Male/female Differences in Radial Arm Water Maze Execution After Chronic Exposure to Noise

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Abstract

Introduction: Noise is one of the main sources of discomfort in modern societies. It affects physiology, behavior, and cognition of exposed subjects. Although the effects of noise on cognition are well known, gender role in noise-cognition relationship remains controversial. Aim: We analyzed the effects of noise on the ability of male and female rats to execute the Radial Arm Water Maze (RAWM) paradigm. Materials and Methods: Male and female Wistar rats were exposed to noise for 3 weeks, and the cognitive effects were assessed at the end of the exposure. RAWM execution included a three-day training phase and a reversal-learning phase conducted on the fourth day. Escape latency, reference memory errors, and working memory errors were quantified and compared between exposed and non-exposed subjects. Results: We found that male rats were in general more affected by noise. Execution during the three-day learning phase evidenced that male exposed rats employed significantly more time to acquire the task than the non-exposed. On the other hand, the exposed females solved the paradigm in latencies similar to control rats. Both, males and females diminished their capacity to execute on the fourth day when re-learning abilities were tested. Conclusion: We conclude that male rats might be less tolerable to noise compared to female ones and that spatial learning may be a cognitive function comparably more vulnerable to noise.

Keywords: Noise, rats, reference memory, sex, spatial learning, working memory

INTRODUCTION

Noise is one of the most important pollutants in the modern world.[1] It is present in work environments, domestic appliances, traffic, loud music, and other sources that often exceed tolerable thresholds.[2] Beyond the well-documented auditory effects, excessive sounds generate stress and interfere with cognitive processes that are essential for adaptation.[3] Negative effects of noise on cognition have been experimentally proved.[4-6] Learning and memory may particularly be affected when sounds are intense, persistent, and/or inescapable.[7-9]

Environmental stressors may alter the normal function of brain structures that are indispensable for cognition. The hippocampus is an example of such a structure.[10] In subjects that are exposed to chronic stress the hippocampal integrity is modified and their ability to solve a spatial memory task is reduced.[11] Due to intrinsic morphological and functional characteristics, the effects of environmental stressors on the hippocampus may vary depending on gender.

To name some of these characteristics, the hippocampus adaptability and plasticity are strongly dependent on the activity of the gonadal steroid hormone receptors.[12] Similarly, some pathological conditions (i.e. Alzheimer disease, schizophrenia) that affect cognitive functions related to hippocampal integrity are influenced by sex.[13]

Gender is also an important modulator of brain activity and behavior. There is a general agreement supporting that females respond different than males in a variety of conditions. Sound processing and stress response are

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well-documented cases of this.\textsuperscript{[14,15]} Women for example have better pure-tone thresholds at frequencies above 1–2 kHz whereas men may be better below these frequencies.\textsuperscript{[16]} Similarly, auditory organs may be more resistant to deterioration over the years in female rats compared to the males.\textsuperscript{[17]} There are also documented sex differences in the neural response to stress elicited by mental arithmetic tasks.\textsuperscript{[18]} Moreover, it has been reported that female rodents show cognitive resilience to chronic stressors that usually impair the males’ cognition.\textsuperscript{[15]} Gender differences may vary from general behavioral or endocrine changes, to basic cognitive functions operating at specific brain circuits.

Water Mazes have been the most used instruments to evaluate cognitive functions related to hippocampal function in rodents. The most commonly used are the Morris Water Maze (MWM) and the Radial Arm Water Maze (RAWM). The MWM accurately evaluates acquisition and memory retrieval, while the RAWM assesses working and reference memory in addition to the spatial learning abilities.\textsuperscript{[19]} The RAWM combines the advantages of a Radial Arm Maze with the accuracy of the MWM. By using these methods, it has been reported that males may outperform females in the acquisition of tasks requiring spatial abilities.\textsuperscript{[15,20]} However, these differences might disappear or even be reversed when subjects are affected by stress.\textsuperscript{[21]}

Since sex differences in the effects of noise on cognition are still controversial, we performed an experiment to evaluate the effects of chronic noise on cognitive abilities of male and female rats. These abilities included spatial, working, and reference memory.

**MATERIALS AND METHODS**

**Animals**

The subjects were 40 adult Swiss Wistar rats from an in-house breeding facility at University of Guadalajara, Mexico. The rats were housed in standard polycarbonate cages and maintained on a 12-hour light-dark cycle, with lights on at 07:00. They were given standard Purina rat chow pellets and purified water ad libitum. Experimental procedures were approved by the Institutional Ethics Commission, according to the US National Institute of Health Guide for the Care and Use of Laboratory Animals. CI. 068-2014.

**Study design**

We evaluated differences between male and female rats in performing RAWM after exposure to noise. We randomly assigned the rats to one of the four conditions that included: Male control (MC) \( n = 10 \), Male noise (MN) \( n = 10 \), Female control (FC) \( n = 10 \), and Female noise (FN) \( n = 10 \). Experimental animals were exposed to noise during 21 days and then, spatial learning, working memory and reference memory abilities were evaluated with the RAWM. Figure 1 illustrates the procedure.

**Noise exposure**

To mimic noisy environments, we employed a rats’ audiogram-fitted adaptation previously reported.\textsuperscript{[8,9]} Audio files containing unpredictable noise events such as aircraft or road traffic noise were applied in intervals ranging from 18 to 39 seconds, followed by silent periods lasting from 20 to 165 seconds. Audio recordings were randomly repeated all day during the 21 experimental days.

Animals were housed in a special sound-isolated acoustic stress chamber afforded with professional tweeters (Steren 80-1088) suspended 60 cm above the solid grid cages and connected to an amplifier (Mackie M1400; freq. 20–70 kHz; 300 W-8 ) arranged with a mixer software that delivered acoustic signals at levels ranging from 60 dB for the background noise to 85–103 dB for the noisy events. To make sure that the sound intensity was homogeneous at all places; noise was measured by placing a sound-level meter (Radio Shack, Mexico) in every corner of the housing box. The boxes were metallic and assembled in a grid to prevent reflection and sound amplification.

**RAWM**

A black round swimming pool (180 cm in diameter, 40 cm height) containing eight radial arms (40 cm high × 43 cm long) was filled with warm water (23 ± 3°C) to a Depth of 32 cm. Fixed to the end of an arm, a submerged platform (10 cm diameter × 30 cm high) was placed, so that it remained hidden from sight. Spatial cues were placed around the pool, which the rat could use to navigate the maze [Figure 1]. To begin a trial, a rat was placed in the water and allowed to swim until it found the hidden platform at the end of the goal arm. Each animal did five trials per session (30 min inter-trial interval) and the sessions were repeated on four consecutive days. On days 1–3, the hidden platform remained in arm 1 (learning phase), switching to arm 4 on the fourth day (reversal learning phase). Each trial started in a different arm and lasted 120 seconds. A rat which could not find the platform was guided to the goal arm and allowed to remain there for 15 second. Then, the animal was removed, dried off, and placed in a holding cage. All these experiments were conducted between 19:00 and 22:00 h on each day. Rats were trained simultaneously on the same day in a closed, sound-proofed room lit with a diffuse, attenuated light. All trials were video-recorded to allow manual analysis. We analyzed the following parameters: Escape latency reflects spatial learning and was quantified as the time required to reach the hidden platform. Reference latency is required for temporally stable representations of objects or places during the search of a goal and was quantified as the number of entries into arms that never contained the platform.\textsuperscript{[22]} Working memory refers to the ability to hold information in mind while manipulating and integrating information to perform some cognitive goal; it was quantified as the number of re-entries into arms that never contained the platform.\textsuperscript{[23]}

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**Fernández-Quezada, et al.: Female rats are less affected than males by environmental noise**
Data analysis

The SPSS statistical software was used to perform the statistical analysis (Systat SPSS, Inc., Chicago, IL). All results are expressed as means ± SEM. A one-way analysis of variance (ANOVA) and Tukey post hoc (Sidak analysis to correct multiple comparisons) was run to determine whether noise exposure resulted in differences between sexes, \( P < 0.05 \) was adopted as statistically significant.

RESULTS

Effect of noise exposure on male execution of RAWM

We found spatial learning and memory impairment in male rats exposed to noise. Impairments were particularly noticeable on latency and reference memory measures. Differences between exposed and non-exposed males were evident on days 1-3 when subjects were trained with a fixed location, and on day 4 when the location was changed. Table 1 illustrates the general performance of rats at the RAWM.

ANOVA analyses revealed that overall latency measures were significantly increased in exposed males when compared to control \[ F(29,240) = 4.06; \ P = 0.001 \]. Tukey’s post hoc analysis indicated that differences between exposed and non-exposed males reached significance on trial 2 (84.44 ± 14.75; \( P = 0.0018 \)), trial 3 (93.44 ± 12.25; \( P = 0.006 \)), and trial 5 (86.89 ± 5.559; \( P = 0.0030 \)) of day 1; in trial 1 (98.67 ± 14.58; \( P = 0.0001 \)) of day 2; and in trial 3 (43.22 ± 14.78; \( P = 0.0289 \)) of day 3.

Figure 2 illustrates latency measures during day 1–3 (learning phase of RAWM).

On the reversal learning day (day 4) when animals had to learn a new strategical route, ANOVA also evidenced that latencies were statistically different between exposed males and control \[ F(9,80) = 5.10; \ P = 0.001 \]. Post hoc analysis showed that differences were statistically significant in four consecutive assays including trial 1 (95.89 ± 13.24; \( P = 0.0001 \)), trial 2 (63.56 ± 13.92; \( P = 0.0366 \)), trial 3 (54.22 ± 13.8; \( P = 0.0190 \)), and trial 4 (58 ± 13.71; \( P = 0.0285 \)). Figure 3 shows latency measures on day 4 of RAWM execution.

Regarding reference memory measures, we found that males exposed to noise made significantly more errors than their own control. Differences for this parameter were significant on day 4 when reversal learning was tested \[ F(29,226) = 0.80; \]
Post hoc analysis evidenced that exposed males committed more errors on trial 1 (3.875 ± 0.875; \( P = 0.0023 \)), trial 3 (2.444 ± 0.7474; \( P = 0.0311 \)), and trial 5 (1.667 ± 0.527; \( P = 0.0385 \)). Figure 4 illustrates reference memory errors during re-learning phase of RAWM. Differences on reference memory were not evident during the first 3 days of training. Figure 5 illustrates reference memory errors during days 1–3.

We found no differences between male exposed and non-exposed subjects when working memory errors were quantified.

### Effect of noise exposure on female execution of RAWM

Female rats exposed to environmental noise also evidenced significant impairments when executed both (learning/reversal learning) phases of the RAWM paradigm. Regarding the training period (days 1–3), ANOVA analyses revealed that exposed females exhibited higher latencies than controls [\( F (29, 240) = 3.10; P = 0.001 \)]. Tukey’s post hoc analysis showed that differences were significant in trial 5 of day 1 (54.56 ± 12.77; \( P = 0.0187 \)) and in trial 3 of day 3 (38.67 ± 7.737; \( P = 0.0103 \)). Figure 2 illustrates escape latencies of all evaluated groups. On this training phase, we found no differences between exposed and non-exposed females when analyzed the number of reference/working memory errors.

Next, when the platform location was changed on day 4, ANOVA analysis evidenced that female exposed rats were slower to learn the new location in comparison to control [\( F (9, 90) = 2.84; P = 0.005 \)]. Tukey’s post hoc analysis showed that differences reached statistical significance on trials 2 (65.7 ± 10.96; \( p = 0.0026 \)) and trial 3 (45.2 ± 13.59; \( p = 0.0252 \)). Figure 3 illustrates this topic. Again, we found no differences between groups when reference/working memory errors were compared on day 4.

### Male vs female differences in RAWM execution after noise exposure

To evidence differences between sexes, we also compared the number of errors and the escape latencies that males and females committed during the execution of both phases of the RAWM paradigm. Table 2 summarizes these parameters.

The one-way ANOVA analysis indicated that there was a main effect of sex when latencies to find the hidden platform were analyzed, since exposed males employed significantly
more time to acquire the task than exposed females [F(29, 240) = 3.50; \( P = 0.001 \)]. No differences were seen at this parameter when male/female controls were compared. Tukey's post hoc test showed that noise exposed males increased their latencies in trial 2 (84.44 ± 14.75; \( P = 0.002 \)) and trial 3 (93.44 ± 12.25; \( P = 0.003 \)) of day 1; and in trial 1 (98.67 ± 14.58; \( P = 0.011 \)) and trial 2 (74.22 ± 14.58; \( P = 0.015 \)) of day 2. Figure 2 illustrates escape latencies of all analyzed groups.

Otherwise when parameters were compared at the reversal learning phase, ANOVA revealed that exposed males also increased their latency numbers compared to exposed females [F (9,70) = 2.30; \( P = 0.024 \)]. Post hoc analysis indicated that differences reached significance in trial 1 (3.875 ± 0.875; \( P = 0.049 \)). Figure 4 shows reference memory errors during the re-learning phase of RAWM.

Finally, we found no differences related to working memory errors between males and females at any phase of the RAWM execution.

**DISCUSSION**

In this study, we used the RAWM paradigm to investigate the effects of noise on the cognitive abilities of male and female rats. We found that both, males and females were negatively affected by audiogenic stress. Spatial learning abilities were affected at the two phases of the RAWM execution; first, when the platform remained in a fixed location and, second, when the task was reconfigured to force the rats to learn a new location. Moreover, we demonstrated that males and females responded different to the acoustic stimulation. Males were more affected than females concerning latencies and reference errors at both phases of the evaluation.
Noise has been considered a public health problem capable to induce damage to the ears and other auditory organs. The most investigated non-auditory health problems are interrupted sleep, cardiovascular disorders, annoyance/stress and more recently, cognitive impairment. Concerning cognition, a growing number of studies conducted in humans are consistently reporting that noise has negative effects on cognitive performance. At experimental level however, results may result controversial trying to replicate or elucidate the conditions behind the noise-induced damage. One of the major problems to explore the effects of environmental noise on laboratory conditions is the lack of “naturalistic” models capable to reproduce or adapt noisy environments to the rat’s audiogram. Most of the employed models stimulate the rat’s ear in a fixed range of frequency (white noise for example) or using artificial broad-band models. Here, we employed a representative model of a noisy environment to stimulate the rat’s ear considering that a rat has better capacity to detect high frequencies (over 8,000 Hz). We presented sounds mimicking the major urban sources of noise (road traffic, aircraft, heavy machinery, etc.) and confirmed that...
Table 3: Sex differences in cognition and brain capacities

| Reference title | Assessment | Conclusion | Agreement/disagreement |
|-----------------|------------|------------|------------------------|
| Animal studies  |            |            |                        |
| Gender dependent alterations in corticosteroid receptor status and spatial performance following 21 days of restraint stress. | Spatial learning and memory on the Morris Water Maze. | Stressed males showed delayed learning and worse memory scores. | Agree with our results. |
| Sex-dependent effects of chronic unpredictable stress in the water maze. | Spatial learning and memory on the Morris Water Maze. | Improved performance of female rats following CUS exposure lasting 3 weeks after the termination of the stress procedures. | Agree with our results. |
| Sex differences in chronic stress effects on cognition in rodents. | Spatial learning and memory on radial arm maze, radial arm water maze, Morris water maze, Y-maze and object placement. | Females show cognitive resilience to chronic stressors that impair male cognitive function. | Agree with our results. |
| Estradiol: Mediator of memories, spine density and cognitive resilience to stress in female rodents. | Hippocampal-dependent memory tasks including Morris and radial arm water mazes. | Estradiol is relevant for the cognitive resilience to chronic stress exhibited by females. | Partially agree with our results. |
| Chronic restraint stress enhances radial arm maze performance in female rats. | Spatial memory in radial arm maze. | Females stressed for 21 days showed enhanced spatial memory performance on the radial arm maze. | Agree with our results. |
| Sex differences in spatial and non-spatial Y-maze performance after chronic stress. | Spatial memory in Y-maze. | Sex differences in response to chronic stress with females exhibiting an ability to recover quickly from deficits in Y-maze performance. | Partially agree with our results. |
| Human studies  |            |            |                        |
| Sex differences in brain organization for verbal and non-verbal functions. | Verbal and non-verbal functions in humans. | The study confirms that there are undoubtedly sex differences in brain organization for a variety of human abilities; however, these sex differences cannot be depicted simply in terms of male brains tending to be more asymmetrically organized than female brains. | Undefined. |
| Sex differences in mental rotation and spatial rotation in a virtual environment. | Paper-and-pencil version of the mental rotations test (MRT) and a virtual environment for investigating rotational ability. | Results replicate sex differences traditionally seen on paper-and-pencil measures, while no sex effects were observed in the virtual environment. Results suggest men may rely more on left hemisphere processing than women when engaged in rotational tasks. | Undefined. |
| Gender-specific hemispheric asymmetry in auditory space perception. | The study investigates gender-related functional asymmetries in monaural sound localization in the vertical plane. | Results show that females were more precise when listening with the left ear, while males did better with the right. However, significant differences in monaural localization performance as a function of gender occurred exclusively when listening with the right ear, with males performing substantially more precisely than females. | Partially agree with our results. |
| Human primary auditory cortex in women and men. | Quantification of cell volume densities and areal borders was used to investigate the size and microstructure of primary auditory cortex. | The total brain volume adjusted of the primary auditory cortex was significantly larger in women than in men bilaterally. | Undefined. |
| Sex differences in prefrontal cortical brain activity during fMRI of auditory verbal working memory. | Functional MRI (fMRI) in prefrontal, parietal, cingulate, and insula regions and auditory verbal WMEM task. | Women exhibited greater signal intensity changes in middle, inferior, and orbital prefrontal cortices than men. | Partially agree with our results. |
| Individual differences in ageing, cognitive status, and sex on susceptibility to the sound-induced flash illusion: A large-scale study. | Susceptibility to the sound-induced flash illusion (SIFI) as a measure of audiovisual temporal integration. | Higher susceptibility to the SIFI was predicted by older age, female sex (at larger temporal asynchronies), and a lower score on the Montreal Cognitive Assessment (MoCA). | Undefined. |
| Sex differences in auditory subcortical function. | Electrophysiological techniques to evaluate the auditory brainstem response to a synthesized stop-consonant speech syllable. | Females have earlier peaks relative to males in the subcomponents of the response representing the onset of the speech sound. Provide a baseline for interpreting the higher incidence of language impairment (e.g. dyslexia, autism, specific language impairment) in males. | Partially agree with our results. |

Summarizes representative studies conducted in animals and humans to elucidate sex differences in cognitive abilities.
adult rats are sensitive and vulnerable to these ubiquitous sources of noise. Our results demonstrate that rats exposed to these stimuli become impaired in their ability to learn, employ, and modify a navigation route to escape from the maze. Since spatial configuration of the RAWM forces the rat to attend external cues, we assume that mainly, the rats in our experiment were affected in their ability to employ allocentric strategies to locate places in the space using independent coordinates; a complex process included in the spatial memory concept. Spatial memory also include the short-term storage of spatial information that keeps it available for immediate access (spatial working memory), and the long-term storage of spatial locations that is involved in obtaining information over the trials (reference memory). Both functional systems are anatomically differentiated and coordinate frontal-parietal and occipital areas (working memory systems) with hippocampal circuits (reference memory circuits) to navigate and solve the task. It is important to make this appointment since results in our experiment showed that errors associated to delayed latencies correspond specifically to reference memory and not to spatial working memory. The later implies that noise stimulation affects more specifically the hippocampus-dependent memory systems, an effect commonly reported in experiments evaluating stress and other aversive conditions. In this line of thought, previous experiments have evidenced that both reference and working memory systems may be differentially affected by sleep deprivation and chronic administration of corticosterone. Interestingly, all of these experiments also reported that reference memory is substantially more vulnerable than working memory to the effects of aversive conditions. In view of this coincidence, we believe that environmental noise as employed in our experiment could exert a focal effect over the hippocampus-dependent memory systems. In support of this, stressing conditions have been widely probed to be particularly aggressive for hippocampal integrity. Moreover, it has been previously demonstrated that chronic exposure to environmental noise may indeed increase the activity of the hypothalamic-pituitary-adrenal axis and exert powerful stress-like effects in rats.

Besides confirmation of impairing effects on spatial memory, we evidenced that males were noticeably more sensible than females to the negative effects of noise. We found that under noisy conditions, female rats were more efficient than males to solve the task.

Differences on male/female cognitive abilities have been investigated both in animals and humans. Table 3 summarizes results of these investigations. It has been established that performance in tasks that require use of spatial cues differs between sexes. Rodent males outperform females in a variety of spatial tasks but recent advances have questioned the extent of this idea. It has been argued that sex differences could be species, task or context specific. Furthermore, it seems that male advantages could vary whether environmental conditions change or whether spatial abilities are fragmented into working/reference memory. It is in this context that our results gain relevance. We demonstrated that under noisy conditions, the male rats’ advantage to solve the spatial task disappeared. If so, we found no difference at basal execution, we indeed found that females outperform males when the execution of RAWM followed noise stimulation. So, it seems that females were cognitively best prepared to deal with noise overstimulation. Herein, studies to compare our own results are scarce since most of the experiments have been conducted in males. Again, reports on the effects of stress over cognitive processes represent the major support for our results. There are numerous experiments showing that chronic stress also elicits sexually differentiated effects on cognition. Most importantly, the majority of these studies are consistent with the idea that females tend to be resilient to those chronic stressors which impair male cognition. Using the MWM or the eight-arm radial maze, researchers have reported that chronic stress generally impairs male execution while females remain unaffected. Reasons for this phenomenon have been referred to hormonal factors since estradiol treatment has been found to exert an activational effect on spatial memory. A series of brilliant experiments earlier demonstrated that the sexually dimorphic effects of estradiol within the hippocampus may explain the cognitive stress resilience seen in females. Then, the resilience effect exhibited by females in our experiment, could be at least in part attributed to these hormonal mechanisms. Therefore, hormonal mechanisms should be investigated in order to prevent damaging effects of noise.

CONCLUSION

Results of this experiment demonstrate that: i) chronic exposure to environmental noise impairs spatial learning and memory of rats and ii) the effects of noise over spatial learning abilities are more pronounced in males.

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Conflicts of interest

There are no conflicts of interest.
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