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Investigation of the behavioral and neurochemical effects of monosodium glutamate on neonatal rats

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Background/aim: The objective of this study was to investigate and analyze the behavioral and neurochemical effects of monosodium glutamate (MSG) injections at various and subsequent dosages on male Wistar rats during the neonatal period.

Materials and methods: In order to determine the behavioral and neurochemical effects of MSG, the experiment was implemented on neonatal male Wistar rats and the test was repeated for various MSG dosages. After completing the experiment, additionally, levels of dopamine, GABA, catecholamine (dopamine, noradrenaline, and adrenaline) and glutamate in the brain cells of the decapitated rats were also measured using the ELISA method.

Results: Considering the results of the behavioral test, when we compared the test values of the control group with the values of the MSG-injected groups we noted that there were significant differences in the statistical figures obtained. Additionally, we found that the statistical figures of some neurochemical parameters were also significantly different when we compared the values of the MSG group with the control values.

Conclusion: MSG injection has a clear effect on the neurochemical parameters, learning memory, and locomotor activities of rats.

Key words: Monosodium glutamate, behavioral analysis, neurochemical analysis

1. Introduction

Glutamate is one of the excitatory amino acids that already exist in the brain's own structure. It serves as a medium for the transmission of the fast synaptic in the whole central nervous system (CNS) (1). Monosodium glutamate (MSG) increases the savor of foods and stimulates the nerves, and so it causes more and more frequent food cravings. The main reason for the use of such an additive is that MSG has a better and more rapid dissolution performance compared to glutamic acid (2,3). Regardless of the dietary sources, all of the glutamate molecules entering the circulation through the gastrointestinal tract are structurally identical (4). A large portion of the glutamate accessing the human body is absorbed through the intestinal lumen (5). It is in the CNS that the presence of a glutamate signaling system was first revealed (6). It is detected that the system has a function in nonneuronal tissues affected by the CNS, such as bone, liver, pancreas, and skin (6,7). Glutamate is considered to be the major excitatory neurotransmitter in the mammalian CNS, where it has a role of mediator during the creation of sensory and cognitive formations such as the usage of sensory information, maintenance of the motor coordination, and retrieval of the memory (8). In the case of a glutamate overdose in mature animals, even if blood–brain barrier penetration prevention is achieved, neuronal cell deaths are induced (9). When MSG is applied to animals during the neonatal period, pyramidal cells in the hippocampal CA1 suffer more histological damage compared to the other regions of the brain comprising the cerebral cortex (9).

The neurotoxin effect of MSG appears as brain cell damage, retinal degeneration, and endocrinal diseases and in some pathological cases such as addiction, stroke, epilepsy, brain trauma, neuropathic pain, schizophrenia, anxiety, depression, Parkinson disease, Alzheimer disease, Huntington disease, and amyotrophic lateral sclerosis (10). Experimental studies related to that subject were first initiated after the determination of neuronal death (necrosis) at the brain's arcuate nucleus hypothalamus, due to orally applied MSG in newborn mice by Olney in 1970 and by Hu et al. in 1998 (11,12). In 1957, in their pioneering studies, Lucas and Newhouse pointed out

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the loss of neuronal cells in the retina and Olney in the hypothalamic arcuate nucleus. They discovered that a high amount of MSG, when implemented in a repetitive and systemic manner, results in neuronal death through a mechanism called excitotoxicity, which is depending on the intracellular Ca^{++} increase triggered by the Glu receptors' (GluR) overactivation (11,13).

Gill (14) and Hughes et al. (15) have concluded that MSG played a key role resulting in the death of neuronal cells. Similar outcomes were obtained by Reistad et al. (16), who have declared that glutamate is the root cause of the death of cerebellar granule cells in the primary cellular culture environment. Additionally, Rogers et al. (5) have proven that MSG was the main cause of apoptosis, necrosis, and learning and memory impairment and they have determined that MSG caused selective neurodegeneration.

The objective of the present study was to investigate the presence of a correlation between injections of MSG and the behaviors and neurochemical parameters of Wistar rats subjected to those injections during the neonatal period.

2. Materials and methods

2.1. Examined animal groups

Six neonatal male Wistar rats were selected as the control group. After completing their behavioral tests, the rats were subjected to injections in the form of intraperitoneal, normal saline, scheduled and implemented as 1 injection per day and 1 day free after each injection, 8 times in total. Eighteen neonatal male Wistar rats were subjected to injections of MSG ($C_5H_8NNaO_4.H_2O$, ≥98.0% (NT), MV: 187.13 g/mol, Sigma, USA) after the completion of their behavioral tests. Injections were scheduled and implemented as 1 injection per day and 1 day free after each, 8 times in total. Injected MSG dosages were as follows:

Group 1: 50 mg/kg per day, 6 rats
Group 2: 100 mg/kg per day, 6 rats
Group 3: 200 mg/kg per day, 6 rats

2.2. Behavioral analysis

Eight-arm radial maze test specifications: In order to test the learning and memory functions of the rats we selected an 8-arm radial maze test setup. Each of the arms has a length of 50 cm and width of 10 cm. Each arm is covered with a housing made of glass. In the middle of the maze setup a central zone is built in order to allow access to other arms of the maze. Animals are unleashed in that central zone and then they are subject to the test. The 8-arm radial maze test setup stands on 3 pillars and its height is 80 cm from the floor. The walls of the room where the maze setup is located are completely painted white. Colored boards are placed around the maze in order to help the animals to find their way in the maze. The test is conducted in a fully silent ambient. The maze is illuminated from top to downward using 3 electrical light sources. Food rewards are placed at the distal end of each arm. During the test, rewards are not refreshed after they are consumed by the animals and at the end of the test each arm is cleaned with alcohol. As we know that learning in the maze is related to starving, food restriction is applied prior to the start of the test until the animals reach 85% of their normal weight, in order to increase their motivation to seek food. The test is scheduled for each animal as 3 trials per day over 14 days.

Training for the 8-arm radial maze test: Prior to the start of the test, the animals are trained in the maze without any MSG injection. The training program includes a warm-up period of 3 days and a training period of 14 days subsequently. The duration of each training session is 10 min. A session is terminated if an animal completes its visit to all of the 8 arms of the maze before the end of the projected 10 min duration. Following the training sessions, starting on days 1, 7, 14, 21, and 28 of MSG injections, the 8-arm radial maze test is performed with the animals.

Application of the 8-arm radial maze test: After the 3-day warm-up period and training period of 14 days, MSG injections of each group are completed. MSG injections consist of 8 dosages in total, each implemented as one dosage in the following order subsequently (day 0, day 1, day 7, day 14, day 21, and day 28). The weight of each animal is measured before injecting the related MSG dosage. After that, similar to the training period, the 8-arm radial maze test is implemented 3 days a day over the next 14 days. Considering the results of the 8-arm radial maze test, calculations are made accordingly in 2 different manners:

Number of correct accesses: Refers to the number of times access to the correct arm was achieved by the animal.

Response latencies: Total time spent in the maze/ Number of arms accessed.

- Definition of the total time spent in the maze:

a) Total time spent by the animal if it has completed all of the 8 accesses in less than 10 min.

b) If the animal did not access all of the 8 arms within 10 min, then the test is terminated. In this case total time spent is taken as 10 min (600 s).

Open field test procedure: The open field test setup consists of a square field with dimensions of 45 × 45 cm and it is illuminated by 3 fluorescent bulbs. The top of the field is open to the ambient and the square field is bordered by white stripes each of 1 cm width on a black background. Each of the experimental animals are brought to the center of the open field setup and released to move freely. Duration of the test is determined as 3 min, during which the rats are observed closely and their behaviors are recorded as line crossing, rearing, and grooming.

2.3. Neurochemical analysis

Following the MSG injections, brain tissues of the decapitated rats were stored in a freezer at –20 °C. The

levels of dopamine, glutamate, GABA, and catecholamine in those brain tissues (frontal lobe for dopamine, GABA, and catecholamine, and cerebellum for glutamate) were measured by the means of a test kit. The test kit was based on an enzyme linked immunosorbent assay (ELISA) (Cusabio, China).

2.4. Statistical analysis

The experimental data compiled during the tests were analyzed by SPSS 21.0. Continuous variables were expressed as mean ± standard deviation and median (minimum–maximum values). Kruskal–Wallis variance analysis was used for intergroup comparisons. The post-hoc Mann–Whitney U test with Bonferroni correction was used when the Kruskal–Wallis variance analysis determined a significant difference. The Wilcoxon signed rank test was used for pre–post comparisons.

3. Results

3.1. Behavioral analysis

Behaviors parameters obtained from the 8-arm radial maze test are given in Table 1.

Following the completion of the training period in the 8-arm radial maze test setup, the control group and the groups subjected to MSG injections at various dosages were monitored on a weekly basis and the results obtained as the number of correct behaviors and response latencies were analyzed statistically by Kruskal–Wallis and Friedman testing methods. Number of mistaken behaviors refers to the frequency at which the rat enters the same section twice. Visits to the same section twice or more are accepted as mistaken behavior. Values taken into consideration are the ones measured prior to the injection and on the following 1, 7, 14, 21, and 28 days after the injection.

In Table 1, MSG 50 refers to the group given 50 mg/kg per day monosodium glutamate, MSG 100 refers to the group given 100 mg/kg per day monosodium glutamate, and MSG 200 refers to the group given 200 mg/kg per day monosodium glutamate. Compared to the control group, in the group subjected to the injection of MSG 200 mg/kg per day, a statistically considerable decrease in the number of correct behaviors was observed on day 7 of the experiment (between MSG 50 dosage and MSG 200 dosage, control group and MSG 200 dosage) ($P < 0.002$) (Figure 1).

On day 14 of the experiment, when the group of MSG 100 is compared with the control group and with the group

Table 1. Effect of MSG on the numbers of correct behaviors and response latency values in the 8-arm radial maze.

	Number of correct behaviors						
	Preinjection	Day 1	Day 7	Day 14	Day 21	Day 28	P
	Median (min–max)	Median (min–max)	Median (min–max)	Median (min–max)	Median (min–max)	Median (min–max)	
Control	7 (6–7)	7 (6–8)	8 (6–8)	8 (6–8)	7 (6–8)	8 (5–8)	0.293
MSG 50	6.5 (6–7)	6.5 (4–8)	8 (7–8)	7 (6–8)	7 (5–8)	7 (4–8)	0.215
MSG 100	6 (6–7)	6 (1–8)	6.5 (5–8)	7 (6–8)	6 (5–7)	6.5 (4–8)	0.594
MSG 200	7 (6–7)	5 (1–6)	5 (5–6)	6 (2–7)	6.5 (5–8)	4.5 (4–8)	0.087
P	0.622	0.075	0.002*	0.1	0.362	0.074	
Response latencies							
	Preinjection	Day 1	Day 7	Day 14	Day 21	Day 28	P
	Median (min–max)	Median (min–max)	Median (min–max)	Median (min–max)	Median (min–max)	Median (min–max)	
Control	19 (12–30)	18 (13–29)	14 (10–51)	15 (11–28)	17.5 (14–41)	11 (6–18)	0.126
MSG50	11.5 (9–20)	18.5 (16–150)	12.5 (9–35)	17 (11–30)	12 (9–18)	14 (8–20)	0.051
MSG100	13.5 (9–21)	30.5 (12– 300)	18.5 (8–46)	45 (42–55)	29 (11–67)	15 (11–20)	0.023*+
MSG200	19 (14–39)	57 (16–200)	18 (16–49)	22 (13–31)	21.5 (12–59)	16 (11–44)	0.089
P	0.118	0.327	0.721	0.004**	0.102	0.259	

Number of correct behaviors; control and MSG 50 are significantly different from MSG 200 ($P < 0.002$*). Response latency; control and MSG 50 are significantly different from MSG 100 ($P < 0.004$**). The values preinjection and on day 7 are significantly different from the values of day 14. In addition, the values on day 14 are significantly different from the values on day 28 at MSG 100 dosage ($P < 0.023$*+).

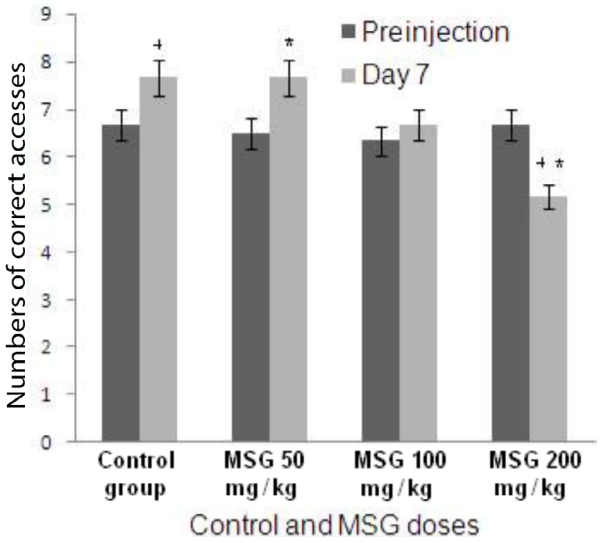

Figure 1. Numbers of correct accesses preinjection are significantly different from the numbers for day 7 for the MSG 50 mg/kg and MSG 200 mg/kg groups ($P < 0.002^{*}$). Additionally, the control group's number is significantly different from the MSG 200 group's value ($P < 0.002^{+}$). Statistically significant value is $P < 0.05$.

of MSG 50, a statistically reasonable decrease is observed in the response latency results ($P < 0.004$) (Figure 2a).

Similarly, again based on the response latency results, statistically reasonable differences are detected in the MSG 100 group when the preinjection values are compared with the values of day 15, the values of day 7 with the values of day 14, and the values of day 14 with the values of day 28 ($P < 0.023$) (Figure 2b).

Response latency results display statistically significant differences according to various MSG dosages applied in Table 1, when compared with the values of preinjection and of day 14, with the values of day 7 and day 14, and with the values of day 14 and day 28 ($P < 0.023$). There is also a statistically significant difference between the results of the response latency of the control group and the MSG 100 group, and between the MSG 50 group and the MSG 100 group as well on day 14 ($P < 0.004$).

Line crossing, rearing, and grooming values are given for the applied MSG dosages before and after the injections in Table 2. A statistically significant difference is determined between the line crossing value when the control group and MSG dosages (MSG 50 - MSG 200) pre- and postinjections are compared ($P < 0.05$). In the postinjection period, only the grooming values of the control group are considered to be significant ($P < 0.05$). Rearing values are determined to be statistically significant for both the preinjection and postinjection periods at all given MGS dosages applied ($P < 0.05$).

3.2. Neurochemical analysis

Dopamine, GABA, glutamate, and catecholamine levels in decapitated rats' brain tissues were measured using ELISA. GABA, dopamine, and other catecholamine levels (noradrenaline and adrenaline) were inspected in the brains' frontal lobe and the level of glutamate was inspected in the cerebellum. For both regions, neurochemical values with respect to relevant MSG dosages are given in ELISA results (Table 3). Significant differences in catecholamine levels (noradrenaline and adrenaline) measured were detected in the following couples of dosage groups when compared with each other: control group and MSG 50 dosage group, control group and MSG 100 dosage group, MSG 100 dosage group and MSG 200 dosage group, and MSG 50 dosage group and MSG 200 dosage group ($P < 0.017$) (Figure 3a). In addition to catecholamine levels, significant differences were also detected in the glutamate levels of the MSG 50 dosage group and the MSG 100 dosage group when compared with the control group's glutamate level ($P < 0.029$) (Figure 3b).

4. Discussion

In recent years, there have been continuous and ongoing discussions about the neurobehavioral effects of MSG. Meanwhile experiments to reveal its possible effects on animals are still being conducted. Artificial flavor enhancers such as MSG may cause animals to display behaviors similar to anxiety, epilepsy, and depression (17–21). Behaviors similar to anxiety can be identified by the means of experimental open field test, light/dark transition test, elevated plus maze test, tail suspension test, forced swim test, and social interaction task (22). Through the open field test, line crossing, rearing, grooming, and defecation number parameters of rats can be assessed (23). In our study, the open field test was conducted with rats both before and after the MSG injections. This allowed us to evaluate and compare the functions of the locomotor activities of rats in the MSG-injected group and the control group.

Dubovickyet et al. (24) revealed that MSG injections during the neonatal period of rats increased locomotor behavior on postnatal days 21 and 65. Ishikawa et al. (9) also stated that MSG injections performed during the neonatal period caused some specific degeneration in the hippocampal CA1 pyramidal cells, which is linked to learning disability. Kiss et al. (25) and López Pérez et al. (26) reported that, on postnatal days 1, 3, 5, 7, and 9, rats who were subjected to MSG injections firstly displayed increased locomotor activities and afterwards hypoactivity, and finally behavioral disorders.

All the above statements taken from previous research support the argument that the degeneration in the hippocampal area of the brain caused by the MSG also

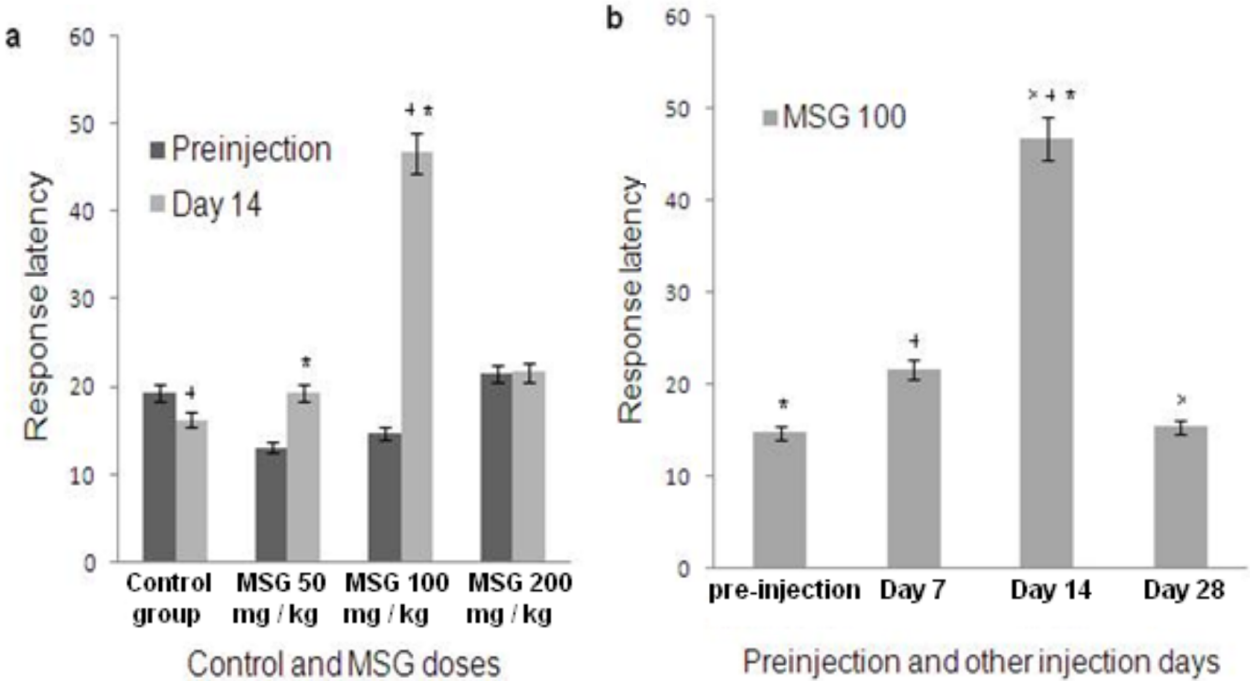

Figure 2. a) Compared to the preinjection phase, response latency values of the MSG 50 mg/kg and MSG 100 mg/kg groups are significantly different from each other on day 14 of the test ($P < 0.004^{*}$). Additionally the control group's value is significantly different from the value of the MSG 100 mg/kg group ($P < 0.004^{+}$). b) Response latency value for MSG 100 mg/kg; values in the preinjection phase and on day 7 are significantly different from the value on day 14 ($P < 0.023^{*,+}$), day 14's value is also significantly different from the value of day 28 ($P < 0.023^{\times}$).

Table 2. Open field test results for various MSG dosages in the preinjection and postinjection periods.

		Preinjection		Postinjection		
		Means ± SEM	Median (min–max)	Means ± SEM	Median (min–max)	P
Line crossing	Control	32.8 ± 17.8	30.5 (10–56)	30.8 ± 17.3	28.5 (9–53)	0.024*
	MSG 50	51.5 ± 7.8	50.5 (43–62)	23.5 ± 10.0	20.0 (13–40)	0.027*
	MSG 100	53.8 ± 23.3	49.0 (34–99)	18.1 ± 9.7	19.5 (4–31)	0.028*
	MSG 200	60.0 ± 12.3	59.0 (42–78)	23.8 ± 7.2	26.5 (11–31)	0.028*
Grooming	Control	20.6 ± 7.6	19.5 (13–34)	14.0 ± 4.3	13.0 (9–20)	0.026*
	MSG 50	19.1 ± 23.0	9.5 (8–66)	52.0 ± 29.5	57.0 (4–84)	0.116
	MSG 100	26.0 ± 29.6	14.0 (0–80)	49.6 ± 24.0	47.5 (19–85)	0.116
	MSG 200	42.1 ± 23.3	38.5 (13–81)	39.5 ± 22.7	34.5 (15–80)	0.917
Rearing	Control	6.0 ± 1.1	6.0 (5–7)	4.7 ± 0.9	4.5 (4–6)	0.059
	MSG 50	11.0 ± 3.5	10.0 (8–17)	4.0 ± 2.6	3.0 (2–9)	0.026*
	MSG 100	8.8 ± 3.0	8.5 (4–13)	2.6 ± 1.2	2.5 (1–4)	0.027*
	MSG 200	13.1 ± 5.9	14.0 (6–19)	3.1 ± 1.4	3.5 (1–5)	0.028*

Significantly different from the preinjection value ($P < 0.05^{*}$).

Table 3. GABA, dopamine, catecholamine, and glutamate levels in rats' brain tissues.

		Mean ± SEM	Median (min–max)	P
GABA (pg/mL)	Control	2.46 ± 3.44	1.18 (0.61–9.46)	0.26
	MSG 50	1.11 ± 0.67	1.17 (0.35–2.2)	
	MSG 100	0.92 ± 0.37	0.94 (0.53–1.36)	
	MSG 200	0.74 ± 0.24	0.73 (0.47–1.02)	
Dopamine (ng/mL)	Control	0.8 ± 0.36	0.63 (0.54–1.46)	0.693
	MSG 50	0.85 ± 0.15	0.91 (0.57–0.97)	
	MSG 100	0.96 ± 0.3	0.97 (0.53–1.43)	
	MSG 200	2.19 ± 2.72	0.7 (0.5–7.19)	
Catecholamine (pg/mL)	Control	32.83 ± 5.31	30.62 (27.31–41.74)	0.017*
	MSG 50	41.45 ± 4.84	40.9 (34.98–47.46)	
	MSG 100	41.19 ± 7.7	41.14 (27.97–50)	
	MSG 200	30.63 ± 4.65	32.14 (21.25–33.54)	
Glutamate (nmol/µL)	Control	0.22 ± 0	0.22 (0.22–0.23)	0.029**
	MSG 50	0.26 ± 0.04	0.23 (0.22–0.31)	
	MSG 100	0.24 ± 0.01	0.24 (0.22–0.24)	
	MSG 200	0.23 ± 0.01	1.23 0.22–0.24)	

Catecholamine levels of the MSG groups (50, 100, 200 mg/kg) are significantly different from the values of the control group ($P < 0.017$)*. Glutamate levels of the MSG (50, 100, 200 mg/kg) groups are significantly different from the values of the control group ($P < 0.029$**).

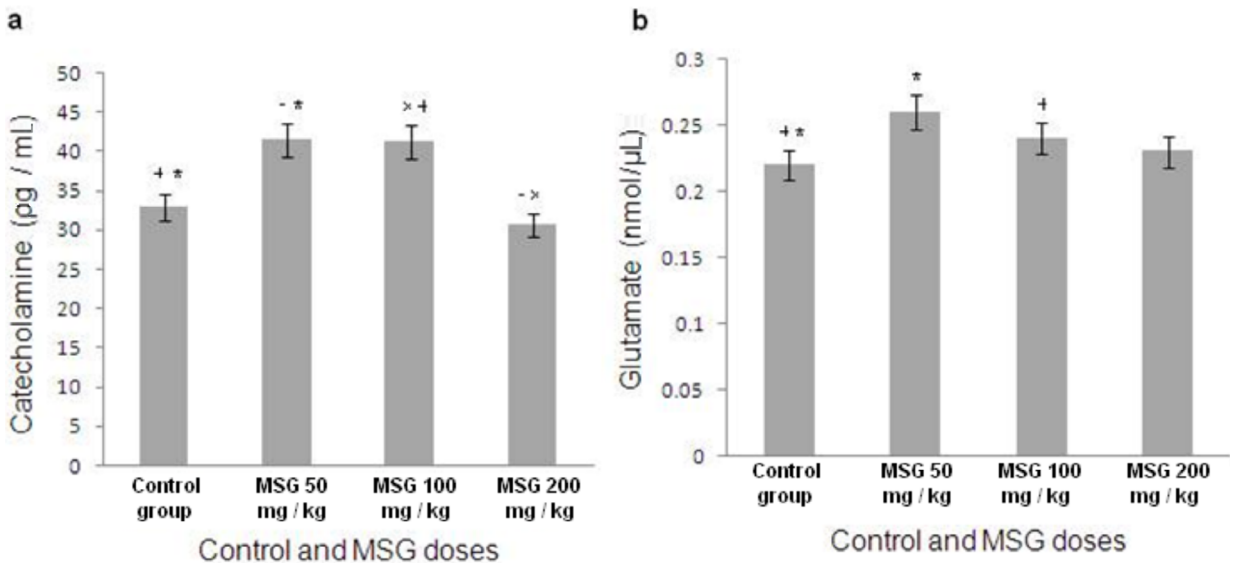

Figure 3. a) Catecholamine level; the values of the MSG 50 mg/kg and MSG 100 mg/kg groups are significantly different from the control group's value. Additionally, the values of the MSG 50 mg/kg and MSG 100 mg/kg groups are significantly different from the value of MSG 200 ($P < 0.017^{*,+,-,\times}$). b) Glutamate level; MSG 50 and MSG 100 were significantly different from the control ($P < 0.029^{*,+}$).

has an effect on behavioral parameters. According to the statistical results of our study, line-crossing values of the rats that were subjected to MSG injections were lower than the control group's values. Similar results showing decreases in motor activities were reported previously (27–29). Additionally, after the MSG injections, an increase in

grooming values and a decrease in rearing values were also observed. Studies by Oliveira de Almeida et al. (30) were the first to report that the animal became more hypoactive and also rooming number increased after MSG injections. They evaluated the expression of dopamine D2 receptor via western blot analysis and implemented an open field test on day 57 of their experiment on rats. Briefly, after 57 days, they observed that following a significant decrease in locomotor activities the rats became less active compared to their initially measured behaviors. However, after day 71 those values returned to their initial numbers. Similarly, in an experimental model based on nicotine, which is known as an oxidant agent, the open field test resulted in reduced motor activity and in an increased level of anxiety in animals (31). On the other hand, in an experimental model built to observe epilepsy at rats, a significant increase in locomotor activities was determined via the measurements made in the open field test (20).

In our study, we tested the learning and memory functions of rats in an 8-arm radial maze, both before and after the MSG injections. In order to determine the appropriate MSG dosage to be injected, the weight of each rat was measured prior to injection.

MSG dosages consisted of 8 subsequent dosages in order to observe its acute and chronic effects on rats. Based on the results of the 8-arm radial maze test, we observed that the number of times the rats chose the correct arm decreased for the MSG-injected rats compared to the control group on day 7. However, response latency increased on day 14. The fact that the increase happens mostly on day 14 of the MSG injections raises the idea that some defects occur in the spatial memory of animals (32). In the radial maze test it was proved that there is a correlation between spatial learning and the density of the brain's receptors. There are two arguments to prove this correlation: first is the increase in the increasing reward system due to the facilitating effect $GABA_A$ in the emission of noradrenalin in the hippocampus; in this case food reward tasks might be the focus. Second is the high positive correlation between the $GABA_A$ receptors, AMPA receptors, and universal receptors (33). GABA is emitted in the brain due to the dopamine level (34). Oliveira de Almeida et al. (30) examined the effect of dopaminergic increase on the locomotor activity of rats and its relation with the expression of dopamine D2 receptor. They argued that the increased expression of dopamine receptor D1 compared to the receptor D2 reduced the locomotor activity and thus caused a decrease in mobilization. Similar findings have also been recorded in other studies (27,35).

In our study, we observed that as the MSG dosages were increased throughout the experiment the level of dopamine increased but the GABA level decreased. Due to that decrease, a feedback inhibition occurred in the cortex and hippocampus, in order to maintain a balanced glutamate/GABA ratio (even the level of dopamine is increased) (36). Those abnormalities in neurochemical levels were also reported previously (37–39). In our study, the increase in glutamate level and decrease in GABA level revealed that the same mechanism exists in the animals subjected to higher MSG dosage injections. Additionally, the presence of a positive correlation between GABA receptor density and spatial learning, according to Schmid et al. (33), is potentially a good explanation for the decrease in the GABA level and the decrease (or change) in the learning parameters observed in the radial maze test results.

On the other hand, it is thought that there is a balance between the excitatory neurotransmitter glutamate levels and the inhibitory neurotransmitter GABA levels and that this balance is of core importance for the hippocampal circuits since any diversion in favor of one of those systems may cause an imbalance in dysfunctions (33).

Excessive glutamate activation allows the neurological circuit to widen its range of effect in infant mice (40) and induces long-term depression in rats (41). Bojanic et al. reported that MSG can act as an 'excitotoxin'; this means that it could overstimulate the neural cells up to the point of damage or even to death (42).

In our study we observed that compared to the control group the glutamate level increased as much as the level of MSG dosage was increased relatively. The reason is that normally the concentration of glutamate released into the synaptic range could reach very high levels but those levels could only be maintained just for a couple of milliseconds. If the duration is extended, overstimulation of neuronal glutamate receptors occurs and neurons face a lethal excitation. During the ischemia, intensive glutamate accumulation stimulates the glutamate receptors, triggering a series of reactions leading to neuronal death. It is assumed that, as a result of ischemia, lower oxygen concentration prevents the recovery of energy dependent glutamate and it causes an increased level of extracellular glutamate (43).

Dopamine and other monoamines such as catecholamine are neuroactive substances that exist abundantly in the CNS (37). Catecholamines are of core importance in building learning abilities and also in the formation of the memory (44). We also observed that the catecholamine level decreased as the dosage of MSG was increased. We think that the decrease in the catecholamine level related to increased MSG dosage is a neurochemical reason for the learning disorder and for the loss of some functions related to memory. From a biochemical point of view (increase in glutamate, decrease in GABA, increase in dopamine, and decrease in the other catecholamines) the results obtained are consistent with the number of

times the correct arm in the radial maze was accessed and with the response latency values. As a result, we concluded that while the MSG dosage is increased, the balance between the ratio of glutamate/GABA and dopamine/catecholamine may have caused the animal to select the right arm in the maze and increase the response latency value. We think that, after the MSG injections, such changes like necrosis and immunopathological expression changes happening in the brain's hippocampus CA1 area and the brain's arcuate nucleus cortex may result in some behavioral changes in the animals, during their acute and chronic periods. Ultimately, cerebral ischemia and reperfusion form mechanisms that affect the spatial memory and motor sensors negatively (45).

In a study in which the effects of the hippocampus, amygdale, and the dorsal striatum on the learning and the memory of animals were investigated, behavioral effects of lesions created in the three areas of the brain were investigated via an 8-arm radial maze (37). According to the results of that study, the hippocampus is responsible for the relations between the stimulants and the events in the nerve system. The results indicate that the hippocampus provides information about the nerve system and also about the behaviors based on important biological events. However, in the same study, it was mentioned that the dorsal striatum contributes to the union of stimulant–reaction improved by the nerve system (37).

Furthermore, the hippocampus also contributes to the operation of a vast variety of neuronal functions including the support and protection of motor neurons in astrocytes. However, in the case where astrocytes are overactivated, their forms and motor neuron functions are also altered. Activation of astrocytes in the brain is a characteristic general pathological situation. In this case, an irregular communication may appear between the motor neurons and astrocytes and ultimately the death of motor neurons is accelerated (46). In a study in which the expressions of the neuronal marker MAP-2 and astroglial marker GFAP were examined, the expression of MAP-2 decreased but the expression of GFAP increased due to an extracellular increase in glutamate (47). When the astrocytes were activated, the expression of GFAP sharply increased (48,49).

In conclusion, MSG negatively affects the learning/memory functions of animals in addition to its effects on their behavioral parameters such as anxiety, depression, and similar behaviors. The neuronal degeneration caused by MSG in the brain's hippocampal area might be considered a reason why such behavioral effects happen. Neurochemical parameters also support those findings and results.

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References

1. Bhattacharya T, Bhakta A, Ghosh SK. Long term effect of monosodium glutamate in liver of albino mice after neo-natal exposure. Nepal Med Coll J 2011; 13: 11-16.
2. Bellisle F, Monneuse MO, Chabert M, Larue-Achagiotis C, Lanteaume MT, Louis-Sylvestre J. Monosodium glutamate as a palatability enhancer in the European diet. Physiol Behav 1991; 49: 869-873.
3. Chevassus H, Renard E, Bertrand G, Mourand I, Puech R, Molinier N, Bockaert J, Petit P, Bringer J. Effects of oral monosodium (L)-glutamate on insulin secretion and glucose tolerance in healthy volunteers. J Clin Pharmacol 2002; 53: 641-643.
4. Geha RS, Beiser A, Ren C, Patterson R, Paul A, Greenberger PA, Leslie C, Grammer LC, Ditto AM, Harris KE et al. Review of alleged reaction to monosodium glutamate and outcome of a multicenter double-blind placebo-controlled study. J Nutr 2000; 130: 1058-1062.
5. Rogers PJ, Blundell JE. Umami and appetite: effects of monosodium glutamate on hunger and food intake in human subjects. Physiol Behav 1990; 48: 801-804.
6. Cirić M, Najman S, Bojanić V, Cekić S, Nesić M, Puskas N. Neonatal influence of monosodium glutamate on the somatometric parameters of rats. Gen Physiol Biophy 2009; 28: 155-161.
7. Skerry TM, Genever PG. Glutamate signaling in nonneuronal tissues. Trends Pharmacol Sci 2001; 22: 174-181.
8. Schwartz JH. Neurotransmitters. In: Kandel ER, Schwartz JH, Jessell TM, editors. Principles of Neural Science. 4th ed. New York, NY, USA: McGraw-Hill; 2000. pp. 280-297.
9. Ishikawa K. Hippocampal degeneration inducing impairment of learning in rats: model of dementia. Behavioral Brain Research 1997; 83: 39-44.
10. Harsha SN, Anilakumar KR. Effect of *Lactuva sativa* leaves extract against monosodium glutamate-induced neurotoxicity and oxidative stress in mice. Journal of Herbal Medicine and Toxicology 2013; 7: 5-13.
11. Olney JW. Brain lesions, obesity and other disturbances in mice treated with monosodium glutamate. Science 1969; 164: 719-721.
12. Hu L, Fernstrom JD, Goldsmithi PC. Exogenous glutamate enhances glutamate receptor subunit expression during selective neuronal injury in the ventral arcuate nucleus of postnatal mice. Neuroendocrinology 1998; 68: 77-88.
13. Lucas DR, Newhouse JP. The toxic effect of sodium L-glutamate on the inner layers of the retina. AMA Arch Ophthalmol 1957; 58: 193-201.

14. Gill SS. Potential target sites in peripheral tissues for excitatory neurotransmission and excitotoxicity. Toxicol Pathol 2000; 28: 277-284.

15. Hughes EG, Maguire JL, McMinn MT, Scholz RE, Sutherland ML. Loss of glial fibrillary acidic protein results in decreased glutamate transport and inhibition of PKA-induced EAAT2 cell surface trafficking. Brain Res Mol Brain Res 2004; 124: 114-123.

16. Reistad T, Mariussen E, Ring A, Fonnum F. In vitro toxicity of tetrabromobisphenol-a on cerebellar granule cells: cell death, free radical formation, calcium influx and extracellular glutamate. Toxicol Sci 2007; 96: 268-278.

17. McCool BA, Chappell AM. Chronic intermittent ethanol inhalation increases ethanol self-administration in both C57BL/6J and DBA/2J mice. Alcohol 2015; 49: 111-120.

18. Quines CB, Rosa SG, Da Rocha JT, Gai BM, Bortolatto CF, Duarte MF, Nogueira CW. Monosodium glutamate, a food additive, induces depressive-like and anxiogenic-like behaviors in young rats. Life Sciences 2014; 107: 27-31.

19. Rosa SG, Quines CB, EC Stangherlin EC, Nogueira CW. Diphenyl diselenide ameliorates monosodium glutamate induced anxiety-like behavior in rats by modulating hippocampal BDNF-Akt pathway and uptake of GABA and serotonin neurotransmitters. Physiol Behav2016; 155: 1-8.

20. Langberg T, Dashek R, Mulvey B, Miller KA, Osting S, Stafstrom CE, Sutula TP. Distinct behavioral phenotypes in novel "fast" kindling-susceptible and "slow" kindling-resistant rat strains selected by stimulation of the hippocampal perforant path. Neurobiol Dis 2016; 85: 122-129.

21. Quines CB, Rosa SG, Velasquez D, Da Rocha JT, Neto JS, Nogueira CW. Diphenyl diselenide elicits antidepressant-like activity in rats exposed to monosodium glutamate: A contribution of serotonin uptake and Na(+), K(+)-ATPase activity. Behav Brain Res 2016; 15: 161-167.

22. Teske JA, Perez-Leighton CE, Noble EE, Wang C, Billington CJ, Kotz CM. Effect of housing types on growth, feeding, physical activity, and anxiety-like behavior in male Sprague-Dawley rats. Frontiers in Nutrition 2016; 3: doi: 10.3389/fnut.2016.00004.

23. Ma P, Liu X, Wu J, Yan B, Zhang Y, Lu Y, Wu Y, Liu C, Guo J, Nanberg E et al. Cognitive deficits and anxiety induced by diisononyl phthalate in mice and the neuroprotective effects of melatonin. Nature 2015; 5: e14676.

24. Dubovicky M, Tokarev D, Skultetyova I, Jezova D. Changes of exploratory behavior and its habituation in rats neonatally treated with monosodium glutamate. Pharmacol Biochem Behav 1996; 56: 565-569.

25. Kiss P, Hauser D, Tamas A, Lubics A, Racz B, Horvath Z, Farkas J, Zimmermann F, Stepien A, Lengvari I et al. Changes in open-field activity and novelty-seeking behavior in preadolescent rats neonatally treated with monosodium glutamate. Neurotox Res 2007; 12: 85-93.

26. López Pérez SJ, Ureña Guerrero ME, Morales Villagrán A. Monosodium glutamate neonatal treatment as a seizure and excitotoxic model. Brain Research 2010; 1317: 246-256.

27. She Y, Xu J, Duan Y, Su N, Sun Y, Cao X, Lao L, Zhang R, Xu S. Possible antidepressant effects and mechanism of electroacupuncture in behaviors and hippocampal synaptic plasticity in a depression rat model. Brain Res 2015; 1629: 291-297.

28. Tyurenkov IN, Gumilevsky BY, Filina IS, Bakulin DA, Volotova EV, Kurkin DV. The influence of the immunity activation and suppression on the outcome of brain ischemia. Zh Nevrol Psikhiatr 2015; 115: 23-29.

29. Zhu XC, Huang HJ, Han QQ, Yue N, Li B, Yang L, Liu Q, Wu GC, Yu J. Effect of electroacupuncture stimulation of "Baihui" (GV 20), etc. on changes of behavior and plasma ghrelin content in depression rats. Zhen Ci Yan Jiu 2015; 40: 283-289.

30. Oliveira de Almeidaa WA, Esteves AM, Almeida Juniora CL, Lee KS, Frank MK, Marianoa MO, Frussa-Filhoa R, Tufika S, Mello MT. The effects of long-term dopaminergic treatment on locomotor behavior in rats. Sleep Sci 2014; 7: 203-208.

31. Motaghinejad M, Fatima S, Karimian M, Ganji S. Protective effects of forced exercise against nicotine-induced anxiety, depression and cognition impairment in rat. J Basic Clin Physiol Pharmacol 2015; 27: 19-27.

32. McDonald RJ, White NM. A triple dissociation of memory systems: hippocampus, amygdala, and dorsal striatum. Behav Neurosci 2013; 127: 835-53.

33. Schmid G, Bonanno G, Raiteri M. Functional evidence for two native $GABA_A$ receptor subtypes in adult rat hippocampus and cerebellum. Neuroscience 1996; 73: 697-704.

34. Chen Y, Xiong M, Zhang SC. Illuminating Parkinson's therapy with optogenetics. Nature Biotechnol 2015; 33: 149-150.

35. Mallick N, Khan RA. Behavioral effects of *Citrus paradisi* in rats. Metab Brain Dis 2016; 31: 329-335.

36. Mishra A, Goel RK. Comparative behavioral and neurochemical analysis of phenytoin and valproate treatment on epilepsy induced learning and memory deficit: search for add on therapy. Metab Brain Dis 2015; 30: 951-958.

37. Mishra A, Goel RK. Psychoneurochemical investigations to reveal neurobiology of memory deficit in epilepsy. Neurochem Res 2013; 38: 2503-2515.

38. Mishra A, Goel RK. Adjuvant anticholinesterase therapy for the management of epilepsy-induced memory deficit: a critical preclinical study. Basic Clin Pharmacol Toxicol 2014; 115: 512-517.

39. Singh D, Mishra A, Goel RK. Effect of saponin fraction from *Ficus religiosa* on memory deficit, and behavioral and biochemical impairments in pentylenetetrazol kindled mice. Epilepsy Behav 2013; 27: 206-211.

40. Goldsmith PC. Neuroglial responses to elevated glutamate in the medial basal hypothalamus of the infant mouse. J Nutr 2000; 130: 1032-1038.

41. Calabresi P, Centonze D, Paolo G, Marfia GA, Bernardi G. Glutamate-triggered events inducing corticostriatal long-term depression. J Neurosci 1999; 19: 6102-6110.

42. Bojanic VV, Bojanic Z, Najman S, Zivanov-Curlis J, Tomin J, Dinoic B, Savic T. Diltiazem prevention of monosodium glutamate toxicity on hypothalamus in Wistar rats. Arch Oncol 2004; 12: 19-20.

43. Kurul S, Gulmez O. Neuroprotection and neuroprotective agents. DEÜ Faculty of Medicine Journal 2007; 21: 119-130.

44. Izquierdo I, Medina JH. Memory formation: the sequence of biochemical events in the hippocampus and its connection to activity in other brain structures. Neurobiol Learn Mem 1997; 68: 285-316.

45. Tao T, Zhao M, Yang W, Bo Y, Li W. Neuroprotective effects of therapeutic hypercapnia on spatial memory and sensorimotor impairment via anti-apoptotic mechanisms after focal cerebral ischemia/reperfusion. Neurosci Lett 2014; 573: 1-6.

46. Donaldson K, Stone V. Current hypotheses on the mechanisms of toxicity of ultrafine particles. Ann Ist Super. Sanit 2003; 39: 405-410.

47. Han F, Shioda N, Moriguchi S, Qin ZH, Fukunaga K. Downregulation of glutamate transporters is associated with elevation in extracellular glutamate concentration following rat microsphere embolism. Neurosci Lett 2008; 430: 275-280.

48. Heales SJ. Neurodegeneration or neuroprotection: the pivotal role of astrocytes. Neurochem Res 2004; 29: 513-519.

49. Middeldorp J, Hol EM. GFAP in health and disease. Prog Neurobiol 2011; 93: 421-443.