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Effect of acute and repeated noise exposure on the behaviour and lipid peroxidation in brain tissue of male and female mice

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Abstract: Despite the increasingly obvious sex differences, male animals are still predominantly used to study stress and related disorders. The aim of this study was to evaluate the effects of repeated noise stress in female and male mice. In the experiment, 12 male and 12 and female SPF/VAF 6-week-old Crl:CD1(lcr) mice were used. After 5 days of acclimatization all animals were placed in the open-field for three consecutive days where each mouse spent 10 min daily without any noise treatment in order to get used to the circumstances of the test.

On day 9 to record baseline behaviour the animals were placed on an open-field for 3 min and a noise mixture was played to them at 90 dB. Video recordings of the tests were made. After testing the animals were returned to their original cages and from then the noise group received noise treatment for 10 h daily. The noise mixture used for the open-field and for habituation contained similar but not identical noises. The open-field test was repeated on day 16 and day 23. At the end of the experiment the animals were weighed and—after intraperitoneal pentobarbital anaesthesia— were bled out, and their brains were removed under icy conditions. Pathological examination and histopathological sampling (spleen, thymus, adrenal glands, gonads) were performed. Because the state of the lipid peroxidation is a sensible indicator of the brain health, after homogenization the brains were frozen, then redox parameters (induced free radical levels, H-donating ability, and induced lipid peroxidation) were determined by luminometric and spectrophotometric methods. During the design and implementation of the study, we considered the EU Directive 2010/63/EU and the Hungarian Government Decree 40/2013 (14.II.) on Animal Experiments. The studies were approved by the Animal Welfare Body of the University of Veterinary Medicine Budapest under PE/EA/1277-5/2017. In our study noise treatment did not cause significant changes in the animals' behaviour. No histopathological lesions were detectable, and the bodyweight of the animals was not affected. In the control groups males had less free radical formation in the brain than females. However, the number of free radicals in the brains of females was significantly reduced by the noise treatment, whereas in males it was practically unchanged.

Our results show that noise habituation and noise exposure in the open-field alone did not induce stress that was manifested in clinical and pathological signs. However, the effects of the treatments were reflected in lipid peroxidation in the brain. Our results also indicate that noise habituation significantly improves noise tolerance in female mice, but not in males.

Key words: Noise, stress, sex, mice, brain, lipid peroxidation

1. Introduction

Noise is one of the most dangerous environmental stressors of our time because it causes somatic changes that go unnoticed. Research confirms that noise above 90 dB is a harmful stressor, affecting the immune and endocrine system. Blood pressure and heart rate increase, ischaemic heart disease develops more quickly and mental changes are observed [1].

It is well known that the stress response leads to the production of reactive oxygen species (ROS) in tissues, which damage receptor proteins, nucleic acids and lipid membranes, damaging cells and tissues both structurally and functionally [2]. McIntosh and Sapolsky [3] found that chronic stress and stress-induced production of glucocorticoids affect ROS formation and increase their amount by approximately 10%. Various stressors are potent in increasing the number of free radicals in the brain [4]. Since the brain contains large amounts of polyunsaturated fatty acids, it is particularly vulnerable to the damaging effects of free radicals, and thus to stress-induced degenerative changes [5]. Accordingly, several previous studies have addressed the relationship between stress and lipid peroxidation processes in the brain [6].

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To neutralise ROS the body uses various enzymatic (e.g., copper, manganese and zinc superoxide dismutase, catalase, glutathione peroxidase) and nonenzymatic antioxidants (e.g., glutathione). Sahin and Gümüslü [7] found higher activity of antioxidant enzymes, decreased glutathione and increased plasma corticosterone levels in rats exposed to different stressors.

It has been shown in several species, including humans, that stress does not affect the two sexes equally. Women are twice as likely as men to develop stress-related major depressive disorder and anxiety [8, 9, 10]. This sex difference extends not only to prevalence but also to symptom severity and treatability [11, 12]. While women have a higher prevalence of anxiety disorders and depression, men are at higher risk of autism and schizophrenia [13, 14]. This phenomenon is likely to be driven by sex hormones and their receptors in the brain [15], but the difference is already evident in the prenatal period [16]. It has also been shown in rodents that the two sexes are not equally sensitive to stress [16, 17, 18]. When exposed to early life stress, female mice exhibited depressive symptoms, but males did not, and female symptoms were treatable by antidepressant administration [19]. Similar results were obtained in mice exposed to subchronic stress, where females showed depressive behaviour, but males did not. In addition, stress in the female brain increased the expression of the enzyme DNA methyltransferase 3a in the nucleus accumbens, which has been shown to be associated with stress-induced depression in humans [20]. In a forced-swim test adult female rats spend more time immobile than males, but separation from other rats, for example, caused depressive symptoms in males but not in females [21]. The defeat stress causes depressive symptoms in adult male rats, but not in females [22, 23]. These data suggest that male rats are more sensitive to social stress than females.

The difference is not just in behaviour, of course. Chronic immobilization stress reduced neurogenesis in the gyrus dentatus of adult female rats, while it enhanced it in males [24]. Adult social stress induced a similar effect in the dorsal hippocampus, with decreased cell proliferation in that area in female rats [25] and increased cell proliferation in males [26]. In the background of the social isolation feeling of females the amylin-calcitonin receptor signalling pathway was found in the medial preoptic area of the forebrain [27]. These findings suggest that stress in female animals is more likely to reduce neurogenesis in adulthood than in males.

Sex hormones have multiple receptors on brain neurons and even on glial cells; therefore, they influence their structure and function at molecular and cellular levels, affecting behaviour, cognitive function, blood pressure regulation, pain perception and may also have neuroprotective effects [28]. In addition, progestins, androgens and glucocorticoids can bind to each other's receptors [29]. In humans, both male and female sex hormones influence mood [30, 31]. But a similar process has also been demonstrated in animals [32]. Moreover, in females sex hormone levels are constantly changing due to cyclical gonadal function. Viau and Meaney [33] found that female rats are most sensitive to stress effects during the proestrous period. However, it is not only sex hormones that affect brain function, but also sex chromosomes [34].

Unfortunately, despite the increasingly obvious sex differences, male animals are still predominantly used to study stress and related disorders. This is due to the cyclical gonadic functioning of females, which introduces considerable variability in studies [35]. Such disregard for the impact of sex differences in scientific experiments is a serious obstacle to translation and makes it difficult to treat many diseases in practice [36]. The aim of this study was to evaluate the effects of repeated noise stress in female and male mice.

2. Materials and methods

In the study 12 male and 12 female SPF/VAF, CD1 mice (Crl:CD1(Icr), Animalab Hungary Kft.) were used. The animals were 3 weeks old at the beginning of the experiment. The mice were housed in T2 type polycarbonate boxes (Acéllabor Kft.), 330 × 160 × 137 mm, and Abedd Aspen Bedding (Animalab Hungary Kft.) wood chipping was used as bedding material. Drinking water and feed (1314 EN Breeding diet for mice and rats, Animalab Hungary Kft.) were available ad libitum. The 2 groups were randomly selected, 6 females and 6 males were assigned to the noise group (N) and 6 females and 6 males to the control group (C). Animals in the same group (6 males and 6 females) were housed in the same room in separate cages. The control group was in a quiet, isolated room, and the noise group listened to a 70 dB noise mix in a different room for 10 h a day (between 8 a.m. and 6 p.m.) from day 9 onwards. The list of noises used is shown in Table 1.

For the first 5 days, no tests were done to let the animals adapt to the new environment. Then, for three consecutive days, the acclimatisation to the open-field (OF) test was carried out by placing each animal individually in the OF for 10 min a day in a quiet room. The vivarium used for the OF test was a 60 × 27.5 × 30 cm glass apparatus.

The first experiment was performed on day 9 (recording of baseline behaviour). Then, the animals were placed one by one on the same OF as during habituation for 3-3 min and a noise mixture was played to them at a sound pressure level of 90 dB. Video recordings of the OF tests were made and analysed using Noldus Observer XT software. After the test, the animals were returned to

Table 1. List of noises used for habituation and open-field tests.

Noises for habituation	Wind and rain	Sewing machine
Ship horn	Smoke detector	Squeaking door and gate
Shovelling snow	Shovelling sand	Wind chime
Factory siren	Coin spinning and dropping	Ducks, geese
Rain and thunder (multiple)	Banging on a door	Waste compactor
Rain	Saw	Dropped metal container
Siren (multiple)	Whiplash	Door knocker
Air alarm	Pencil and eraser sound	Rain sound on umbrella
Zipper	Shattering glass	Garage door opening
Crickets	Phone ringing (multiple)	Knocking on a door
Frogs	Bees	Forest fire
Compressor	Electric can opener	Broom sweeping
Beer mug sliding on the counter	Wind blowing	Human heartbeat
Fireworks	Sneezing woman	Knocking on wooden door
Bell	Blender	Elevator bell
Laughing man and woman	Digital printer	Morse code
Puma	Chimpanzees fighting	Rocket
Opening, closing briefcase	Computer keyboard	Scratching
Closing a filing cabinet	Hammer	Shower
Screaming woman	Irrigation system	Waste disposal
Doorbell	Snoring man	Vacuum cleaner
Noises for the open-field tests		
Siren	Hammer	Passing helicopter
Factory siren	Rain and thunder	Aircraft taking off
Telephone ringing		

their original location and from then on, the noise group received noise treatment for 10 h a day. The noise mixture used for the OF and for the habituation contained similar but not identical noises. The OF test was repeated under the same conditions on day 16 (week 1) and day 23 (week 2). During the tests, we observed the position of the animals in the OF (centre, corner, edge), their locomotor activity (moving, sitting, rearing) and some other behavioural cues (listening, sniffing, self-grooming).

After the final OF tests, the mice were weighed, then bled out after anaesthesia with i.p. pentobarbital injections (Euthanimal 40%, 400 mg/mL, Alfasan Nederland BV), and finally the brains were removed under icy conditions. Dissection was performed, during which histological samples were taken from the thymus, spleen, adrenal glands, testes, and ovaries.

Whole mouse brains were homogenized in ice-cold isotonic KCl solution with Potter-Elvehjem homogenizer and protein content was set to 10 mg/mL and bovine serum albumin was used as a standard. Protein concentration was measured by the method of Lowry et al. [37] using serum albumin and 50–100 µL volumes were taken from these samples.

For the total scavenger capacity measurement the reaction mixtures consist of H_2O_2/·OH, microperoxidase and luminol. In detail, the composition of the reaction mixture is as follows: 300 µL hydrogen peroxide (10,000 dilutions), 300 µL microperoxidase ($3 \times 10 - 7$ M) 50 µL luminol ($7 \times 10 - 7$ M), the sample is in 50 or 100 µL. Total volume is 850 µL. The intensity of the chemiluminescence light is given as the relative light unit (RLU) reduced by tissue homogenate. In this system, free radicals are generated and the examined brain homogenate antioxidant capacities inhibit oxidative reactions. Measurements is carried out with Berthold Lumat 9501 luminometer in 30 s reaction time [38].

The H-donor activities of samples were determined with the help of 1,1-diphenyl-2-picrylhydrazyl (DPPH) stable free radical by the modified spectrophotometric method of Hatano et al. [39]. For the determination, the 50 µL brain homogenate (protein content: 10 mg/mL) was adjusted with 950 µL bidistilled water to 1 mL volume. After, 1 mL methanol was added to it. After this 500 µL methanolic DPPH-solution (9 mg DPPH was dissolved in 100 mL methanol) was added, and stirred. The reaction mixture was incubated for 30 min at 37 °C. Ten-minute centrifuging was carried out (3000 rpm) and the absorbance was determined at 517 nm with methanol blank.

The modified Ottolenghi [40] method was used to study the induced lipid peroxidation in brain homogenates. The reaction mixture contains tris-maleate buffer (0.05 M, pH 6.8), KH_2PO_4 (1 mM) and brain homogenate with 1 mg/mL protein content, Fe^{2+} (0.2 mM), and ascorbic acid (5×10^{-3} M) to induce lipid peroxidation (LPO). Total volume is 0.5 mL (adjusted with bidistilled water). Samples were incubated at 37 °C for 20 min. After induction of lipid peroxidation 0.4 mL was mixed with 2 mL 2-thiobarbituric acid solution (1%). The samples were placed in boiling water for 20 min, then centrifuged (3000 rpm) at 4 °C. The extinction of supernatant was determined spectrophotometrically at 535 nm.

For the tests serum bovine albumin, luminol, hydrogen peroxide, microperoxidase, 1,1-diphenyl-2-picrylhydrazyl (DPPH), maleic acid were obtained from Sigma-Aldrich (St. Louis, MO, USA). All the other reagents in the analytical grade were purchased from Reanal (Budapest, Hungary).

The normal distribution of raw data was evaluated using the Kolmogorov-Smirnov test. Comparative statistical analysis was carried out by one-way ANOVA followed by posthoc Tukey HSD test based on the theory described by Petrie and Watson [41]. The statistical software "R" has been used. Differences between the groups were considered significant in all cases at the level of $p < 0.05$.

During the design and implementation of the study, we considered the EU Directive 2010/63/EU and the Hungarian Government Decree 40/2013 (14.II.) on Animal Experiments. The studies were approved by the Animal Welfare Body of the University of Veterinary Medicine Budapest under PE/EA/1277-5/2017.

3. Results and discussion

3.1. Results

Our results show that the three-week noise treatment did not cause clinically manifested stress in the animals. Animal growth was not affected by the noise treatment. Males weighed 8.9 ± 2.5 g more than females in the control group and 9 ± 1.8 g more than females in the noise-treated group, which is natural in mice. No significant differences in organ weights were found between the groups (Table 2). Neither pathological nor histological examination revealed any stress-induced lesions in the animals. Figure 1 shows the histological structure of the adrenal glands, while Figure 2 shows the spleen.

The treatment affected the lipid peroxidation status of the brain. In the control group, fewer free radicals were formed in the brains of male animals than in females. At the same time, noise habituation resulted in lower levels of lipid peroxidation in the brains of females than in control females but had no effect in males (Figure 3).

In the open-field tests, the animals spent most of their time in the corner of the open-field and least in the edge of the apparatus. No significant differences were found between groups in terms of positioning on the OF (Table 3).

For other behavioural elements, no significant differences were found between treatment groups and the different time points of the OF tests. The exact data is shown in Table 4.

3.2. Discussion

Research to date has shown that the adverse effects of noise are clear, it acts as a stressor. Therefore, noise is an additional variable in scientific research, the control of which is essential to meet the requirements of the third R, refinement. In animal houses, there are many noise sources: the various machines (from ventilation systems to computers and telephones), the animals, the staff working there, and the various operations all generate noise to a greater or lesser extent. But noise can also come from outside of the building, whether artificial (e.g., the sound of transport) or natural (e.g., a storm). The main source of noise in animal housing, however, is the human activity; animal housing areas are usually quiet when people are not present [42]. The aim of the present study was to gain insight into the effects of prolonged noise treatment on

Table 2. Weight of animals and absolute weight of organs at the end of the experiment (grams, mean ± SD).

Group	Weight	Thymus	Testis	Spleen	Liver
Control female	27.3 ± 1.6	0.16 ± 0.07		0.14 ± 0.03	1.46 ± 0.21
Control male	36.2 ± 3.4	0.18 ± 0.07	0.41 ± 0.09	0.13 ± 0.03	1.90 ± 0.25
Noise female	28.5 ± 1.5	0.10 ± 0.04		0.13 ± 0.01	1.22 ± 0.29
Noise male	37.5 ± 2.1	0.21 ± 0.05	0.47 ± 0.06	0.11 ± 0.02	2.12 ± 0.27

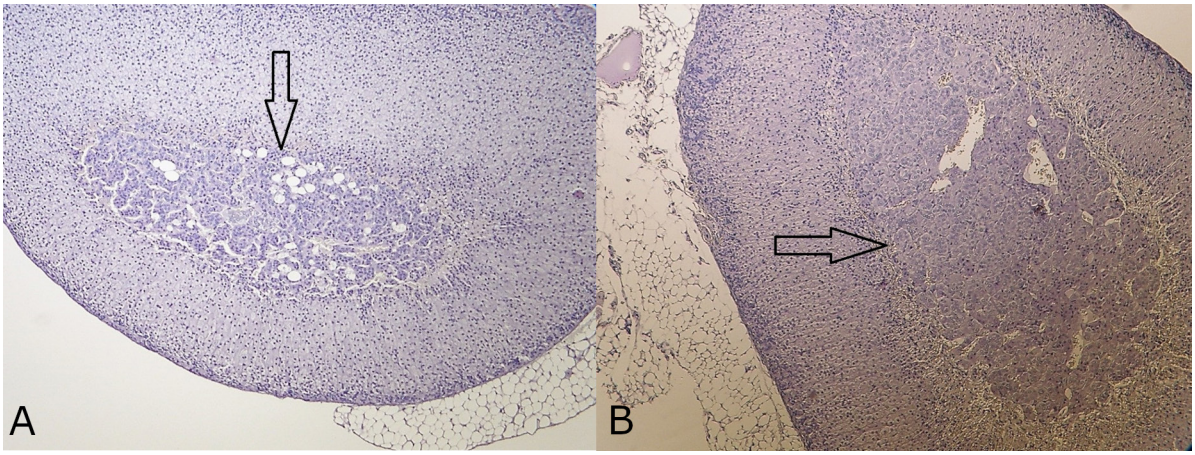


Figure 1. Histological picture of the adrenal glands of noise treated (A) and control (B) males. The arrow marks the border between the cortex and medulla. Hematoxylin and eosin staining, original magnification ×100.

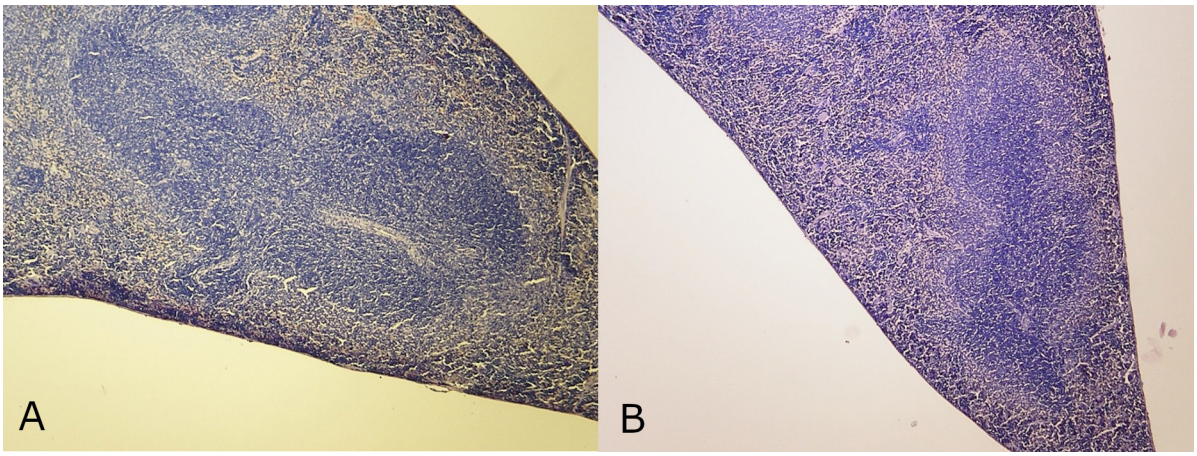
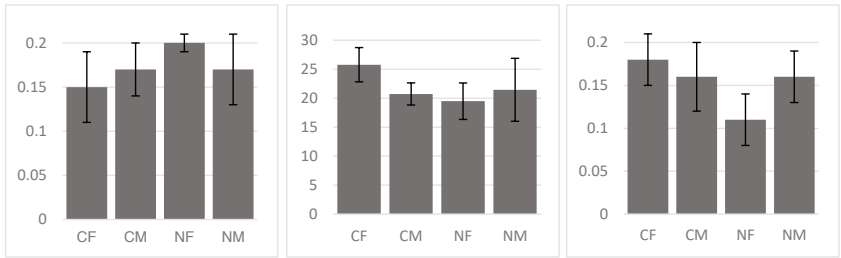


Figure 2. Histological picture of the spleen of noise treated (A) and control (B) males. Hematoxylin and eosin staining, original magnification ×100.

animal behaviour, noise tolerance and the formation of free radicals in the brain.

Stress activates the sympathetic nervous system and increases the synthesis of catecholamines [43]. During the metabolism of catecholamines such as dopamine or noradrenaline in the brain, free radicals are produced [44], and these compounds can undergo autooxidation, which releases electrons that promote ROS formation [45]. To neutralise these, the body produces enzymatic (e.g., copper and zinc superoxide dismutase, catalase, glutathione peroxidase) and nonenzymatic antioxidants (e.g., glutathione). Sahin and Gümüslü [46] compared oxidative parameters of different organs in animals exposed to cold stress. Among the organs studied (brain, liver, kidney, heart), the highest catalase activity was measured in the brain. In their later study, the activity of antioxidant enzymes increased in the brain of animals exposed to different stresses, while the concentration of glutathione decreased [7].

Free radical levels in the brain correlate with neurotransmitter synthesis. Mental stress in mice increases lipid peroxidation activity in the brain, but no such change is measured in the liver or serum [47]. These findings suggest that lipid peroxidation in the brain might be one of the first measurable signs of stress. Nitric oxide (NO) is one of the signal transmitting molecules of the central nervous system and chronic stress enhances its synthesis by increasing NOS (nitric oxide synthase) expression [6], as stress triggers the overproduction of the neurotransmitter glutamate [48]. Overproduction of NO is toxic to cells because it may form peroxynitrite with superoxide radicals, a highly reactive molecule that damages DNA, lipids, and proteins. Estradiol has a regulatory role in the production of NO by reducing the expression of NOS in



	DPPH		Ind. Chemilum.		Malonaldehyde	
CF	0.15 ± 0.04	a	25774657 ± 2956030	b	0.18 ± 0.03	b
CM	0.17 ± 0.03	ab	20729282 ± 1910157	ab	0.16 ± 0.04	ab
NF	0.20 ± 0.01	b	19477381 ± 3143630	a	0.11 ± 0.03	a
NM	0.17 ± 0.04	ab	21445720 ± 5430886	ab	0.16 ± 0.03	ab
Post-hoc	Df = 3,19 F = 3.304 P < 0.05 CF vs. NF P < 0.05		Df = 3,20 F = 3.47 P < 0.05 CF vs. NF P < 0.05		Df = 3,20 F = 5.075 P < 0.01 CF vs. NF p = 0.01	

Figure 3. Effect of noise on lipid peroxidation in the brain. DPPH: H-donor activity (Abs 517 nm), Ind. Chemilum.: induced chemiluminescence intensity (RLU), Malonaldehyde: level of lipid peroxidation (Abs 535 nm). CF = control female, CM = control male, NF = noise treated female, NM = noise treated male.
Statistics: one-way ANOVA and posthoc Tukey HSD test. Different letter markings indicate significant differences ($p \leq 0.05$).

Table 3. Time spent in the different parts of the open-field during the three tests (seconds, mean ± SD).

Basic behaviour	Corner	Edge	Middle
Control female	105.31 ± 12.67	58.71 ± 10.16	15.98 ± 7.72
Control male	104.26 ± 25.09	63.09 ± 19.48	12.65 ± 6.57
Noise female	100.20 ± 11.91	67.97 ± 12.25	11.84 ± 3.59
Noise male	94.08 ± 22.97	69.12 ± 18.16	16.80 ± 9.02
Week 1			
Control female	116.74 ± 29.65	57.66 ± 26.46	5.60 ± 4.10
Control male	98.07 ± 27.30	72.05 ± 23.26	9.87 ± 6.05
Noise female	100.64 ± 13.63	70.88 ± 9.42	8.48 ± 6.72
Noise male	107.37 ± 34.53	63.44 ± 27.57	9.19 ± 11.15
Week 2			
Control female	114.25 ± 27.90	61.04 ± 27.75	4.71 ± 4.11
Control male	91.59 ± 16.53	82.79 ± 18.34	5.62 ± 2.55
Noise female	116.29 ± 13.84	56.93 ± 12.57	6.79 ± 4.69
Noise male	88.47 ± 26.43	75.16 ± 22.50	16.37 ± 8.70

the hippocampus via beta estrogen receptors. In a study by Hu et al. [49] stress increased glucocorticoid-dependent NO production in the hippocampus of males but not females. Moreover, estrogen itself has antioxidant effects [50], which also indicates that stress-induced brain lipid peroxidation may show sex differences.

In this study we used whole brain homogenates, because according to Baek et al. [51], the sensitivity of different brain areas to oxidative stress is different, so the sampling location would greatly influence the results. Therefore, it is common practice to homogenize the entire brain tissue in one homogenate [2, 6, 7].

Table 4. Effect of treatment on the incidence of the different behavioural elements (seconds, mean ± SD).

Basic behaviour	Listening	Grooming	Sniffing	Moving	Sitting	Rearing
Control female	29.82 ± 9.09	7.60 ± 3.32	19.15 ± 9.78	99.21 ± 15.73	24.26 ± 12.74	56.53 ± 17.58
Control male	19.15 ± 15.10	12.78 ± 6.90	14.16 ± 3.77	91.48 ± 17.04	20.70 ± 12.85	67.82 ± 19.89
Noise female	20.81 ± 15.03	4.14 ± 3.04	13.06 ± 7.96	93.82 ± 15.10	12.19 ± 8.72	73.98 ± 12.29
Noise male	23.94 ± 13.13	9.73 ± 7.90	11.13 ± 2.48	100.21 ± 7.52	17.64 ± 13.56	62.15 ± 16.56
Week 1						
Control female	24.51 ± 6.54	13.91 ± 11.61	14.16 ± 8.79	84.99 ± 17.64	42.87 ± 19.94	52.14 ± 16.64
Control male	15.08 ± 0.11	14.13 ± 16.26	24.18 ± 11.74	86.98 ± 20.51	25.87 ± 20.95	67.15 ± 25.56
Noise female	12.47 ± 4.99	7.43 ± 4.06	16.70 ± 13.39	90.62 ± 20.42	27.73 ± 19.17	61.64 ± 14.49
Noise male	25.97 ± 13.93	12.71 ± 6.82	12.03 ± 4.79	97.29 ± 20.88	31.48 ± 17.78	51.23 ± 17.17
Week 2						
Control female	25.32 ± 17.74	7.56 ± 5.29	12.77 ± 7.91	90.26 ± 39.87	35.58 ± 36.86	54.16 ± 20.97
Control male	15.71 ± 4.66	6.70 ± 3.94	28.98 ± 12.76	102.36 ± 6.23	17.15 ± 10.70	60.49 ± 11.89
Noise female	27.38 ± 19.84	10.04 ± 5.32	25.10 ± 12.11	109.08 ± 25.33	28.72 ± 20.08	42.20 ± 19.23
Noise male	26.44 ± 14.60	8.33 ± 4.77	20.18 ± 9.76	106.32 ± 12.20	25.43 ± 21.72	48.25 ± 18.74

When examining the brain tissue, we found that more free radicals were formed in the brains of female control mice than in control males, indicated by lower H-donor activity and increased chemiluminescence intensity and malonaldehyde concentration (Figure 3). This supports the hypothesis that females are generally more sensitive to acute stress effects and that the baseline glucocorticoid levels in females are initially higher than in males [52]. It is also a fact that females have more corticosteroid-binding globulin (CBG) in the plasma, which to some extent may compensate the higher glucocorticoid levels [53]. In addition, the amount of CBG is regulated by estrogen [54]. In female rats, ACTH levels are more elevated following stress than in males [55], and consequently, the increase in corticosterone levels following stress is also more pronounced in this sex [56].

No difference in the degree of lipid peroxidation in the brain was found in the control and noise-trained male mice. However, there was a significantly lower degree of lipid peroxidation in the brains of noise-treated females than in the brains of control females. The values of noise-treated females were lower than those of noise-treated or control males, although the difference was not significant. This means that the habituation to quieter but persistent noise reduced the negative effect in females, to the extent that it reversed the original trend of females having a higher concentration of these products in the brain than males.

It is well-known that males and females (including humans) adapt differently to environmental challenges; therefore, the response to stress may be different. These differences can be attributed to different gene expression patterns, which can be observed in the hippocampus, prefrontal cortex and nucleus accumbens [57, 47]. While the hippocampus-dependent memory of female rats improves under chronic stress, that of males deteriorates [58]. But females show enhanced cognitive performance only following mild stressors [59]. Conversely, in male rats, combined movement restriction and pain (intermittent tail shock) improved performance in the classical blink conditioning test but worsened in females. At the same time, this effect was not observed in neutered females and could be due to the presence of estrogen [60, 61]. Doremus-Fitzwater et al. [62] found that plasma cortisol levels in females returned to baseline by the end of 5 days of immobilization stress, but not in males. In contrast, chronic immobilization stress (22 days) significantly increased cortisol levels in female rats, but this was not observed in males [24]. Meanwhile, one week of immobilization in early adulthood attenuated HPA axis reactivity in male rats but not in females. Thus, early adulthood stress causes a decrease in HPA axis activity in males [63], whereas it causes an increase in HPA axis activity in females [24]. Overall, the sensitivity of females to stress is higher [64] but compensatory mechanisms are also stronger, which explains our results in the present study.

Additionally, females exposed to early stress later show anhedonia (decreased sugar preference), increased immobility time in tests measuring learned helplessness and increased food intake in new environments compared to males. However, locomotor activity and exploration skills did not differ between the two sexes, suggesting that there was no difference in anxiety between males and females [19]. A similar result was obtained by Hodes

et al. [20], where females showed depressive symptoms in response to subchronic stress and even differences in brain transcription, but the locomotor activity and anxiety measured in the elevated maze showed no difference between either sexes or treatment groups. In our own study we also found no differences between groups in locomotor activity and behaviour as measured by the open-field test. In case of substantial stress, there is an increase in the suprarenal gland activity and a lymphocyte depletion in the lympoid tissues like spleen [65]. The intact histological pictures show that the stress level in this experiment was low.

3.3. Conclusion

The significant role of the sex in stress tolerance, in the development of stress disorders and in the coping strategies can be seen. When stress-induced changes are not detected by behavioural, pathological or histopathological tests, changes in brain lipid peroxidation processes are already evident, with significant sex differences. The stress coping strategies of the two sexes show significant differences in both severity and the time line of symptoms, but also in compensatory mechanisms. Given this phenomenon, it is particularly worrying that researchers have used mainly male animals in a large proportion of psychological studies. In the future it is important to ensure that translation is equally efficient in females and males, and to extend the studies to females, including the influence of the cycle, since hormonal changes greatly affect stress mechanisms as well.

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Conflict of interest

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