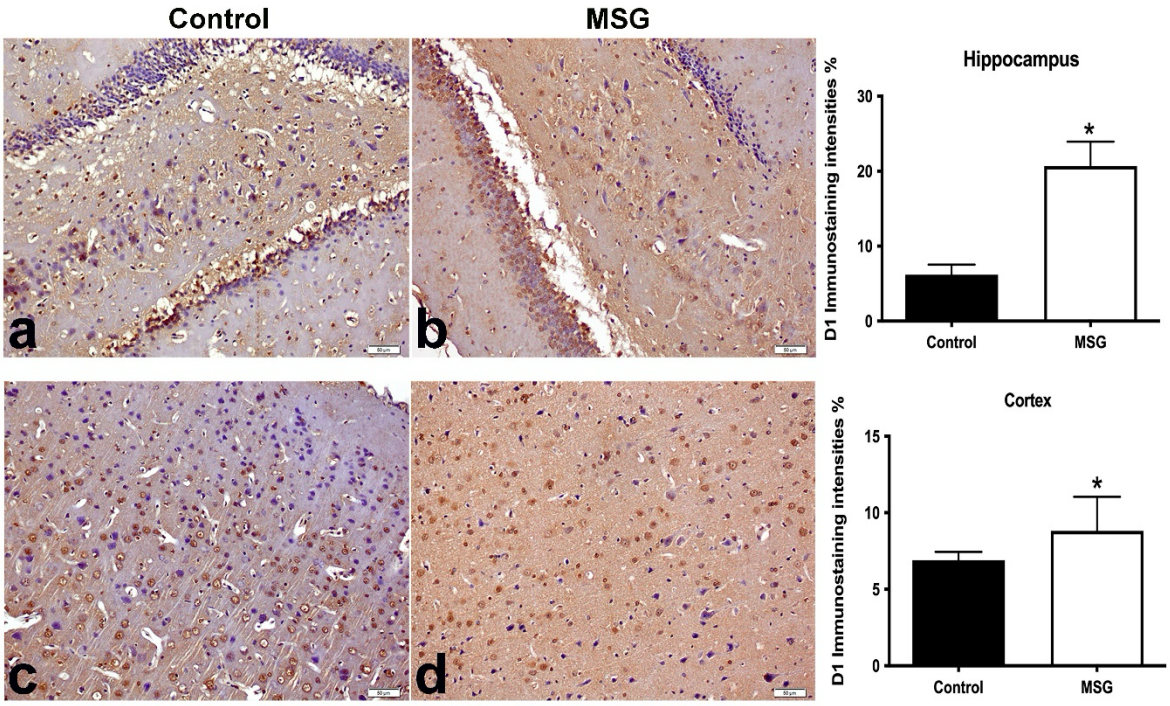


Figure 2. The pictures come from the hippocampus and the cortex. Anti-D1 immunostaining images in the brain tissues of experimental groups. Brown stained areas were considered immunoreactive areas. An independent sample t-test was applied. Data shown in histogram bars are expressed as mean±SD. In the hippocampus, *p<0.0001 and in the cortex, *p=0.0490 represents a statistically significant difference. Magnification 20X, scale bar: 50µm.

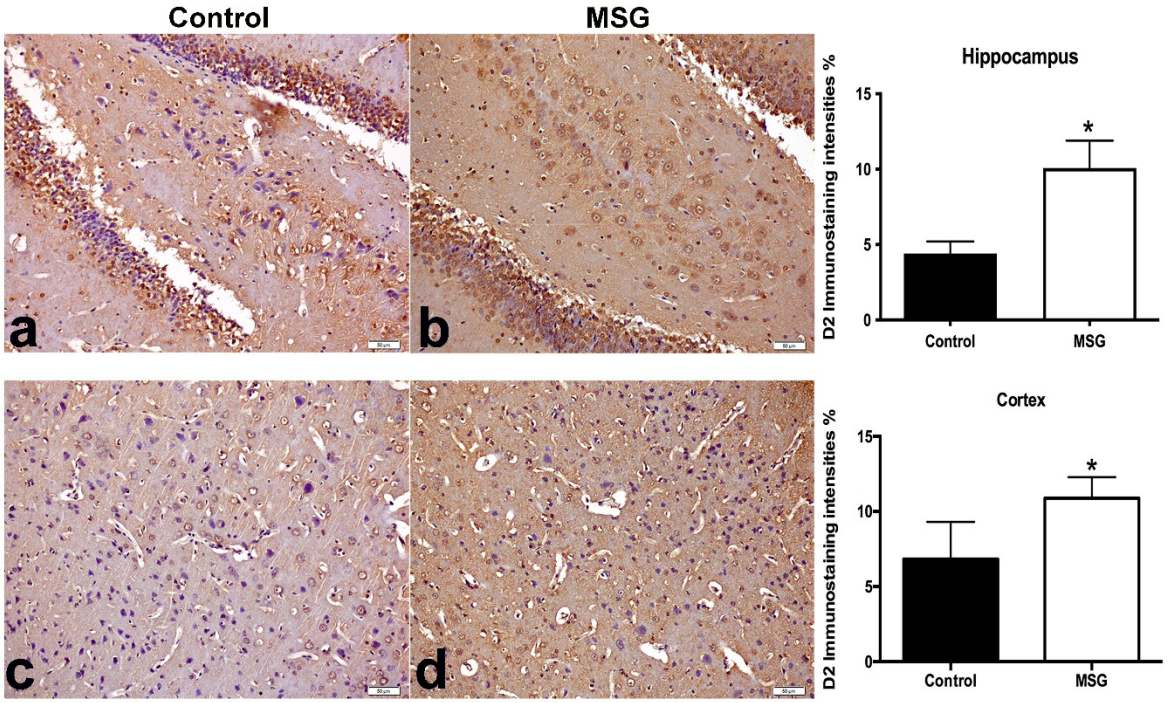


Figure 3. The pictures come from the hippocampus and the cortex. Anti-D2 immunostaining images in the brain tissues of experimental groups. Brown stained areas were considered immunoreactive areas. An independent sample t-test was applied. Data shown in histogram bars are expressed as mean ± SD. In the hippocampus, *p<0.0004 and in the cortex, *p=0,0157 represent a statistically significant difference. Magnification 20X, scale bar: 50µm.

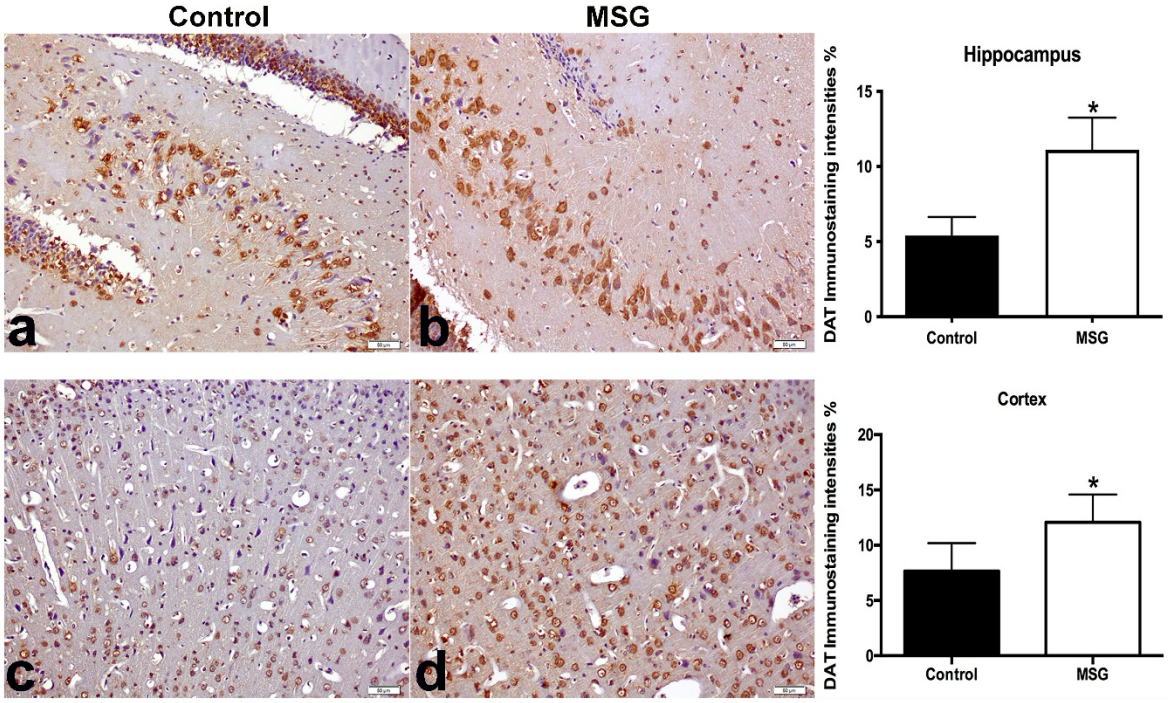


Figure 4. The pictures come from the hippocampus and the cortex. Anti-DAT immunostaining images in the brain tissues of experimental groups. Brown stained areas were considered immunoreactive areas. An independent sample t-test was applied. Data shown in histogram bars are expressed as mean ± SD. In the hippocampus, *p<0.0095 and in the cortex, *p=0,0146 represent a statistically significant difference. Magnification 20X, scale bar: 50µm.

4. DISCUSSION

In the data we obtained from the study, we observed a significant increase in D1, D2, and DAT expression in both cortex and hippocampus tissues in the MSG group. In the histopathological examination, there was no significant difference related to dilated vasculature in the hippocampus and cortex, but we detected significant neuronal degeneration in the cortical tissue. These data provide new molecular insights by emphasizing that exposure to MSG leads to changes in dopaminergic activity in the aging brain.

Wallace and Dawson (1990) investigated the effects of MSG administration, which is known to have neurotoxic effects, on monoamines in rats. In the data obtained, it has been shown that the administration of MSG caused a decrease in dopamine content in the posterior cortex of the brain and an increase in the striatum tissue in adult rats. However, it has been reported that dopamine is decreased both in the posterior cortex and in the striatum in aged rats (25). These data show that MSG administration leads to a decrease in dopamine levels in advanced age, changing with age. Our data, on the other hand, show that D1, D2, and DAT expressions in cortex and hippocampus tissues increase as a result of MSG exposure in aged rats.

In the study of López-Pérez et al. (2005), it was reported that MSG administration caused a decrease in D1, D2, and DAT markers in the striatum of neonatal male rats (26). In addition, in another study, it was determined that dopamine levels in the median eminence in the neurohypophysis of female rats tended to decrease as a result of MSG administration (27). Oxidative stress serves as both a key underlying mechanism in neuropathy development and a significant biological driver of the overall aging process(28). These findings show that MSG exposure during early development causes a decrease in dopaminergic markers. Our data suggest that exposure to MSG leads to an increase in dopaminergic markers in aged brain tissue. In this direction, further studies should be conducted in animals in a wide age spectrum and in groups where behavioral/cognitive changes are also examined in order to fully understand the effects of MSG.

When the amounts of pretranslational and posttranslational DAT were investigated, it was reported that the pretranslational DAT form accumulated in the Golgi body, while the posttranslational DAT decreased in the striatal terminals. In addition, DAT protein levels and mRNA levels were found to decrease in aging rats (29). These findings indicate that compensatory mechanisms are activated in striatal neurons in response to dopaminergic activity that decreases with ageing. Data from Salvatore et al. (2003) emphasized that age-related decreases in striatal DAT function and ligand binding are associated with decreased plasma membrane expression of DAT. In addition, it has been shown that there is no loss of steady-state levels of the DAT protein or any component of dopaminergic neuronal networks (30). In addition to the data obtained from experimental animals and rodents in the literature, DAT-related studies have been carried out in clinical studies in recent years. Li et al. (2020) investigated the relationship between age-related striatal DAT changes and cognitive function. In the data they obtained, it was shown that the changes in striatal DAT functioning that change with age are related to verbal functions, and especially the caudate nucleus plays an important role in this relationship (31). In another study, the relationship between age-related decline in DAT binding and motor function in the elderly was investigated by PET scanning. It has been

reported that the findings obtained may have a protective effect against possible loss of motor function of DAT expression, which decreases with aging (32). These data suggest that striatal DAT changes may affect motor and cognitive abilities with age. In the data we obtained, we observed that MSG exposure with age led to increased DAT expression in the cortex and hippocampus tissue in rats. These data may be related to declining cognitive abilities and changing behavioral patterns with aging. In a study conducted in 2021, a selective DAT inhibitor agent was produced. It has been reported that a highly specific DAT inhibitor (S, S)-CE-158, which increases dopamine levels in cognition-related brain regions, restores cognitive activities at the synaptic level in aging rats (33). Similar to our data, molecular investigation of changes in specific regions of the brain within the scope of MSG may provide new perspectives in terms of developing new therapeutic approaches that can preserve cognitive and motor abilities.

In a study conducted in 2020, it was shown that the coordination between the presence of D1 receptors and hemodynamic signalling in cortical areas known to be associated with face recognition may be impaired in the elderly and may be associated with impaired facial recognition ability (34). These data suggest that D1 may be associated with deterioration in cognitive and memory functions in old age. In another study on humans using PET imaging and ligand, age-related loss of D1 receptors in striatal, mesolimbic, and cortical areas was shown with age. It has also been shown that striatal attachment potentials are weakened between limbic and neocortical regions (35). In the study of MacDonald et al. (2012), decreased D1 binding was found in the anterior cingulate gyrus, dorsolateral prefrontal cortex, and parietal cortex with increasing age. It has been reported that decreased D1 binding in these regions with aging is associated with receptor loss and causes cognitive changes (36). These data from clinical and imaging studies show that D1 receptor changes that occur with age lead to cognitive and behavioural changes. Keeler et al. (2016) reported that D1 receptor expression levels in the striatum and spinal cord of mice increase with age (37). In our data, D1 expression in aged rats increased significantly in the hippocampus and cortex tissue compared to the control group of aged rats after MSG administration. Considering the data in the literature, this change coincides with the cognitive abilities and behavioral patterns that are affected in old age. Accordingly, more detailed molecular investigations of the possible role of MSG in altered cognitive and behavioral abilities may be beneficial.

In the study of Sakata et al. (1992), it was shown that while the level of D2 receptor mRNA in the striatal region did not change with age in rats, the protein levels in the striatal membranes decreased. In line with these data, it has been reported that this change can be achieved by posttranscriptional mechanisms (38). Considering these data, studies need to be molecularly expanded to update the conflicting results in the literature. In the study by Saroja et al. (2014), age-related changes in the levels of a monoamine receptor complex, including D1 and D2 receptors, were investigated. In their data, it was reported that age-related reduction of co-localized complexes of D1 and D2 receptors led to spatial memory-related changes in mice (39). Another study showed that genotypes associated with high levels of striatal synaptic DAT and extrastriatal D2 receptors are associated with memory processing and retrieval-associated episodic memory. It has been reported that these phenotypic functions may be affected by the decreasing gene-gene relationship with age (40). These data show that age-related changes in dopaminergic activity associated with DAT, D2, and D1 receptors, especially D2 receptors,

may be associated with deterioration in cognitive functions such as memory and memory processing. In addition, the effect of chronic MSG exposure, depending on dietary habits, on dopaminergic activity, which changes with advancing age, should not be ignored.

In our study, we investigated the expressions of DAT, D1, and D2 receptors in the cortex and hippocampus tissues of MSG-treated aged rats by immunohistochemistry. In our findings, DAT, D1, and D2 expressions were significantly increased in the hippocampus and cortex in the MSG administered group compared to the rats in the control group. It may be beneficial to include young and adult rats in the evaluation in addition to aged rats in order to obtain more comprehensive data and to clarify the conflicting results in the literature. The fact that the data we obtained does not include mRNA levels and protein levels can be considered as one of the limitations of the study. In addition, another limitation is that the changes in expression of dopaminergic components in the data we obtained were not supported by changes in motor and cognitive abilities in animals. Discussion of molecular changes in specific regions of the brain by evaluating motor movement and cognitive tests in further studies may contribute to the literature in order to emphasize the phenotypic changes that will occur.

5. CONCLUSION

As a result, it is known that deteriorations in motor functions and cognitive abilities occur as a result of aging. In recent years, clinical and imaging studies have emphasized that these deteriorations occur due to the change in dopaminergic activity. In addition, the data in the literature suggest that dopaminergic activity, which is affected in the aging process, may be closely related to MSG exposure. However, the molecular mechanism has not yet been fully elucidated. Our data show increased expression of DAT, D1, and D2 receptors in the hippocampus and cortex tissues of aged rats treated with MSG. Our study provides information that can form a molecular basis for aging and the deterioration of MSG exposure. In future studies, molecular investigation of the change in dopaminergic activity in specific regions of the brain and comprehensive evaluation of animals from different age groups may contribute to our understanding of the basic mechanisms underlying the effects of aging and MSG exposure on the dopaminergic system. Nevertheless, the research has a limitation. The lack of behavioral tests assessing dopaminergic system effects in this study is a limitation; future studies should include neurobehavioral assessments such as the Morris Water Maze and Y-maze.

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Author Contrıbutıons

AO, GB, ZD, TK, AKU, EA, SO designed the experiments and synthesized the applied substance. GB, TK and SO performed the experiments. AO, ZD, AKU and EA wrote the article.

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Availability of data and materials

Not applicable.

Competing interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Consent for publication

Not applicable.

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