The Relation Between the Noise Stress Effects and Neurotransmitters, Sex Hormones Levels in the Male and Female Balb/c Mice

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ABSTRACT

Many studies suggest that noise stress could damage many body systems such as nervous and endocrine systems, so the goal of this study was to demonstrate how the levels of neurotransmitters and sex hormones in mature male and female mice are affected by different noise stress exposure durations? In this study, 60 Balb/C mice (males and females) were used. Fifty mice were exposed to 90dB noise stress for 4h/daily and were scarified in different time points; 1,7,14,21, and 28 days while the rest 10 mice were not exposed to noise stress and they serve as control. After the end of the experiment, the blood was collected and the serum samples were used to determine neurotransmitters levels, serotonin (5-HT) and noradrenaline (NA), and hormones levels included testosterone, estrogen and cortisol levels. The findings showed that neurotransmitters levels were significantly higher in mice exposed to noise stress for 1,8,14, and 21 days which was non-significant decrease compared to the control in the 28-day stress group. While the levels of cortisone were continuously rising in the exposed noise stress mice groups with the length of duration, the levels of estrogen in female and testosterone in male were continuously decreasing. Interestingly, the levels of neurotransmitters and cortisol were significantly higher in females than males in all noise stress groups. Additionally, these effects completely differ after 28 days of noise exposure, which needs more future studies to prove the risk of exposure to this type of stress on the body systems.

KEYWORDS: Noise stress, noradrenaline, serotonin, testosterone, estrogen.

INTRODUCTION

Stress is a condition in which the body is subjected for competing external or internal forces that alter the homeostasis of the body [1]. Noise, the unwanted and dangerous sound levels [2], is one of the world's most common causes of environmental stress due to global technological developments such as the rise in vehicles, aircraft, factories, and others that cause noise stress [3]. The unit used to measure the sound volume is Decibel (dB). It is expressed by Frequency, which refers to the number of vibrations of air per second in which the sound propagates, in Hertz (Hz) [4]. Noise exposure is one of the major causes of acquired hearing loss in adults and children [5]. The damaging effect of noise, however, is not limited in the auditory system, but extended in many other systems [6] such as the nervous system...
since it was clearly demonstrated that the differences in medial prefrontal cortex mPFC remodeling and disruption of frontal lobe-mediated activities, such as spatial working memory, result from chronic stress [7]. Since the state of the brain determines the impact of noise on the central nervous system, it differs from person to person, for example, the compensatory mechanisms are weaker in the exhausted individual than in the rested individual [8, 9], but in general, noise activates the body's sympathetic nervous system. It may cause variation in neurotransmitter concentrations, which may lead to elevated blood pressure and heart rate and affect reproduction, mood, and body weight. These probably changes in neurotransmitters can trigger perturbed GnRH release. Chronic intermittent noise stress in mice induced ultra-structural changes in catecholamine granules in the adrenal medulla and in the fascial area of the adrenal cortex, followed by increased levels of plasma corticosterone [10]. Furthermore, Acute exposure to sound pressure over 90 dB may increase secretions of cortisol [11]. The primary sign of depression is excess cortisol, caused by an imbalance in the pituitary-adrenal (HPA) axis, resulting from frequent chronic stress. This imbalance in the HPA axis inhibits its role to interrupt the secretion of neurotransmitters and also affects the endocrine system, thus disrupting the body's balance, which may lead to the development of many stress-related diseases [12]. The natural hormone estrogen naturally declines over the menstrual cycle and its peak levels might be decreased by psychological stress. When the physical and emotional effects of stress combine, testosterone levels can drop to hypogonadism levels and the pituitary gland typically is unable to fully compensate for this drop in testosterone levels under stressful circumstances [13]. This study attempted to show how adult male and female mice's neurotransmitters and sex hormones levels were affected by 90 dB noise stress for four hours each day for 1, 8, 14, 21 and 28 consecutive days.

MATERIALS AND METHODOLOGIES
In this study which was achieved between 1st November 2019 and 1st March 2020, 60 adult Balb/C mice (male and female) were used. The Ministry of Higher Education and Scientific Research/Iraqi Center for Cancer Research and Medical Genetic supplied the mice. These mice were 6 to 8 weeks old and weighed 20 to 28 g. Fifty mice were subjected to broad band white noise (20-20,000Hz) at 90 dB intensity after two weeks of acclimatization under standard settings, including steady temperature and a 12-hour light-dark cycle [2], by an android application which generate the frequency of sound and measure its intensity by sound level meter. Then the multimedia speaker (BK_868) was fixed 30 cm above the animal cages and connected with the phone by Bluetooth. These animals remained in the stress room during their period of exposure to stress in order to spare them from further stress that wasn't necessary. However, 10 mice (5male and 5male) were left undisturbed without exposure to stress and served as the control group.

Mice exposed the noise stress were divided into 5 groups (n=10, 5male and 5female): mice were exposure the noise stress for 4 hours and sacrificed at the same day; for 8 days and sacrificed at the 9th day; for 14 days and sacrificed at the 15th day; for 21 days and the mice were sacrificed at the 22th day; for 28 days and sacrificed at the 29th day. After the end of the experiment, mice were sacrificed by cervical dislocation in different time points as mentioned above. Blood samples were taken from the eyes and placed in sterile gel tubes that allowed for coagulation. The serum was separated from the blood sample by centrifuging it at 10,000 rpm for 10 to 15 minutes. It was maintained at -20 C until the hormone and neurotransmitter levels were determined.

The neurotransmitters study included the determination of the Serotonin and Noradrenaline levels in the mice serum by sandwich enzyme-linked immune sorbent assay technique using serotonin and noradrenaline kits from shanghai biological company/China.

The hormonal study included the determination of serum levels of testosterone in males and estrogen in females and cortisol in both gender by Cobas e 411 analyzer using the kit from Roshe Company/Germany.

RESULTS AND DISCUSSION
Table 1 shows the comparison of the mean ± SD Noradrenalin (NA) and Serotonin (5-HT) among different study groups by comparing the control group with other groups exposure stress for the different duration; 1, 8, 14, 21, and 28 days and by comparing the male with female groups.
There was a highly significant (P≤0.01) increase in NA levels in all stress groups. Interestingly, NA levels increased gradually in the stress groups; 1, 8, and 14 days (241.9±30.0, 245.9±17.4, and 257.9±9.9 ng/ml, respectively in male and 371.9±78.6, 406.9±30.6, and 500.2±191.9, respectively in female) until they reach their highest point in the stress group (21 days) (276.6±64.4 ng/ml in male and 536.4±110.8 in female), then return to decreased in 28 days stress group (236.8±19.3 ng/ml in male and 257.9±9.9 in male and 536.4±110.8 in female) compared to control group (197.4±22.1 ng/ml in male and 204.4±50.3 in female).

Comparable results were obtained at the level of 5-HT at the same time points. 5-HT levels increased significantly (P≤0.01) in all stress groups. 5-HT levels increase gradually in the stress groups; 1, 8, and 14 days (37.1±8.2 in female) compared to control group (23.4±3.3 ng/ml in male and 30.6±5.5 in female). Interestingly, the levels of NA and 5-HT were about 1 to 2 times higher in female compared to male in all noise stress groups.

Table 2 shows the comparison of the mean ± SD Cortisol in males and females among different study groups by comparing control group with other groups under noise stress for different time duration; 1, 8, 14, 21, and 28 days and by comparing female with male groups. Data in these tables clearly showed highly significance (P<0.01) increased in cortisol levels in all stress groups; 1, 8, 14, 21, and 28 days stress group (3.810±1.310, 4.220±1.315, 9.570±0.907, 13.448±1.606, 19.480±3.348 nmol/L, respectively in male and 13.072±1.792, 22.304±1.380, 16.994±0.504, 45.782±1.505, 39.462±1.912 nmol/L, respectively in female) compared to control groups (3.378±1.167 in male and 3.984±1.484 in female). These dates were significantly (P<0.01) higher in female groups compared to male groups.
stress group (1.891±0.86, 1.261±0.015, 1.464±0.035, 0.166±0.003, 0.025±0.001 ng/ml, respectively) comparing with control group (2.615±0.011 ng/ml). Estrogen level was decreased gradually in all female stress group; 1, 8, 14, 21 and 28 days stress groups (7.780±1.418, 5.390±0.560, 4.700±1.335, 4.320±1.231 and 3.130±0.351 pg/ml, respectively) compared to the control group (9.900±1.460 pg/ml).

| Table 3. Effect of the Stress on the levels of testosterone in the Male and estrogen in Female Mice |
|---|---|---|---|
| Groups | Estrogen pg/ml in female (mean ± SD) | Testosterone ng/ml in male (mean ± SD) | P value |
| Control | 9.900±1.460 e | 2.615±0.011 a | <0.01 |
| Stress Exposure groups | | | |
| 1 day | 7.780±1.418 d | 1.891±0.086 b | |
| 8 days | 5.390±0.560 c | 1.261±0.015 b | |
| 14 days | 4.700±1.335 b | 1.464±0.035 c | |
| 21 days | 4.320±1.231 b | 0.166±0.003 d | |
| 28 days | 3.130±0.351 a | 0.025±0.001 c | |
| LSD | 0.180 | 0.095 | |

A significant difference between means in columns is shown by a different letter (p≤0.01), but a non-significant difference between means in columns is indicated by a similar letter (p>0.05)

DISCUSSION SECTION
Stress is known to enhance the function of the hypothalamic-pituitary-adrenal axis and contribute to increase corticosteroid secretion from the adrenal cortex, so in both mice and humans, cortisol is utilized as a measure of the activation of stress [14, 15]. It is well known that the stress status effect on sensory nervous which stimulates HPA axis to release corticosterone as end production. Shafiei et al., (2017) demonstrated that the exposure groups' corticosterone levels were higher than those of the control group [16]. Additionally, the level of corticosterone raised as noise stress intensity increased. The rate at which the cortisol levels changed suggested that exposure to noise stress can have negative effects on mice and elevate their stress levels. Also, Kaiser et al., (2015) reported that stress is increased secretion, of cortisol hormone and heart rate in tree frogs [17]. Further research revealed that exposure to noise exceeding 90 dB raised blood sugar and cortisol levels in mice [18]. These evidences agreed with present results of cortisol levels which indicated an increase with the stress levels. These results also agreed with Yin et al., (2007) as they found that after 21 days of unexpected stress, rats were much more likely to have higher plasma cortisol levels [19]. Also, it is agreed with the observation of Bhat et al., (2007) who observed that after 21 days of chronic restraint stress in female mice, blood cortisol levels increased in rats [20]. On the other hand, noise pollution impacts the testicular tissue's morphometric characteristics, including ACTH, cortisol, and testosterone. It increases cortisol and ACTH level and decreases testosterone level as reported by Farzadinia et al., (2016) who demonstrated that noise stress reduced testosterone levels and raised ACTH and cortisol in male rats exposed to 95, 105, and 115 dB noise [21]. In the same study, a histological slice of the testis demonstrated a slight but non-significant decrease in the diameter of the seminiferous tubules. However, Chamkori et al., (2016) also reported that the problem of sterility and infertility resulting from noise pollution (119 dB group) caused significantly reduced concentration of the testosterone hormone and significantly increased the concentration of the cortisol and ACTH hormones compared to the control group [22]. These results are similar to results of current study which showed decrease in testosterone of the male mice group exposed noise stress. Moreover, the increased levels of cortisol during times of stress cause the decline in testosterone levels because of the activity of glucocorticoid receptors in Leydig cells and because of the cells' diminished response to LH. These increased levels are caused by the secretion of ACTH, which is stimulated by neurotransmitters which increased in the mice exposed noise stress as it was shown in the current study. Germ cell atrophy is caused by the decreased testosterone levels, which halt and alter spermatozoa maturation [23]. The finding of other study shows that increased levels of the stress hormone cortisol in the bloodstream suppress gonadotropin-releasing hormone (GnRH) production and stimulate the production of the gonadotropin inhibitory hormone rfmide related peptide (RERP), which also suppresses GnRH production and decreases pituitary secretion of LH and FSH [24]. These in turn cause decrease levels of the estrogen and progesterone in the
experimental exposed group, moreover at the time of implantation, noise pollution alters ovarian histological follicles and endometrial receptors [16]. In the current study, the estrogen hormone concentrations in female mice decline with increase the days of exposure to stress, these findings were similar to other study that showed a significant decrease in the level of estrogen in mice groups exposed noise stress compared to control groups [16]. Thus, a common response to stress in the modern world is an alteration in the serum levels of different hormones, such as testosterone, estrogen, progesterone, LH, and FSH. However, long-term exposure to noise stress can have numerous negative effects, including the development of various endocrine problems [16]. The explanation mentioned before regarding hormones is agreed with results of current study where increased cortisol and decrease testosterone, and estrogen with stress levels. Hajj-Dahmane, and Shen (2011) demonstrated that corticosteroid hormones interact with various neurotransmitter systems, e.g., serotonin, dopamine, endocannabinoids, and noradrenaline [25]. Because corticosteroid hormones are very lipophilic and therefore easily pass the blood-brain barrier, in principle reaching all cells and stimulates neurotransmitters production [26]. Interestingly, depending on the facts that: 1) NA has role reorienting of attention and in cognitive flexibility by enhances the processing of sensory in puts, the attention, the formation and retrieval of both long term and working memory, and the ability of brain respond to inputs by changing the activity pattern in the prefrontal cortex and other areas [27]. 2) Serotonin is an important neurotransmitter in the central nervous system where it modulates circuits involved in mood, cognition, movement, arousal, and autonomic function [28]. Serotonin is involved in regulating mood and emotion, it also contributes to feeling of well-being and happiness [29]. Thus, in case of flight to flight action that occurs during distress, the interaction of cortisol and these neurotransmitters enhances the body to be more focused on the surrounding environment, by stimulating senses memory, sight, hearing, and other senses become sharper [27]. The same interpretation was suggested by Kirby et al., 2013 who suggested that the combination of neurotransmitters and hormones that stress produces, including estrogen, testosterone, dopamine, and serotonin, as well as adrenaline, cortisol, and norepinephrine, could help people perform better under pressure and explained that as: The brain receives more oxygen, which boosts alertness and improves memory, sight, hearing, and other senses. Since the combination of these hormones and neurotransmitters enhances the body’s response to challenging circumstances, moderate stress is beneficial. It may even encourage the creation of new brain cells in the learning centers [30]. All these evidences could explain the increase levels of NA and 5-TH in the mice exposure noise stress until 21 days showed in present study. However, Kirby et al., 2013 showed that after 30 days of stress, long-term exposure to stress reduced levels of the neurotransmitter, which was consistent with the findings in current study [30]. This resulted in extremely severe disorders such attention deficit hyperactivity disorder (ADHD), depression, and hypotension (very low blood pressure) [31]. This study showed difference in the changing of cortisol and neurotransmitters levels in the female more than male, as these parameters were increased in the female than male groups under stress. Numerous studies have found evidence of sex variations in the hypothalamus's activation. Zavala et al (2011) claimed that when compared to men, female rats showed increased activation in the hypothalamus following both acute and long-term stress [32]. It is well known that the difference between genders is the sex hormones especially, estrogen in the females and testosterone in males. Many researchers explain the role of estrogen in the stress condition of body which does not happen with testosterone. Iwasaki-Sekino et al (2009) demonstrated how the HPA axis is affected by the estrous cycle. In contrast to males, female rats in the proestrus phase, which is marked by greater serum levels of progesterone and estradiol, exhibit excessive activity in the paraventricular nucleus of the hypothalamus following an acute stressor [33]. Weiser and Handa (2009) provided the following explanation for this fact: Estradiol elevates blood ACTH and corticosterone while weakening dexamethasone suppression through the hypothalamic estrogen receptor, and the estrogen receptor α isoforms seem to play a significant role in HPA control [34]. Sierksma et al. (2013) demonstrated that corticosterone neuroendocrine secretion appears to be sex-specific because female
mice had significantly greater plasma corticosterone concentrations under resting, stressed, and recovery conditions than male mice, regardless of exposure to stress [35]. Additionally, evidence suggests that estrogen influences neurotransmission, synaptic plasticity, and neurogenesis [36]. The hippocampus is known as one of the important areas of the so-called emotional brain due to its function in modulating anxiety levels, in addition to cognitive processes and emotional reactions via the hippocampus [37]. According to several studies, gender differences in the brain's arousal regions predispose women to diseases where hyperarousal is a key sign of stress. The locus coeruleus (LC), which regulates arousal levels by releasing norepinephrine (NE) into forebrain regions, is one candidate site for such sex differences [38]. These evidences could explain the differences in cortisol and neurotransmitters levels that were shown in this study since these levels were higher than 2 folders in the females compared to male.

**CONCLUSIONS**

By the end of this study, it is concluded that:

1. The noise stress effect on sensory nerves which stimulate HPA axis to raise cortisol level in the blood which lead to endocrine disorders which result in a continuous decrease in testosterone and estrogen hormone levels.

2. The noise stress has a sympathetic hyperactivation effect by increasing the levels of NA and 5-HT, but the long-term exposure of noise stress, more than 28 days, could cause sympathetic hypoactivation by decreasing NA and 5-HT.

3. Female groups were more affected by stress than male groups, since the cortisol and neurotransmitters levels were significantly higher in the female compared to male.

These findings need further research, especially on the effect of chronic stress. Since the results of neurotransmitter levels in 28 days stress group were headed in a different direction than the shorter stress exposure groups, longer period than what has been studied in this work need to be studied to prove the risk of exposure to this type of stress and adaptation occurs with increasing stress duration.

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**REFERENCES**


