Original Article

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Male rats exhibit higher pro-BDNF, c-Fos and dendritic tree changes after chronic acoustic stress

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Summary **Prolonged or intense exposure to environmental noise (EN) has been associated with a number of changes in auditory organs as well as other brain structures. Notably, males and females have shown different susceptibilities to acoustic damage as well as different responses to environmental stressors. Rodent models have evidence of sex-specific changes in brain structures involved in noise and sound processing. As a common effect, experimental models have demonstrated that dendrite arborizations reconfigure in response to aversive conditions in several brain regions. Here, we examined the effect of chronic noise on dendritic reorganization and c-Fos expression patterns of both sexes. During 21 days male and female rats were exposed to a rats' audiogram-fitted adaptation of a noisy environment. Golgi-Cox and c-Fos staining were performed at auditory cortices (AC) and hippocampal regions. Sholl analysis and c-Fos counts were conducted for evidence of intersex differences. In addition, pro-BDNF serum levels were also measured. We found different patterns of c-Fos expression in hippocampus and AC. While in AC expression levels showed rapid and intense increases starting at 2 h, hippocampal areas showed slower rises that reached the highest levels at 21 days. Sholl analysis also evidenced regional differences in response to noise. Dendritic trees were reduced after 21 days in hippocampus but not in AC. Meanwhile, pro-BDNF levels augmented after EN exposure. In all analyzed variables, exposed males were the most affected. These findings suggest that noise may exert differential effects on male and female brains and that males could be more vulnerable to the chronic effects of noise.**

Keywords: Noise, hippocampus, auditory cortex, sex differences, Golgi-Cox

1. Introduction

Noise represents a growing health problem for industrialized and developing countries (*1*). Recreational, occupational or environmental noise (EN) has long been known to induce damage in classic auditory structures including cochlear hair cells, auditory nerve fiber terminals and superior cortical structures (*2*). At the central level, changes in spontaneous firing rates, neural synchrony, tonotopic map reorganization, cell death, abnormal neural coding and axonal sprouting have been reported affecting neurons in auditory cortices (AC) (*3-6*). Several organs and functions beyond the auditory system, can also be affected by noise (*7*). EN may affect non-auditory brain regions such as the hippocampus, a limbic structure that receives direct and/or indirect neuronal projections from the auditory system (*8*). As an environmental stressor, EN may also affect hippocampal integrity by inducing dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis since the hippocampus contains one of the higher distributions of the stress hormone receptors in the brain: the glucocorticoid (GR) and mineralocorticoid receptors (MR) (*9*).

Accumulated evidence previously demonstrated that noise might affect hippocampal-related cognition (*10-12*), cell proliferation, neurotransmitter function and neurogenesis (*13-16*). The most consistent data depicting the effect of environmental stressors over the brain has been reported as structural changes affecting the plastic properties of neurons in the hippocampus and other stress-related structures. The protein product

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of the Fos proto-oncogene, has been frequently used to evidence changes in neuronal activity associated to environmental threats (*17*). Studies assessing effects of stress have evidenced changes on c-Fos levels after acute and chronic exposures (*18-20*). Also, it has also been consistently reported a causal relationship between stress and atrophy of dendrite arbors in specific subregions of the hippocampus (*21-24*). Yet, expansion of dendrites and reversible dendritic remodeling over a time frame of days or weeks has also been described inside and outside the hippocampus (*25,26*). Since the patterning of dendrites and the overall shape of the dendritic arbor depends on synaptic input, it follows that changes in the structure of the dendritic tree may instead reflect changes in the plastic/adaptive properties of neurons (*27*). So, conditions challenging the adaptive capability of the brain should affect and reshape dendritic complexity at specific regions of the brain.

Mechanisms underlying stress-induced dendritic remodeling have also been investigated. It has been suggested that glucocorticoids, excitatory amino acid release, serotonin and some neurotrophic factors could be critical mediators of this effect (*28*). Accounting for neurotrophins, it has been documented that stress effects on structural plasticity of neurons are related to changes in brain-derived neurotrophic factor (BDNF) signaling (*29*). While BDNF promotes plasticity by enhancing survival, differentiation or dendrite growing, its precursor pro-BDNF has been associated with debranching of dendritic arbors (*30-32*). Then, changes on BDNF or its precursor pro-BDNF could be associated with structural changes induced by environmental stressors.

In the last years, sex has also been recognized as an important determinant of brain susceptibility to environmental threats (*33-35*). Since the susceptibility to noise may differ among individuals, investigators in this area have begun to suspect that gender may indeed be a main condition determining the neurobiological effects of noise. Concerning other stressors, a growing number of investigations have established that males and females present different amounts of susceptibility to threatening conditions (*34*). Moreover, it has been established that most of the stress-related disorders that affect the hippocampus integrity show sex differences in severity of symptoms. While depression or Alzheimer disease shows greater severity in females, schizophrenia and other diseases are more severe in males (*36-38*). Therefore, it could be expected that changes induced by environmental stressors on the integrity of hippocampus and/or AC, deeply vary from one sex to another.

To analyze these differences, we designed an experiment to compare Golgi-stained dendrites and c-Fos activity patterns in AC and hippocampal neurons from male and female rats exposed to environmental noise. Fluctuations of serum pro-BDNF levels were also investigated in order to support the expected morphological changes.

2. Materials and Methods

2.1. *Experimental animals*

In order to compare differences, we used 40 adult Wistar male and female rats (age 90 days old) obtained from the in-house breeding facility at the West Center for Biomedical Research, Guadalajara, México. These animals were randomly divided to evaluate the effects of noise on c-Fos expression levels (n = 12 for each sex) and dendritic arborizations (n = 8 for each sex). All groups were maintained in a 12:12 light-darkness cycle with lights on at 07:00. Temperature in the experimental room was maintained at 22 ± 2°C and humidity at 70%. We guaranteed free access to tap water and balanced food. All animal experiments complied with National Institutes of Health guide (NIH Publications No. 8023, revised 1978) for the care and use of Laboratory animals.

2.2. *Noise exposure*

To produce a noisy environment, we disposed a rats' audiogram-fitted adaptation provided with representative sounds of urban environments (*i.e.*, turbines, hooters, horns, *etc*) as described by Rabat (*39*). The administered sounds considered the rats' lower capacity to detect low frequencies (under 500 Hz) and its greater capacity to perceive high frequencies (over 8,000 Hz). We used metal grid cages to avoid sound refraction and housed the animals in groups of 4 in a soundproofed room. Professional tweeters (Yamaha, Inc. Japan) were placed 1 m above the cages and were powered by a Mackie amplifier (Mackie M1400; freq. 20 Hz to 70 kHz; 300 W at 8 Ω). The speaker and tweeter characteristics allowed sound delivery between 20 and 50,000 Hz. Audio files containing unpredictable noise events were presented in random tracks that alternated noisy events (18-39 s of turbine, hooter or horn sounds) with silent intervals ranging from 20 to 165 s. Soundtracks were presented with mixer software that transmitted the signal at levels ranging from 70 dB(A) to 103 dB(A).

To avoid housing effects, both, control and experimental rats were transferred to the testing room 48 hours before the start of the stimuli. A few minutes before the speakers were activated, control rats were transferred to the surgical room for further sacrifice.

Prior to sacrifice, female rats were examined to determine estrous phase. Vaginal lavages were achieved and exfoliate cytology was observed under light microscopy. Estrous phase was determined based on the morphology of cells present and the day of sacrifice was chosen avoiding proestrus and estrus since these phases could generate confounding results.

2.3. *pro-BDNF assays*

To identify changes on circulating pro-BDNF levels, we collected a blood sample from each rat before sacrifice. pro-BDNF levels were measured using an enzyme immunoassay kit (Aviva Systems Biology OKAG00197). Blood samples were obtained immediately after the noise was ended in day 21 and from tail veins at day 7 (always between 07:00 and 08:00 hours, in order to avoid circadian variation).

2.4. *c-Fos immunohistochemistry*

The rats received an *i.p.* injection of sodium pentobarbital (60 mg/kg) and were perfused through the left cardiac ventricle with 150 ml of saline solution followed by 200 ml of 3.8% paraformaldehyde in 0.1 M phosphate buffer saline (PBS), pH 7.4. After perfusion, brains were removed and sectioned in coronal slices (40 μm) using a vibratome Leica VT1000E.

To evaluate the neuronal activity on the exposed subjects, the protein product of the proto-oncogene c-Fos was immunohistochemically analyzed. We perfused the animals at the acute phase of 2h (n = 4/sex), and, at chronic phase of 21d (n = 4/sex). The 0h group (n = 4/sex) was considered the control for all groups.

We selected one of every third section spanning from Bregma -2.1mm to Bregma -3.8mm to conduct immunohistochemistry (*40*). Sections were blocked with 10% normal goat serum (NGS) in Tris-buffered saline (0.05M, 0.9%, pH 7.4) plus 0.3% of Triton X-100 (TBST) for 1 h at room temperature. After blocking, tissues were incubated overnight with rabbit anti c-Fos primary antibody at a 1:1000 dilution (Santacruz Biotechnology, Santa Cruz, CA) in TBST + 1% NGS at 4°C with 50 rpm shaking. On the next day, sections were rinsed three times for 10 min with TBST and incubated for 2 h at room temperature with biotinylated goat anti-rabbit secondary antibody (1:500; Vector Labs, Burlingame, CA). Next, tissues were incubated in avidin-biotin-peroxidase complex (Elite ABC kit, Vector labs) for 1 h and revealed with diaminobenzidine 0.05% as chromogen. Once revealed, sections were rinsed and mounted in permount-mounting medium. c-Fos immunoreactive nuclei per 540 μm (40x microscopic field) were counted using a Leica DMi8 microscope.

2.5. *Golgi-Cox Stainning*

When the animals completed the 3 weeks in the noise room, both exposed (n = 4/sex) and control (n = 4/sex) rats were anesthesiated and decapitated. The skulls were opened, the brains quickly removed, cut with a blade into 1 cm thick slabs and processed using a FD Rapid GolgiStain™ kit (FD Neuro Technologies, Ellicott city, MD, USA). On each procedure, blocks from 1 control and 1 exposed rat were put in the dark at room temperature into a mixture of solutions (provided by the kit producer) for the next 2 weeks. The tissues were then transferred into a protectant solution C (0.1 M phosphate buffer, sucrose, polyvinylpyrrolidone and ethylene glycol) and stored for 48 hours in the dark at 4°C. The tissues were sectioned into 200μm slices using a vibratome (Leica, VT1000 S). Each section was mounted with protectant solution on gelatin-coated microscope slides. Sections were then dried at room temperature in the dark for a couple of weeks. For the next procedure the slides were collocated into the staining solution D and E (ammonia and sodium thiosulfate). Then the tissues were dehydrated in 50%, 75%, 95% and 100% ethanol and cleared in xylene. The tissues were coverslipped in Permount™ Mounting Medium. The slides were finally viewed under a Leica DMi8 microscope.

2.6. *Morphological analysis*

Sections containing the hippocampus and the auditory cortex (AC) were delineated according to anatomical atlases and with the help of c-Fos stained sections.

Sholl analysis was employed to assess dendritic trees as described by Kutzing (2010). We quantified the number of intersections in concentric rings at 3 μm intervals. We also quantified the total number of dendrites and the total length of dendrites.

To conduct the Sholl analysis, photographs were obtained using bright-field microscopy (Leica DMi8 microscope) 20× magnification connected to a DFC 7000T camera, which transmitted the microscopic image to a PC coupled with MATLAB (MathWorks), NeuroJ pluggin for ImajeJ and NeuroStudio. Spatial information related to the position of dendritic segments in relation to soma was acquired with ImageJ/NeuroJ, identification of branch and endpoints was realized with NeuroStudio, and writing of scripts to convert data was conducted in MATLAB. We analyzed 400 neurons on the hippocampus and 200 neurons in AC. To select neurons, we observed that Golgi impregnation was consistent, the neurons presented at least some tertiary branches, were visualized in isolation from other neurons, and the soma was visible and centrally located.

2.7. *Statistical analysis*

The particular measurements from selected neurons were averaged to get a single value of the number of intersections, the dendritic length and the number of segments for one animal and group means were obtained from 4 subjects. The significance of the differences between morphological or serological data from exposed and control groups of rats were tested by ANOVA. All statistical analysis was performed using GraphPad (GraphPad, version prism 8). Results were

expressed as mean ± SEM. Post hoc test (Sidak analysis to correct multiple comparisons) was employed to explore differences in single time points between male and female exposed rats as well as in comparison with the control group. Differences were considered statistically significant at a value *$p < 0.05$ (**$p < 0.01$, ***$p < 0.001$); (*) control *vs*. male noise; (°) control *vs*. female noise, (&) male *vs*. female.

3. Results

3.1. *Serum pro-BDNF concentrations*

ANOVA analysis evidenced main differences for serum pro-BDNF levels [$F(5,6) = 10.15$; $p < 0.006$]. Post hoc comparisons showed that noise-exposed males increased their pro-BDNF levels after 7 ($t = 5.994$; $p < 0.001$) and 21 days ($t = 5.691$; $p < 0.010$) compared to their own control. Female differences did not reach significance when compared to controls. We found differences between exposed males and females when comparisons were made on day 21 ($t = 5.994$; $p < 0.001$). Figure 1 illustrates pro-BDNF differences.

3.2. *c-Fos immunohistochemistry*

3.2.1. *Auditory Cortex*

Exposed males increased their expression levels after 2h ($t = 18.51$; $p < 0.001$) and 21d ($t = 9.432$; $p < 0.001$) when compared to control. Females also showed significant increases at 2h ($t = 11.67$; $p < 0.001$) and 21d ($t = 6.691$; $p < 0.001$). Intersex comparisons showed that males outnumbered females at 2h ($t = 6.855$; $p < 0.001$) and 21days ($t = 5.766$; $p < 0.001$). Figure 2 illustrates AC differences in c-Fos counting.

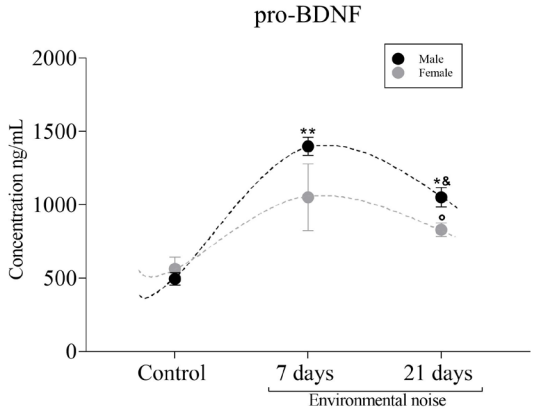

Figure 1. Serum pro-BDNF levels in male and female rats exposed to acoustic stress. Shows results of samples collected on 7 seven and 21 days of exposure. Data represent the mean ± SEM. *$p < 0.05$ (**$p < 0.01$, ***$p < 0.001$); (*) control *vs*. exposed males; (°) control *vs*. exposed females, (&) exposed males *vs*. exposed females.

3.2.2. *Hippocampus*

Male rats exposed to environmental noise showed higher amounts of c-Fos+ cells at 2h ($t = 12.56$; $p < 0.001$) and 21d ($t = 15.03$; $p < 0.001$) when compared with control. Also, females displayed higher expression levels at 2h ($t = 9.897$; $p < 0.001$), and 21d ($t = 14.90$; $p < 0.001$). Analysis of differences between sexes, showed that male increases were higher than females at 2h ($t = 6.635$; $p < 0.001$), and 21d ($t = 9.985$; $p < 0.001$). Figure 3 illustrates these differences.

3.3. *Dendritic complexity in auditory cortex (AC)*

Sholl analysis was employed to assess the dendritic branching tree, the number of segments and the total length of dendrites as shown in Figure 4.

No differences in the number of intersections (Figure 5), branches (Figure 6) and dendrite lengths (Figure 7) were found when comparing exposed groups with their respective control or in intersex comparisons. However, we noted that basal numbers of branches were higher in females ($t = 13.19$; $p < 0.001$), and that basal dendrite lengths were higher in males ($t = 4.761$; $p < 0.001$). Such patterns changed under EN since males decreased

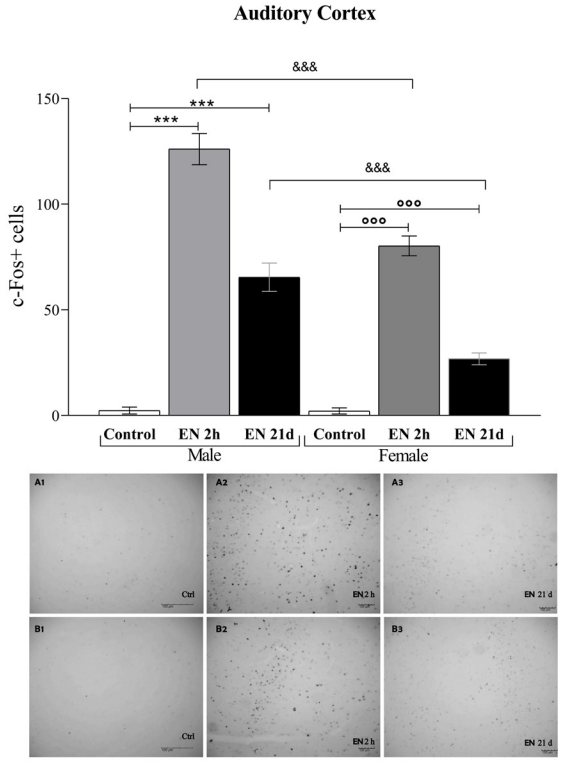

Figure 2. c-Fos expression levels in AC of male and female rats exposed acoustic stress. Shows results of c-Fos counts after 2 hour and 21 days of environmental noise. Data represent the mean ± SEM. *$p < 0.05$ (**$p < 0.01$, ***$p < 0.001$) (*) control *vs*. exposed males; (°) control *vs*. exposed females, (&) exposed males *vs*. exposed females. Representative sections of c-Fos expression in Auditory cortex (AC). Figures A1-A3 represent male groups. Figures B1-B3 female groups. Magnification 10X, scale bars indicate 100 μm.

their numbers of branches (t = 10.89; $p < 0.001$) but increased their dendrite lengths (t = 3.878; $p < 0.001$).

3.4. Dendritic complexity in hippocampus

ANOVA analysis revealed significant differences in Sholl analyses [F(3, 342) = 10.84; $p < 0.001$]. Figure 8 illustrates this. Compared to control, exposed males reduced the total number of intersections (t = 3.951; $p < 0.001$). Those reductions were significant for branches (t = 4.366; $p < 0.001$) (Figure 9) but not for dendrite lengths (Figure 10). Females in the other hand showed no differences in the number of intersections but exhibited reductions in branches (t = 4.694; $p < 0.001$) that were compensated with increases in lengths (t = 9.338; $p < 0.001$). Intersex comparisons exhibited that male reductions were significant for the number of branches (t = 2.915; $p < 0.001$).

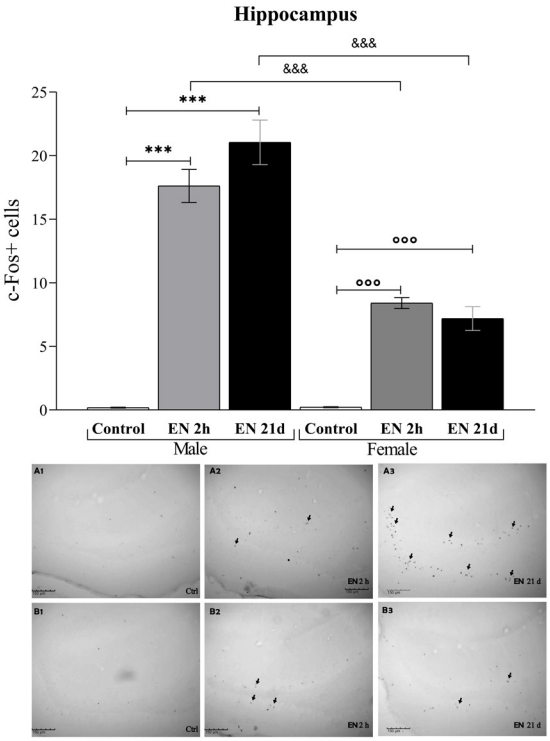

Figure 3. c-Fos expression levels in hippocampus of male and female rats exposed to acoustic stress. Shows results of c-Fos counts after 2 hour and 21 days of environmental noise. Data represent the mean ± SEM. *$p < 0.05$ (**$p < 0.01$, ***$p < 0.001$) (*) control *vs*. exposed males; (°) control *vs*. exposed females, (&) exposed males *vs*. exposed females. Representative sections of c-Fos expression in hippocampus. Figures A1-A3 represent male groups. Figures B1-B3 female groups. Magnification 10X, scale bars indicate 100 µm.

4. Discussion

Our data demonstrated that EN differentially increased c-Fos expression levels, induced structural changes in dendritic trees, and elevated serum levels of pro-BDNF. While c-Fos quantification showed strong 2h-increases that diminished at 21 days in auditory cortex, the hippocampus showed the opposite effect by registering bigger increases after 21days. In contrast, the analysis of dendritic trees showed no main differences in the complexity of the dendrite arbors of AC, but intense reductions in the dendritic trees of hippocampal neurons. The differences were significantly higher in the hippocampus and markedly affected the group of exposed males. Those changes were also accompanied by differential increases in the serum levels of pro-BDNF.

Given the probed utility of c-Fos to evidence neural responses to environmental threats, it was expected that auditory cortices reacted to environmental noise with regional increases of c-Fos expression (*41*). As expected, our results demonstrated that the protein product c-Fos quickly increased its expression patterns after acute exposures. Beyond this, we noted that patterns of c-Fos activation include not only peaks in the range of the first 2 hours affecting auditory structures, but also retarded increased expressions

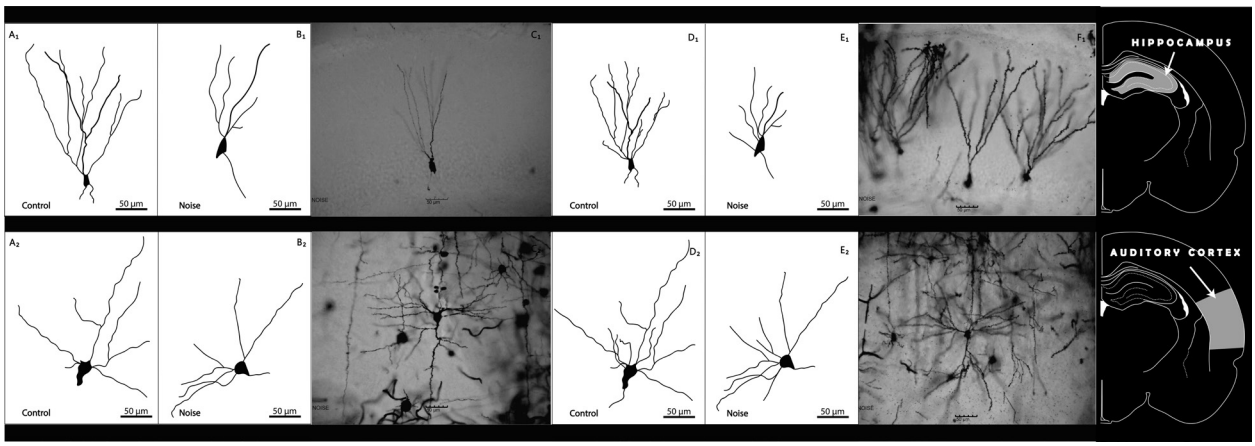

Figure 4. Morphometric analysis in AC and hippocampus of male and female rats exposed to acoustic stress. Figure above shows hippocampal representative neurons. Panel below shows auditory cortex representative neurons. Male groups are displayed with letters **A**, **B** and **C**, females with **D**, **E** and **F**. $\mathbf{C_1}$, $\mathbf{C_3}$, $\mathbf{F_1}$, $\mathbf{F_2}$ illustrates 21 days of EN exposure. Magnification 20×, scale bars indicate 50 µm.

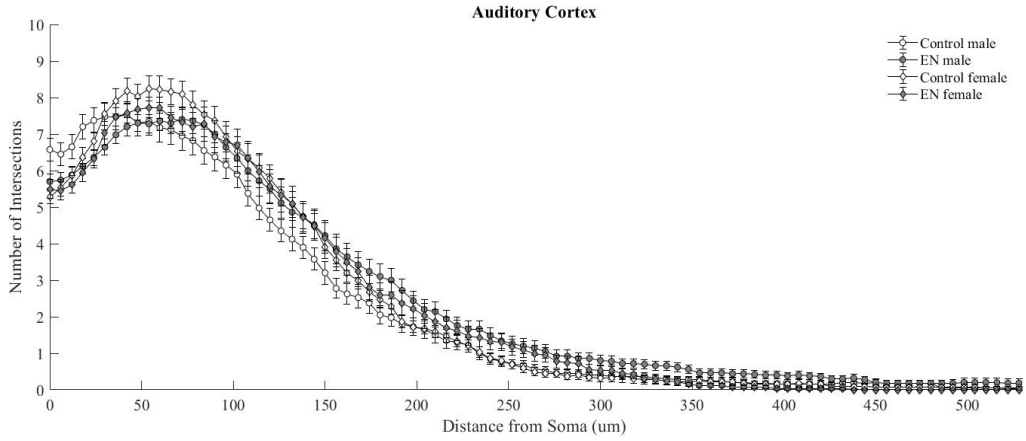

Figure 5. Sholl analysis in AC of male and female rats exposed to acoustic stress. Illustrates the number of intersections found in 200 neurons of auditory cortex. Data represent the mean ± SEM. *$p < 0.05$ (**$p < 0.01$, ***$p < 0.001$); (*) control *vs*. exposed males; (°) control *vs*. exposed females, (&) exposed males *vs*. exposed females.

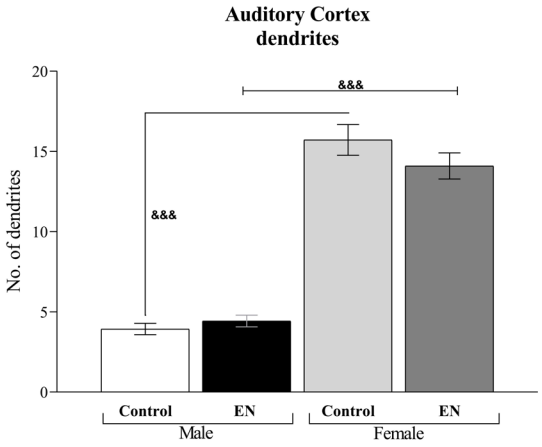

Figure 6. Total number of dendrites in neurons of AC from male and female rats exposed to acoustic stress. Illustrates the number of dendrites counted per neuron in auditory cortex of male and female rats exposed to 21 days of environmental noise. Data represent the mean ± SEM. *$p < 0.05$ (**$p < 0.01$, ***$p < 0.001$); (*) control *vs*. exposed males; (°) control *vs*. exposed females, (&) exposed males *vs*. exposed females.

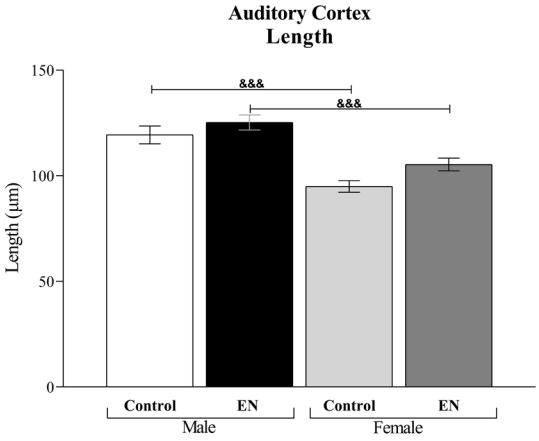

Figure 7. Length of dendrites in neurons of AC from male and female rats exposed to acoustic stress. Illustrates the length of dendrites registered in neurons of auditory cortex of male and female rats exposed to 21 days of environmental noise. Data represent the mean ± SEM. *$p < 0.05$ (**$p < 0.01$, ***$p < 0.001$); (*) control *vs*. exposed males; (°) control *vs*. exposed females, (&) exposed males *vs*. exposed females.

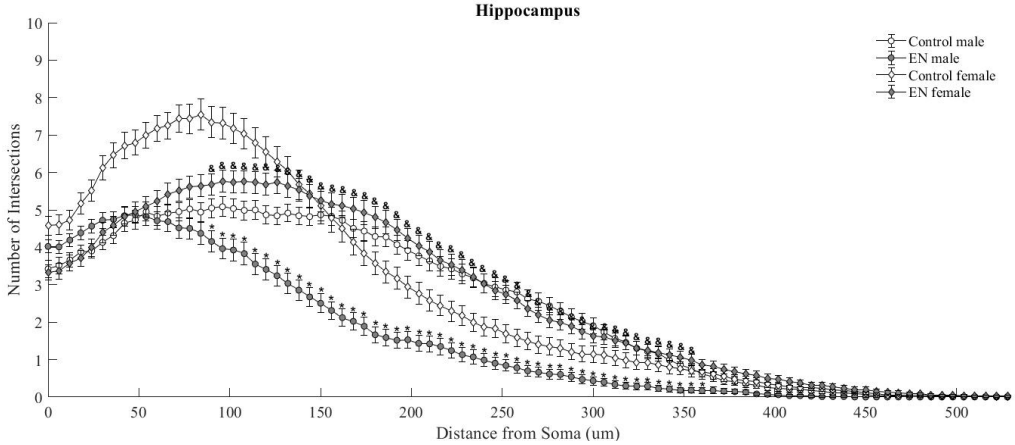

Figure 8. Sholl analysis in hippocampus of male and female rats exposed to acoustic stress. Illustrates the number of intersections found in 400 neurons of hippocampal area. Data represent the mean ± SEM. *$p < 0.05$ (**$p < 0.01$, ***$p < 0.001$); (*) control *vs*. exposed males; (°) control *vs*. exposed females, (&) exposed males *vs*. exposed females.

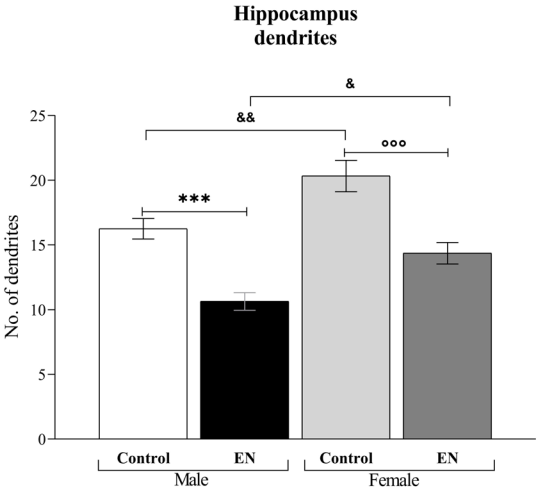

Figure 9. Total number of dendrites in neurons of the hippocampus from male and female rats exposed to acoustic stress. Illustrates the number of dendrites counted per neuron in the hippocampus of male and female rats exposed to 21 days of environmental noise. Data represent the mean ± SEM. *$p < 0.05$ (**$p < 0.01$, ***$p < 0.001$); (*) control *vs*. exposed males; (°) control *vs*. exposed females, (&) exposed males *vs*. exposed females.

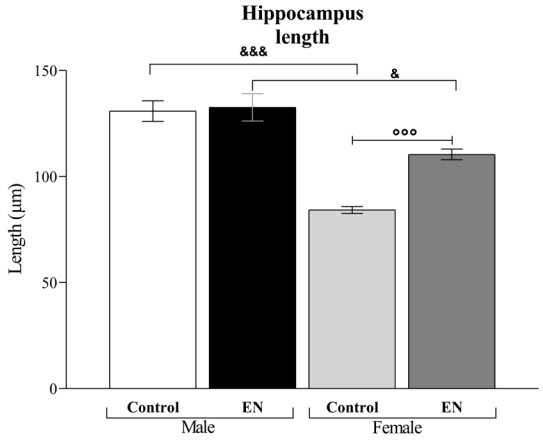

Figure 10. Length of dendrites in neurons of the hippocampus from male and female rats exposed to acoustic stress. Illustrates the length of dendrites registered in neurons of hippocampal areas of male and female rats exposed to 21 days of environmental noise. Data represent the mean ± SEM. *$p < 0.05$ (**$p < 0.01$, ***$p < 0.001$); (*) control *vs*. exposed males; (°) control *vs*. exposed females, (&) exposed males *vs*. exposed females.

affecting the hippocampus (*42*). Then, our experiment demonstrated regional differences with expression patterns differing as a function of time and structure. In support of this, time and region differences in expression patterns of c-Fos have been previously described. Reported patterns include fast general rises after a few hours affecting large regions of the brain, and retarded or sustained elevations affecting restricted areas of the limbic system (*i.e*. prefrontal cortex or other limbic structures) (*20,42,43*). Since this seems to be the case in our results (the hippocampus became lately activated), we may suppose in agreement with these authors that while some structures may be critical for the rapid processing of noise, other areas may be more dedicated to the chronic adaptation to the stimuli. Then, beyond the confirmation that neural activation varies in a time and region-dependent manner, our data also support the hypothesis that hippocampal regions and perhaps other limbic structures could be critical to respond to persistent noisy environments.

Sholl analysis confirmed that EN, is capable of inducing enduring structural changes affecting limbic extra-auditory structures. Recent evidence supports our results by showing that when exposing rats to other models of noise, the dendritic trees of hippocampal neurons became diminished (*44,45*). Since changes affecting dendritic arborizations may correspond to changes in firing properties of the hippocampal neurons (*27*), it could be supposed that noise-induced debranchings should reduce the subjects ability to process hippocampus-dependent information and to adapt to challenging conditions. Studies in the stress area support this assumption by showing that dendritic retractions have indirect functional consequences on spatial memory and the regulatory capacity of the HPA axis (*28*). According to this idea, some studies have demonstrated that noise-induced structural changes affecting the hippocampus are accompanied by deficits in learning and memory abilities (*14,15,46-48*). Given this coincidence, the chronic effects of noise over the hippocampal dendrites, could in great part be attributed to the stressing properties of noise. In this respect, it was previously demonstrated that the model of noise employed here is capable of exacerbating the activity of the HPA axis hormones (*46*). Therefore, besides the well documented effects on hearing structures (*i.e*. noise induced hearing loss -NIHL-), our study provides evidence linking noise exposure to activational and morphological effects outside the classical auditory structures. In view of this, it should be now considered that noise induced damage may also include brain structures and mechanisms different from that previously described for NIHL. Given the similarity with results in experimental stress, mechanisms of stress response could be responsible for some of the noise-induced damage outside the auditory system. Unlike common stressors that transiently affect the subject's life, it must be considered that environmental noise is an omnipresent stimuli affecting almost every part of human life.

In line with this, our results showed that hippocampal neurons could be more affected than AC neurons when noisy environments persist over longer periods. Recent evidence offers support for this idea. The main group of data has been provided by Cheng and colleagues (*49,50*) in a series of experiments designed to assess peroxidation levels and tau phosphorylation in time lapses of 1, 3 and 6

weeks. After exposing rodents to moderate levels of noise, investigators suggested that hippocampal cells could be more vulnerable since oxidative damage and tau hyperphosphorylation appeared faster and stronger than appreciated in AC. Otherwise, results obtained in a parallel experiment conducted in our lab, showed that dendritic trees belonging to AC neurons were increased after 7 days of noise, but turned-back to basal values after 21 days. On the contrary, neurons in hippocampal regions showed no alterations after 7 days but exhibited strong reductions after 21 days (*51*). Then, in addition to the hypothesis sustaining that chronic effects of noise could be largely explained by its stressing properties, it could be added now the fact that the hippocampus may also exhibit more vulnerability than AC under chronic mild-level circumstances.

Apart from the above discussed, our results showed that males and females were differently affected by EN since males exhibited higher c-Fos expression, larger dendritic tree reductions and higher pro-BDNF increases. About c-Fos, reports exist demonstrating different patterns of expression in male and female rats. According to available evidence, females should be more sensitive to the effects of both, acute and chronic stress (*35*). However, our results found the opposite effect since males exhibited higher expression levels. In line with our results, greater increases in males were also reported in a recent experiment that evaluated the acute effect of restraint stress over c-Fos expression levels (*35*). Accordingly, chronically stressed females also showed less c-Fos expressions after a novel acute stressor (*52*). Contrasted with our own data, it seems that unlike other commonly used stressors *i.e*. foot and tail shock (*53*), neonatal handling (*54*), restraint (*55*) and immune challenge (*56*), environmental noise provokes a stressor-specific response that strongly affects males. Babb and colleagues supported this idea by demonstrating that contrary to males, females show less HPA-axis and c-Fos changes after noise than after restraint (*57*).

Similar patterns were observed in our Sholl analysis. Males exhibited lower number of intersections, shorter dendritic lengths, and a smaller number of segments. Then, the observed dendrite retractions confirmed the above suggested increased vulnerability from males to acoustic stress. Since other experiments have showed that females also exhibit less dendritic remodeling after chronic stress (*58*) it seems clear that plastic capabilities of females are more efficient dealing with some environmental stressors. To support this, previous studies have established that auditory organs of females are more resistant to deterioration over the years (*59*). Moreover, human observations have also suggested that men are more susceptible compared to women in hearing loss induced by long-term occupational exposure (*60*). Then, in order to optimize noise-induced treatment and prevention strategies, sex bias must be considered as well. In our experiment, resilience or adaptive capability of females was illustrated by data showing that even when reductions occurred in the number of dendrites, compensatory increases in the length of arborizations were activated to ameliorate the impact of aversive stimulation. Results on serum pro-BDNF also support this idea. While females exhibited non-significant increases, males significantly augmented this parameter at both assessments. As previously mentioned, reduced dendritic trees may at least in part be due to exacerbated p75NTR signaling since elevated pro-BDNF levels has been associated with dendrite retraction (*32*). Results showing that blood BDNF measurements are reliable predictors of brain BDNF offers additional support for our results (*61*). Moreover, there is also the possibility that BDNF conducts differential signaling cascades in male and females under physiological or pathological settings, offering for this mechanism novel opportunities for BDNF-based therapeutic strategies.

Limitations of our study include the lack of assessments for molecular mechanisms linking BDNF to dendrite retractions and c-Fos expression. By including sex steroids in the analysis, future studies could obtain reliable explanations for sex differences. Such mechanisms must be explored in new studies. Increasing the size of the sample, future studies could produce stronger results.

In conclusion, we generated evidence supporting that chronic acoustic stress may affect adaptive capabilities of subjects by inducing regional morphological changes outside the auditory structures. Our results indicate that hippocampal neurons could be particularly sensitive to longer effects of noise, and markedly, these effects could be gender biased with higher probabilities of damage in males.

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