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Original Article

Urban traffic noise pollution disturbs spatial learning and memory and increases anxiety-like behavior in adult male rats

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Abstract

Introduction: Noise pollution is an unwanted inevitable distribution of the modern and industrialized life of mankind. With the expansion of urban life, humans are daily exposed to noise pollution which can cause anxiety and disorders in cognitive activities. The present study was aimed to investigate the impact of sub-chronic urban traffic noise pollution on learning, memory and anxiety-like behavior in adult male rats.

Methods: Thirty two adult male Wistar rats (weighing 275-300g) were used in the present experimental study. The animals were divided into two groups: the control and the noise-exposed. The rats in the test group were exposed to a 90dB noise recorded from a crowded street traffic for 6h/10 days. Control rats were intact. Morris water maze (MWM) and an elevated plus maze (EPM) were used to assess spatial learning and memory and anxiety-like behavior in rats.

Results: The findings displayed that both control and noise-exposed group improved their maze steering over 4 days of experiment in MWM; however, noise-exposed group had more latency and traveled-distance in MWM to find the hidden platform in probe trial compared to those of control ($P<0.05$). Moreover, noise-exposed group showed a significant increase in weight gain compared to the control group ($P<0.05$). In addition, the spent time in open arm of the EPM was significantly decreased compared to controls ($P<0.05$).

Conclusion: Urban traffic noise pollution for a short-term period causes a meaningful increase on weight gain, disorders in retrieval memory and increase in anxiety-like behavior in rats.

Keywords:

Noise pollution;
Learning;
Anxiety;
Male rats

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Introduction

Among all causes of anxiety, noise pollution is a

strong and prevalent environmental stress for both humans and animals. This is considered as a critical concerning issue of the industrialized societies. What is being called as noise pollution refers to unwanted

waves which effect functions of living beings (especially humans) in particular time and place conditions. The results of such noise pollution doesn't only include hearing loss, but it also embraces disorders in our sleeping habits, work experience and daily interaction, the signs of which are later physically projected in our body (Basner et al., 2014). There is an increasing evidence which shows that noise pollution causes damage to the auditory (Basner et al., 2014), endocrine and cardiovascular systems (Babisch, 2003; Munzel et al., 2018). The signs of negative impact were observed on cognitive functions e.g. anxiety, learning, memory and other individual's behavior in human (Rooszendaal, 2002; Clark et al., 2012; Tzivian et al., 2016) and rodents (Cheng et al., 2011; Tao et al., 2015; Badache et al., 2017).

In developed countries, despite the fact that environmental noises caused by factors such as road traffic noises, air or rail traffic and construction are so common, but the experts cars and motorcycles blowing horns to be the main causes of noise pollution in metropolitan areas. Since the learning conditions and a suitable environment play an important role in learning, these factors can influence on learning functions of students and impede learning (Rooszendaal, 2002; Clark et al., 2012; Tzivian et al., 2016). According to researchers, with the increase in noise pollution, learning pace slows down; to have a distinct perception, for each 10 dB of loud noise, learning reading comprehension is decelerated for month; if the amount of loud is doubled, learning is slowed for two months (Haines et al., 2003; Evans and Higge, 2010). Moreover, the children exposed to noise pollution not only lose their attention and have a slowed learning process, but also they show a decreased amount of resistance to diseases (Prior, 2002).

For the survival of animals, the ability to remember information is considered to be very important. It is very important for animals to remember where the food source is located. The ability to remember location with the application of the signs in the physical environment is called spatial memory. In the human brain and rodents, this process takes shape in the hippocampus (Eichenbaum, 2001; Gorchetnikov and Grossberg, 2007). Previous studies have shown that exposing pregnant mothers to noise can disrupt the development of the central nervous system of

their offspring. At the behavioral levels, adult offspring showed cognitive impairments such as learning and memory, disorder in social behavior and the appearance of pseudo-anxiety behaviors (Kim et al., 2006; Barzegar et al., 2015); moreover, their neurogenesis of the hippocampal neurons is defective (Kim et al., 2006). Chronic exposure to noise pollution can cause a decrease in the volume of hypothalamus, stress as well as learning and memory disorder (Kujawa and Liberman, 2015). Additionally, the sound causing stress in pregnant rats raises vulnerability to stress in their offspring (Barzegar et al., 2015; Badache et al., 2017). Similar to other stress factors (Nishio et al., 2006), noise pollution induces an activation of hypothalamic-pituitary-adrenal axis following the increase in blood glucocorticoids of pregnant rats (Barzegar et al., 2015). Other impacts of being exposed to noise (average 82-85dB for 8 hours a day) are less capability of problem solving in adult rats and 66% of weight loss in rat fetus (Haines et al., 2003).

Many studies have shown that the exposure of rodents to noise pollution causes learning and memory dysfunctions and increased anxiety like behaviors (Prior, 2002; Kim et al., 2006; Manikandan et al., 2006; Cui et al., 2009; Cheng et al., 2011; Cui et al., 2011; Barzegar et al., 2015; Tao et al., 2015; Liu et al., 2016). These studies vary in many aspects such as the intensity of the sound, the amount of exposure to noise, the type of animal species and its developmental stage, as well as the type of learning which is being evaluated. With regards to the significance of this critical concern and the existence of reports indicating that long-term approach to noise pollution can cause spatial learning and memory disorder (Prior, 2002; Manikandan et al., 2006; Cui et al., 2009; Barzegar et al., 2015), we evaluated the impacts of short-term exposure to noise pollution caused by urban traffic on spatial learning and memory in Morris water maze (MWM) as well as anxiety-like behaviors in rats; this can enhance our perception and knowledge in the field of external factors involved with cognitive behavior in animal models so that the findings could be ascribed to human in consequence.

Materials and methods

Animals

This study used thirty two male Wistar rats provided from National Center of laboratory Animal Breeding and Maintenance center (Kerman, Iran). The animals were kept in standard conditions (12h light/dark cycle) and had open access to food and water. In order to prevent from congestion stress, four big cages type 3 (8 rats in each cage) were used. Before and after the study, all rats were carefully weighed. The animals were randomly divided in two groups: the noise-exposed and the control. The noise-exposed: sixteen animals were exposed to traffic noise (90dB) from 8:00-14:00 daily for ten successive days. The control group: sixteen animals had no intervention.

Noise induction

First of all, the recorded broad band traffic from a high-traffic square in Kerman, Iran by a standard recorder (Samsung, Yv120) was saved on a flash memory. A sound amplifier and its speaker (creative zen NEEON, 300W) were located 30cm above the cages. A precision sound level decibel meter (Extech Instruments, MA) was utilized in order to modify the sound intensity to 90dB uniformly in the cage. The animals from the noise-exposed group were daily exposed to the noise (08:00–14:00). The rat home cages were situated in a separate room adjacent to the animal house (Barzegar et al., 2015). The control group was kept in the same room for the same period of time without noise switched on. Behavioral analysis was performed on both the control and noise-exposed groups 24h after the last noise stress session in order to examine the spatial learning and memory and the anxiety like behavior.

Behavioral tests

The elevated plus-maze task (EPM)

An EPM was used in order to evaluate anxiety-like behavior. Eight from sixteen rats in the noise-exposed group and eight from sixteen rats in the control group were randomly subjected to EPM test. EPM is made of aluminum with two open arms (50×10cm) and two close arms (50×10×40cm) which are traversed at the center. The similar named arms are placed opposite to each other and the height of the maze is 50cm. Before the test, each rodent was placed in the lab environment for 30 minutes. As the test started, each rat was moved to the open arm of the maze. The number of entering into open and

close arms, and spent time in the open and close arms was recorded for 5 minutes.

The MWM task

For evaluating learning and spatial memory, a Morris water maze was utilized. Eight rats in the noise-exposed group and eight rats in the control group were used for MWM test. The behavioral test is widely used for evaluation of spatial learning and memory. In this test, a circular pool (160cm diameter, 80cm high) is conceptually divided in four equal quadrants. The water temperature was kept in standard heat of $21\pm 2^{\circ}\text{C}$. First, the pool was conceptually divided to four quadrants. In a fixed area through the study, a platform (10cm diameter) is hidden under water. The study is performed in a dim lighted room. The rodent can use pictures spatially or by the help of objects which are constantly placed around the maze (such as computer) in order to remember the place of the platform. All rat's movements are recorded with a placed camera.

Training phase: In the training sessions each rat was left in each of the conceptual quadrants of the pool (4 trails) in the three blocks. A maximum 90s chance was given to each animal to find the hidden platform under the surface of the water to rest in each trial. If the animal was unable to find the platform, we leaded him with our hand. In any case, the animal could rest (30-35s) on the platform after reaching it; then it rested in the cage under the lamp for 60 seconds. The distance moved to find the hidden platform of the animal was recorded using a commercial software (Noldus, Netherlands; version:6 XT).

Probe test: The probe trials were done following the training phase. The platform was removed from the pool and each rat was placed into the pool from the opposite quadrant. This phase consisted of 60s free swim period without a platform. The distance moved to find the former platform, time spent in the target quadrant, the number of times rats crossed the former platform and rat velocity was also recorded (Saber Moghadam et al., 2013; Sepehri et al., 2014).

Statistical analysis

The results of the present study were analyzed by Prism software 6.0. After distinguishing the normality of the data using Shapiro normality test, if the data was normal, it was evaluated with unpaired or paired Student t tests; otherwise, nonparametric

equivalent was utilized to compare the data. It shall be noted that repeated ANOVA-measurement was used to compare the traveled distance in MWM to find the platform in the training phase. The data was displayed in the form of row data, median or mean±SEM. $P<0.05$ was considered as the criteria.

Results

Weighing gain

The results of the present study showed that the control group rats had no change in weight gain before and after doing behavioral tests. The finally weight gain of rats in the noise-exposed group showed a significant increase compared to their primary weight gain (paired Student's t-test, $P<0.001$) and also, compared to the finally weight gain of rats in the control group (unpaired Student's t-test, $P<0.001$) significantly (Fig. 1).

The elevated plus-maze

In the EPM, we found that the noise-exposed group has fewer times of traveling between the open and close arms compared to those of control (in order, median=4.5 vs. median=7) which signified a meaningful difference between the two groups (Fig. 2A, $P<0.001$). The rats in the noise-exposed group had fewer number of entering both the open and close arms, especially this difference was more significant in the number of entering the close arm compared to the control group (in order, median =3.5

vs. median=5) showing a significant difference (Fig 2C, $P<0.01$). Moreover, the results showed that 50% of the rats in the noise-exposed group, compared to the control group, spent a longer time duration in the close arms (in order, median=12 vs. median=24; Fig. 2D), and these rats have never experienced the open arms (Fig. 2E).

The Morris water maze

In spatial learning evaluation of male rats in MWM, we found that during subsequent blocks of learning, the distance moved to find the hidden platform changes in noise traffic exposed group and the control group changes ($F_{2, 192}=4.6$, $P=0.01$). More analysis distinguished that the distance moved to find the hidden platform decreases in the control group; this decrease has a significant difference in block III compared to block I, significantly ($P<0.05$). Such a trend does not exist in noise traffic exposed group. There was not a significance for group factors ($F_{1, 192}=0.05$, $P=0.84$) and the block×group interaction ($F_{2, 192}=1.49$, $P=0.22$; Fig. 3).

In the probe trial which is used to evaluate the spatial memory of male rats in MWM, we found that the distance moved to find the former platform in the maze by male rats in the noise-exposed group showed a significant increase compared to those of control (in order, median=407, median=364, $P<0.05$; Fig. 4A). Moreover, the time spent in the target quadrant for the rats in noise-exposed group to find the former platform had a significant increase

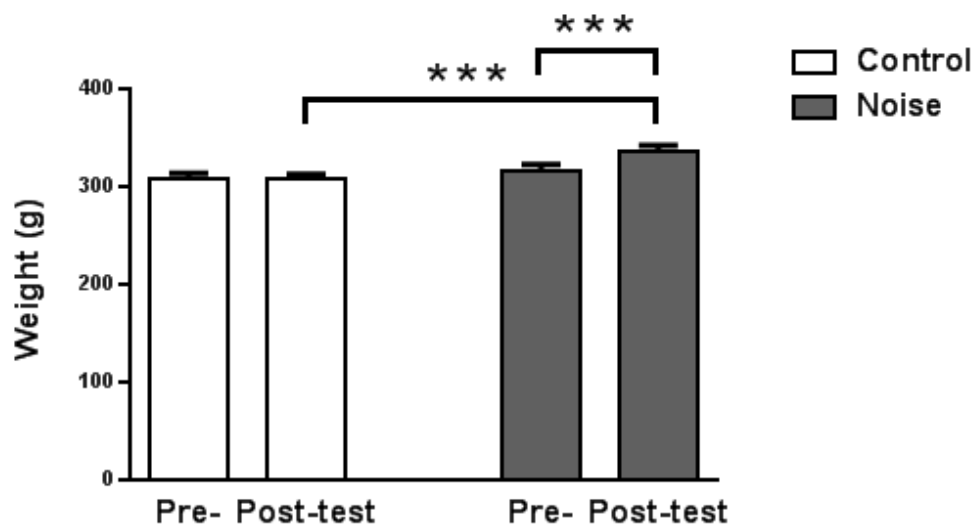


Fig.1. The impact of traffic noise on the weight changes in both control group and the exposed-noise group before and after behavioral tests. The symbol (*) shows a significant statistical difference between exposed-noise group and the control group. The data is show in form of mean±SEM; *** $P<0.001$, n=8.

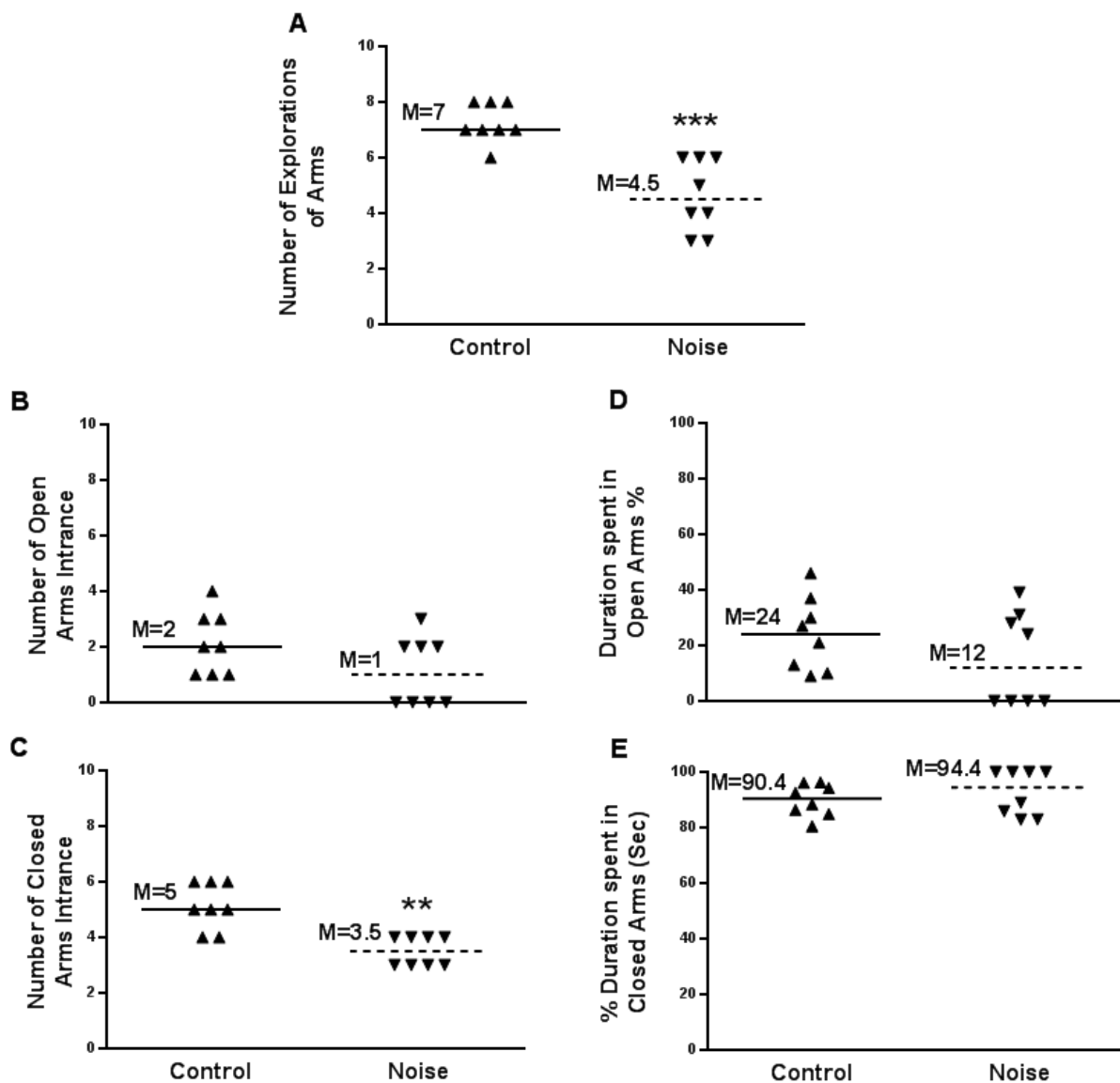


Fig.2. The evaluation of anxiety-like behavior in elevated-plus maze. The impact of traffic noise on the total number of exploration of open and close arms (A), the number of entering open and close arms (C and D), and spent time in the open and close maze (D and E) by male rats in the noise traffic exposed group compared to those of control in the elevated-plus maze; the data is shown in the form of mean and median. The symbol (*) shows a significant statistical difference between the noise-exposed group compared to the control group; ** $P < 0.01$ and *** $P < 0.001$, $n = 8$.

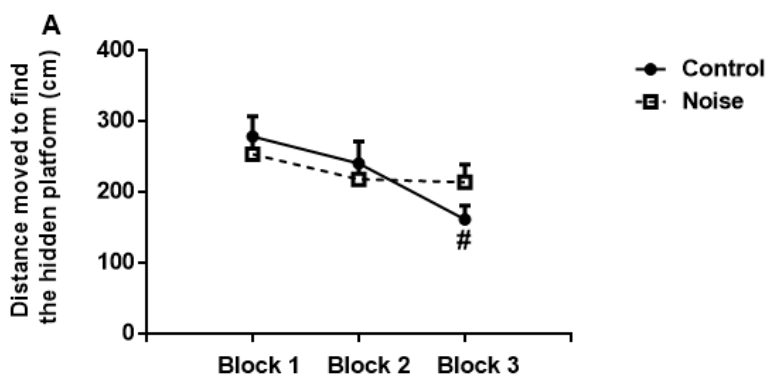


Fig.3. The evaluation of training learning phase in MWM. The effect of traffic noise on the distance moved to find the hidden platform in MWM by male rats in the exposed-noise group compared to the control group. The data is show in form of mean \pm SEM. The symbol (#) shows a significant statistical difference between block III compared to block I in the control group: # $P < 0.01$, $n = 8$.

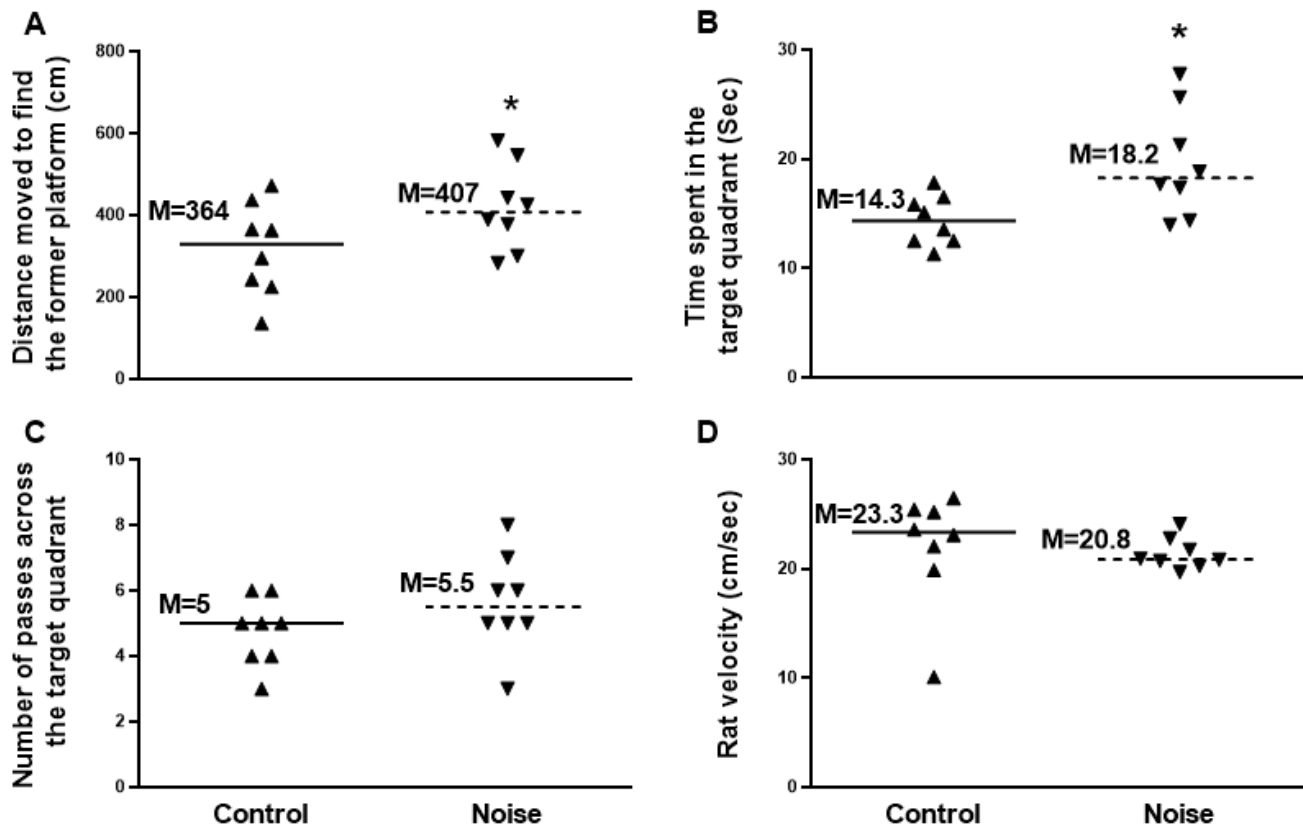


Fig.4. The evaluation of spatial memory during a 60s probe trial in MWM. The effect of traffic noise on the distance moved to find the former platform (A), time spent in the target quadrant (B), the number of times rats crossed the former platform (C) and rat velocity (D) on male rats in order to find the hidden platform in MWM in the noise-exposed group compared to the control group. The data is shown in the form of mean and median. The symbol (*) shows a significant statistical difference between the noise-exposed group compared to the control group; * $P < 0.05$, $n = 8$.

compared to the control group (in order, median=18.2, median=14.3, $P < 0.05$; Fig 4B). The number of times rats crossed the former platform and the rat velocity variables had no significant difference between the traffic noise-exposed group and those of control (Figs. 4C and 4D).

Discussion

According to the findings of the study, being exposed to traffic noise in a 10 day period can cause gaining weight in male rats. The new discoveries show that noise pollution can be an important factor for obesity as a concerning issue of today world. In fact, constant exposure to noise and various disturbs sounds results in increasing activation of brain functions which cause hunger and postpone satiety signals. The considerable note lies on increasing tendency to consume sweet and greasy food at this time; this is because noise can cause secretion of stress hormone which usually results in polyphagia (Ising

and Braun, 2000; Oladehin et al., 2007). In congruence with our present study, it is discovered that sleep deprivation caused by extreme noise pollution (less than 24 hours) does not affect weight gain and energy loss in rats; however it lasts in a chronic form (more than 3 hours), it leads to polyphagia and outbreak of obesity in rats. The presence of interrupting noises during the daytime, while the corticosterones and anti-anxiety hormones are at the minimum level, can cause a decrease in deep-sleep hours, activity, and energy and metabolism which lead to outbreak of obesity (Prior, 2002).

In the present study, the non-involved parameters in learning such as the difference in physical activity of the groups have no intervention in the amount of spatial learning in the rats. This finding is confirmed by lack of difference in the swimming velocity of rats in traffic noise exposed and the control group. In the control group, it is observed that with the decrease the distance moved to find the hidden platform during

training phase in the MWM. The decrease in traveled distance to reach the hidden platform in block III compared to block I give evidence to this note. Such a trend is not found in the rats of traffic noise exposed group which indicates that spatial learning in this group of rats is disrupted compared to the control group. Besides, spatial memory of rats in traffic noise-exposed group is also interrupted that can cause more duration and distance of travel to find the hidden platform in MWM according to Probe trials.

In 2006, Manikandan and colleagues observed that the rats' working memory in the radial maze was disturbed when the rats underwent a relatively high noise of 100dB for 4 hours per day for 30 days. In these rats, enzymes associated with oxidative stress, corticosteroid levels and also acetylcholinesterase activity is increased. They reported that this increase could disrupt working memory in the radial maze. The number of dendrite bundles in the hippocampal neurons also decreased (Manikandan et al., 2006). In addition to the type of maze used, that study is different in the type and intensity of noise from the present study. Moreover, we studied the anxiety of the rats in the elevated plus maze.

Cheng et al., (2011) subjected the mice to mild noise 80dB for two hours during one, three and six weeks. The 3 and 6 weeks animals under noise showed a reduced performance in MWM. This mentioned study is different from our study in the type of animal breeds and also the noise profile. In congruence with our present study, Barzegar et al. used urban pollution noise in their study. In their study, pregnant rats were subjected to 95dB noise for 1, 2 and 4 hours during a week. It was observed that the concentration of corticosterone in pregnant rats increased under noise. Pups were tested for behavior in postnatal days to 50. In addition to lowering of performance in MWM, induction of long-term potentiation in the hippocampus would be impaired. Also, the anxiety of their rats was increased in the elevated plus maze (Barzegar et al., 2015). The difference between the explained study and the present study was that our rats in the present study were directly subjected to urban pollution noise for 21 days.

In contrast, Liu et al., (2016) used noises with a high sound level of 123dB in their study. They studied short-term effects of 123dB intense noise for only 2 hours on spatial learning and memory of rats at age

of 6 and 8 weeks. Such noise was also used in another study (Tao et al., 2015). The effects of 2h exposure of pregnant rats to their 75-day-old pups were investigated. Liu et al., (2016) and Tao et al., (2015) observed that the spatial learning and memory of small mice are interrupted in MWM. As noted, many studies have been conducted on the effects of noise in spatial learning and memory disabilities. These studies vary in terms of different types of animal species, their age and their growth period, and also the intensity of noise and its type and the duration exposure of animals exposed to noise. In addition to the destructive effects of noise on spatial learning and memory, the present study also investigated the anxiety behaviors of rats. Previous studies (Manikandan et al., 2006; Barzegar et al., 2015; Liu et al., 2016) have shown that exposing animals to noise can lead to increased levels of corticosteroids; however we did not measure serum cortisol levels, which is one of the limitations of this study.

In all the noise pollution studies, it is emphasized that encountering any sound more than 90dB is known as a source of stress. A study by Ising and Braun reported that responses such as changes in heartbeat, blood pressure, breathing pace, gastrointestinal movements and hormone secretion from endocrines and finally the memory and learning disorders are followed by auditory stress. The impact of short-term noise exposure are usually temporary but chronic exposure to noise can cause outbreak of liver toxicity, reducing antioxidant power and increase in blood peroxidase lipid parameters of male rats (Ising and Braun, 2000; Munzel et al., 2018). The significant note in studies show that if rats are exposed to pleasurable sounds such as Mozart classical music, their spatial memory is meaningfully increased (Xing et al., 2016); therefore, aside from the intensity and frequency of a sound, each sound's type can also make a divergence of various impacts on the central nervous system. Through a complicated process, mild music can significantly stimulate auditory cortex and enhance memory and learning. The main reason for this efficacy is that these pieces are composed to draw individuals' attention and therefore, people's attention is unconsciously attracted to the music. Musical pieces with verses and vocals motivate the listener to accompany and this can disrupt learning (Jian Goa et

al., 2009). Moreover, it is discovered that long term exposure to noise pollution can cause a decreased hippocampus volume in lab animal models. These neuroanatomical findings indicates that reported learning and memory disorders caused by noise pollution can be permanent and persistent which could indeed be dangerous; hence there is an essential need to control and manage noise pollution in metropolitan areas (Prior, 2002; Manikandan et al., 2006; Cui et al., 2009).

From another point of view, animals' anxiety-like behavior in EPM showed that the rats exposed to traffic noise spend less time in the open maze and they express less curiosity which is a contribution of increasing anxiety in animals. Long-term exposure to noise pollution causes an increase in density of stress hormone such as corticosterone and cortisol in blood through increasing hormonal activity of hypothalamus, pituitary and adrenal gland (Toukh et al., 2014). Moreover, auditory stress has irrecoverable impacts on the fetus during pregnancy (Niemtzow, 1993; Haines et al., 2003). Spatial learning and memory disorder and increased anxiety is observed among neonatal rat who were born from mothers exposed to noise pollution during pregnancy; a possible reason for this prevalence could be changes in glucocorticoid receptors in centers for anxiety control (e.g. amygdala) and learning and memory process (e.g. hippocampus) due to being exposed to high corticosterone (Barzegar et al., 2015; Eraslan et al., 2015).

Conclusion

In conclusion, according to the results of this study it was distinguished that being exposed to an even short term noise pollution causes a significant increase in weight gain and disorders in spatial learning and memory, and anxiety-like behavior in male rats. Therefore, controlling noise pollution in our residential and working environment should seriously be considered as the essential presidency by civil engineers, occupational and industrial hygiene and environmental experts.

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Conflict of interest

The authors declare no conflict of interest.

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