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Long-term recovery from hippocampal-related behavioral and biochemical abnormalities induced by noise exposure during brain development. Evaluation of auditory pathway integrity

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ABSTRACT

Sound is an important part of man's contact with the environment and has served as critical means for survival throughout his evolution. As a result of exposure to noise, physiological functions such as those involving structures of the auditory and non-auditory systems might be damaged.

We have previously reported that noise-exposed developing rats elicited hippocampal-related histological, biochemical and behavioral changes. However, no data about the time lapse of these changes were reported. Moreover, measurements of auditory pathway function were not performed in exposed animals. Therefore, with the present work, we aim to test the onset and the persistence of the different extra-auditory abnormalities observed in noise-exposed rats and to evaluate auditory pathway integrity.

Male Wistar rats of 15 days were exposed to moderate noise levels (95–97 dB SPL, 2 h a day) during one day (acute noise exposure, ANE) or during 15 days (sub-acute noise exposure, SANE). Hippocampal biochemical determinations as well as short (ST) and long term (LT) behavioral assessments were performed. In addition, histological and functional evaluations of the auditory pathway were carried out in exposed animals.

Our results show that hippocampal-related behavioral and biochemical changes (impairments in habituation, recognition and associative memories as well as distortion of anxiety-related behavior, decreases in reactive oxygen species (ROS) levels and increases in antioxidant enzymes activities) induced by noise exposure were almost completely restored by PND 90. In addition, auditory evaluation shows that increased cochlear thresholds observed in exposed rats were re-established at PND 90, although with a remarkable supra-threshold amplitude reduction.

These data suggest that noise-induced hippocampal and auditory-related alterations are mostly transient and that the effects of noise on the hippocampus might be, at least in part, mediated by the damage on the auditory pathway. However, we cannot exclude that a different mechanism might be responsible for the observed hippocampal-related changes.

1. Introduction

Sound above a certain range of sound-pressure level (SPL), referred to as "unwanted sound" or noise, can produce hearing loss as well as different types of extra-auditory alterations (Trapanotto et al., 2004; Fonseca et al., 2012). Although usually the effects of noise on living organisms are reversible in the short term, some can cause long-lasting or even permanent damage. However, the consequences of noisy stimuli coming from the environment on auditory and extra-auditory structures are largely underestimated in the public health setting.

Different extra-auditory alterations can be found in the literature. It has been reported that noise can produce serious

Abbreviations: HC, hippocampus; ANE, acute noise exposure; SANE, sub-acute noise exposure; Ct, control; CNS, Central Nervous System; PNS, Peripheral Nervous System; PND, postnatal day; ROS, reactive oxygen species; Cat, catalase; SOD, superoxide dismutase; OF, open field; OR, object recognition; ST, short term; LT, long term; IHC, inner hair cell; OHC, outer hair cell; ABRs, auditory brainstem responses; DPOAEs, distortion product otoacoustic emissions.

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behavioral disturbances in eating (Krebs et al., 1996) and sleep (Rabat et al., 2004). Besides, a variety of CNS-related symptoms, including emotional stress, increase in social conflicts and general psychiatric disorders (Rabat, 2007) were observed after noise exposure, together with an increase in aggressive behavior and anxiety (Stansfeld and Matheson, 2003).

Previous results from our laboratory (Uran et al., 2010, 2012) and from others (Manikandan et al., 2006; Cui et al., 2009, 2013) demonstrated that the Central Nervous System (CNS) might be a target for noise exposure damage. Specifically, several behavioral and biochemical extra-auditory abnormalities were observed in noise-exposed animals. Manikandan et al. (2006) and Cui et al. (2009, 2013) showed a deficit in spatial memory in noise-exposed animals. In our previous work, we found alterations in a CNS structure, the hippocampus (HC), that include an oxidative imbalance at PND 30 (decrease in hippocampal ROS levels, increase of antioxidant enzymes Cat and SOD activities) together with several behavioral abnormalities (including deficits in habituation, associative and recognition memories, mainly at LT in SANE rats, as well as a decrease in anxiety-like behaviors) and histological changes (increase in the number of pyknotic cells in all hippocampal layers, see Uran et al., 2010, 2012).

In the cochlea, noise-induced injury typically includes structural damage to hair cells and supporting cells as well as swollen postsynaptic terminals of the spiral ganglion neurons (Saunders et al., 1985). It has become increasingly evident that even occasional exposure to loud sounds in occupational or recreational settings can cause irreversible damage to the cochlea, although the resulting partial loss of hearing sensitivity could disappear within hours or days after exposure (Bohne, 1977; Hamernik et al., 1984; Hu and Zheng, 2008; Pienkowski and Eggermont, 2012). Conversely, recent work has revealed that exposure to moderate noise levels, causing transient threshold elevation, can cause degeneration of afferent terminals, without loss of hair cells (Kujawa and Liberman, 2009; Chen et al., 2012; Maison et al., 2013).

Therefore, as different functions might be affected without causing evident auditory damage, studying the effect of moderate noise levels on extra-auditory structures acquires relevance. Moreover, long-term reversal of moderate noise-induced damage could take place.

Unfortunately, few data are available concerning the different extra-auditory effects that result after exposure to noise of various intensities (Uran et al., 2010, 2012; Cui et al., 2013). In particular, much is still unknown about the effect of noise on extra-auditory regions of the CNS, outside the classical auditory pathway. Since the HC is able to respond to auditory stimuli through a non-classical pathway (Xi et al., 1994; Sakurai, 2002), this structure could be indirectly affected by noise (Kraus et al., 2010). Alternatively, since different tissues might be affected by the vibration provoked by noise, it should not be discarded that noise might impact the HC through a direct mechanism. Recently, Säljö et al. (2011) concluded that the scalp, skull bone and cerebrospinal fluid, which separate the brain from the surrounding air, do not constitute an appreciable protection for the brain against noise. In consequence, the transmission of sound into the brain appears to be highly efficient. Interestingly, soldiers and other personnel exposed to extremely high-level noise (170 dB SPL, such as blast waves or explosions) often suffer cognitive and memory impairments. Similarly, rats exposed to shock waves at 10 kPa (174 dB SPL) showed poor cognitive function on the Morris water maze test, which seems to be most likely the result of a direct injury to the brain caused by acoustic overpressure (Säljö et al., 2002).

The developing brain is in general considered more plastic than the adult brain. Disruption of normal developmental trajectory can be affected with a relatively short sound exposure period and with more lasting effects when compared with exposure of adult individuals.

Different structures within the Peripheral Nervous System (PNS) and the CNS have overlapping developmental periods, such as the rat auditory system (from PND 11 to PND 13, de Villers-Sidani et al., 2008) and the HC (from GD 18 to PND 15, Winer and Lee, 2007; Munoz-Lopez et al., 2010). For this reason, it resulted interesting to analyze what happens to both structures when a developing animal is exposed to a harmful agent at this critical developmental period (Winer and Lee, 2007; de Villers-Sidani et al., 2008; Munoz-Lopez et al., 2010). Therefore, since the maturation process of rat' HC occurs within a few weeks after birth, early chronic or intense exposure to noxious events might affect hippocampal development.

In particular, the HC has been proposed to be a potential extra-auditory target for the deleterious effects of noise. It has been shown that chronic and/or intense exposure to noise has the ability to impair hippocampus-dependent memory (Rabat, 2007; Manikandan et al., 2006; Uran et al., 2010, 2012) and to reduce the number of hippocampal neurons and their ramifications (Jáuregui-Huerta et al., 2011).

Even though we have previously reported that developing rats exposed to acute (ANE) or sub-acute (SANE) moderate white noise levels elicited changes in the HC that take place after several weeks, no data concerning the early changes that could be triggered by noise were reported. Besides, the occurrence of a long-term recovery from the disturbances induced after noise exposure has not been explored yet. Since noise exposure started after the beginning of rat hearing, verification of auditory pathway function was necessary to discard possible effects of noise on auditory structures that might influence the extra-auditory effects. With the present work, the onset and persistence of the different extra-auditory abnormalities observed in noise-exposed rats were tested. In addition, auditory pathway integrity through histological and functional studies was evaluated.

2. Materials and methods

2.1. Animals

Healthy male and female albino Wistar rats were obtained from the animal facilities of the Biochemistry and Pharmacy School, University of Buenos Aires, Argentina. A total of 20 females and 10 males were used for mating procedures. Pregnant rats were isolated and left undisturbed until delivery. The day of birth was designated as postnatal day (PND) 0. Only male rats were used for the different experimental procedures.

One hundred and eighty five PND 15 rats were used for the experiments, randomly assigned into three groups: control (Ct), acute noise exposed (ANE) and sub-acute noise exposed (SANE). In each experimental group, 4–7 animals were used for each parameter measured. Therefore, a subset of rats ($n = 121$) was exposed to noise (71 to ANE and 50 to SANE) and another subset – the Ct, sham-exposed rats ($n = 64$) – was placed in the same box as noise-exposed rats, but without being exposed.

Animals were handled and sacrificed according to the Institutional Committee for the Use and Care of Laboratory Animal rules (CICUAL, School of Medicine, University of Buenos Aires, Argentina) and this Committee, under resolution 503/10, approved our experimental protocol. The CICUAL adheres to the rules of the "Guide for the Care and Use of Laboratory Animals" (NIH) (2011 revision) and to the EC Directive 86/609/EEC (2010 revision) for animal experiments.

To avoid circadian rhythm alterations, noise exposures were performed in the intermediate phase of the light cycle, between 10 A.M. and 2 P.M. Moreover, behavioral tests were performed at the same time in each session. Behavioral tests were performed at PND 30 or 90.

2.2. Noise exposure

Animals were kept in their home wire-mesh cages (40 cm × 25 cm × 16 cm), so that they were not handled throughout all noise exposure periods. The cages were introduced in an "ad hoc" wooden sound chamber of 1 m × 1 m × 1 m fitted with a ventilated top as reported by Cui et al. (2009). 2–4 rats per cage were exposed simultaneously.

Computer software (TrueRTA) was chosen to produce white noise, using a bandwidth from 20 Hz to 20,000 Hz in octave bands. For sound amplification, we used an active 2 way monitor (SKP, SK150A, 40 W RMS per channel) located 30 cm above the

animal cage placed in the sound chamber. We measured noise intensity using an omnidirectional measurement condenser microphone (Behringer ECM 8000) each day prior to animal exposure, by positioning the microphone in the sound chamber at several locations, and taking an average of the different readings.

PND 15 animals were exposed to white noise at 95–97 dB SPL (20–20000 Hz), 2 h a day, either in a single (ANE) or in multiple exposures (15 consecutive days, SANE). Ct animals were placed in the same box of noise-exposed animals for the same period of time, but without being exposed to noise. Background noise level ranged from 50 to 55 dB SPL. This interval is the suggested as not harmful by the WHO guidelines (NIOSH, 1998) and by different authors (Campeau, 2002; Sasse et al., 2008). Lighting was provided by a 20 W lamp suspended above the sound chamber. In addition, to soundproof the exposure room, the chamber was provided with a sound attenuation system made with Celotex™.

2.3. Behavioral assessment

2.3.1. Open field task (OF)

We used repeated sessions in an open field device to analyze habituation memory. Habituation to a novel environment is believed to be one of the forms of non-associative learning, known to depend on the HC (Vianna et al., 2000; Barros et al., 2006). In this task, the repeated exposure to the same environment induces a reduction in the exploratory behavior. A decrease in the number of squares crossed in the second session, when compared with the first session, was taken as a measure of habituation memory retention (Vianna et al., 2000; Pereira et al., 2011). Open field device consisted of a 50 cm × 50 cm × 50 cm dimly illuminated wooden box, with a floor divided into 25 equal squares by black lines. Prior to exposure, rats were allowed to habituate to the behavioral room for 3 min in their home cages. After that, rats were withdrawn from the cage, placed on the left rear quadrant of the OF box and allowed to freely explore the box for 6 min (first session). The number of lines crossed was recorded over the session. In a second session, either at ST (1 h intertrial interval) or at LT (24 h intertrial interval), animals were left to explore the apparatus for another 6 min and the number of lines crossed was recorded again to evaluate habituation to the device (Barros et al., 2006). Finally, we compared between the numbers of lines crossed by each group during the first session (maximal novelty) with the number of lines crossed during the second session (when the environment became familiar). Activity was recorded using a camcorder. To minimize the olfactory stimulus, the floor of the box was cleaned with a 10% ethanol solution between sessions.

2.3.2. Object recognition task (OR)

The object recognition task was performed according to the protocol described by Bevins and Besheer (2006). Object recognition task evaluates visual hippocampal functions (Ennaceur and Delacour, 1988; Bevins and Besheer, 2006) and is used to assess the non-spatial recognition memory performance of rodents (Heldt et al., 2007; Clark et al., 2000). The task was performed in the same wooden box used in the OF task (50 cm × 50 cm × 50 cm). In the habituation session, the rat was placed in the box and allowed to freely explore the apparatus for 5 min. During the training session (first session), two identical objects were placed in the box. The session started when the rat was placed in the apparatus facing the wall at the middle of the front segment and allowed to explore the box and the objects. At the end of session, the rat was immediately put back in its home cage. In the testing session (second session), one object was replaced by a novel, non-familiar, object. Exploration time was assessed in training and testing sessions. Exploration was defined as directing the nose to the object at a distance of no more than 2 cm and/or touching the object with the nose. Sitting on the object was not considered exploration. Testing session was performed either at ST or at LT after training. Total exploration time was 5 min for each session. Animals' activity was recorded using a camcorder. As a convention, rats were not allowed to displace the objects. Experiments made in a separate cohort of animals demonstrated that rats had no preference for either object or location in the box. To minimize the olfactory stimulus, the box and objects were cleaned with a 10% ethanol solution between sessions. Different groups of rats were used for each intertrial interval.

2.3.3. Elevated plus maze (EPM)

This task is used to evaluate anxiety-related behaviors (Brenes et al., 2009). The wooden apparatus consisted of four arms of equal dimensions (50 cm × 10 cm) and raised 50 cm above the floor. Two arms, enclosed by walls 40 cm high, were perpendicular to the two other opposed open arms. To avoid falls, the open arms were surrounded by a Formica rim 0.5 cm high. The EPM was dimly illuminated with a lamp located 200 cm above the maze. Rats were placed in the center of the maze, facing one of the closed arms, and were recorded for 5 min using a camcorder. The percent of time spent on each arm was scored. Maze was cleaned between sessions with a 10% alcohol solution. Some rats fell down when walking in open arms. These animals were excluded from the study.

2.3.4. Inhibitory avoidance task (IA)

Inhibitory avoidance task measures the memory of an aversive experience through the simple avoidance of a location in which the unpleasant experience occurred. This task depends heavily on the dorsal HC (Ennaceur and Delacour, 1988; Izquierdo and Medina, 1997).

- Apparatus: we used an inhibitory avoidance apparatus as described by Roozendaal (2002). It consists of a box (60 cm × 60 cm × 40 cm), divided into two compartments: one is illuminated, while the other is equipped with a removable cover to allow it to be dark. A removable partition divided the two compartments. The floor of the dark compartment consisted of a stainless steel grid at the bottom, through which a continuous current could be delivered.
- Habituation session: the rat was placed into the lit box and allowed to freely explore the apparatus. Either after passing 3 times to the dark side or after 3 min spent in the dark side, the rat was removed from the apparatus. After 10 min, the rat was placed again in the lit side and when it entered the dark, the doors closed and the rat was retained for 10 s in this side.
- Training session: each rat was placed in the lit compartment, facing away from the dark compartment and the latency to move into the dark compartment was recorded. When the rat stepped with all four paws into the dark compartment, a foot shock (1.2 mA, 2 s duration) was delivered. The rat was then removed from the apparatus and returned to its home cage.
- Retention session: retention was tested after 24 h following a procedure similar to the training session, except that no shock was delivered. The relationship between the latency of the rat to move into the dark compartment in the *retention* and the *training* sessions (*T*1 and *T*2) was taken as a measure of associative memory retention.

To minimize the olfactory stimulus, the box and objects were cleaned with a 10% ethanol solution between sessions.

2.4. Biochemical procedures

2.4.1. ROS determination

The levels of hippocampal and cerebellar ROS were determined by a method described by Driver et al. (2000). Briefly, tissues were homogenized in ice Locke's solution (0.5 mg of tissue/ml). Aliquots of the homogenate were taken and left to warm at room temperature for 5 min. After that, 10 μL of dichloro-fluorescein diacetate (0.97 mg DCFH/ml in methanol) was added (10 μM final concentration) and the mixture was incubated at room temperature for 15 minutes. Finally, fluorescence was measured at 485 nm (excitation) and 530 nm (emission). A standard curve was performed using oxidized dichloro-fluorescein (DCF). Results were calculated as pmol DCF/mg tissue/min and expressed as mean values ± SEM.

2.4.2. Antioxidant enzymes assays

2.4.2.1. SOD activity measurement. The activity of hippocampal and cerebellar SOD was determined according to McCord and Fridovich (1969). Briefly, tissues were homogenized at 10% (w/v) in a 216 nM, pH 7.8 phosphate buffer solution and were centrifuged at 900 × *g* for 10 min. A supernatant aliquot was mixed with 216 nM, pH 7.8 phosphate buffer, 10.7 mM EDTA, 1.1 mM C Cytochrome and 0.108 mM Xanthine at 25 °C. Reaction started with the addition of 0.1 ml of xanthine oxidase (XO) enzyme solution (2 U/ml). The increase in the absorbance at 550 nm for 5 min was registered. One unit of SOD is defined as the amount that inhibits the rate of reduction of cytochrome c by 50% in a coupled system, using xanthine and xanthine oxidase at pH 7.8 at 25 °C in a 3.0 ml reaction volume. Results were calculated as SOD units/mg tissue and expressed as mean ± SEM.

2.4.2.2. Cat activity measurement. Cat activity was determined according to Beers and Sizer (1952). Briefly, 10% (w/v) tissue homogenates were made in 50 mM phosphate buffer and were centrifuged at 42,000 × *g* for 15 min. A supernatant aliquot was incubated with 0.036% (w/w) hydrogen peroxide solution (H_2O_2). The time required to decrease the absorbance at 240 nm from 0.45 to 0.40 absorbance units was registered. One unit will decompose 1.0 μmole of H_2O_2 per minute at pH 7.0 at 25 °C. Results were calculated as Cat units/mg tissue and expressed as mean ± SEM.

2.5. Auditory pathway assessment

2.5.1. Cochlear function tests

We used two different techniques for evaluating cochlear function and the degree of noise-induced threshold shift. Together, they allow differential diagnosis of outer hair cells (OHCs) versus inner hair cells (IHCs)/neuronal dysfunction throughout the cochlea. The auditory brainstem responses (ABRs) are sound-evoked potentials generated by neuronal circuits in the ascending auditory pathways and consequently require functional integrity of hair cells, as well as their afferent neurons. The first ABR peak represents the summed activity of the first cochlear synapse between IHCs and afferent neurons (Melcher and Kiang, 1996). Distortion product otoacoustic emissions (DPOAEs) can be measured in the ear-canal sound pressure and arise from normal cochlear nonlinearities generated by transduction in OHCs (Shera and Guinan, 1999; Lukashkin et al., 2002). ABRs and DPOAEs can be recorded noninvasively at different post-exposure times. The comparison of their threshold shifts can give us important information about the site(s) of dysfunction.

Animals were anesthetized with ketamine (100 mg/kg, i.p.) and xylazine (20 mg/kg, i.p.) and placed in an acoustically electrically shielded room maintained at 30 °C. Methods for measuring ABRs and DPOAEs were essentially as described in Kujawa and Liberman (2009) and Maison et al. (2013). Briefly, acoustic stimuli were delivered through an acoustic system consisting of two miniature dynamic

earphones used as sound sources and an electret condenser microphone coupled to a probe tube to measure sound pressure near the eardrum. Digital stimulus generation and response processing were handled by digital I-O boards from National Instruments driven by custom software written in LabVIEW. ABRs were recorded with needle electrodes inserted at vertex and pinna with a ground reference near the tail. Auditory responses were evoked with 5 ms tone pips, amplified (10,000×), filtered (0.1–3 kHz), and acquired on a computer. Sound level was raised in 5 dB steps and 'threshold' was defined as the lowest SPL level at which a wave is detected. For DPOAEs, stimuli were two primary tones, $f1$ and $f2$ ($f2/f1 = 1.2$), with the $f1$ level 10 dB above the $f2$ level. Primaries were swept in 5 dB steps from 20 to 80 dB SPL (for $f2$). Measurements were performed using an ER-10C (Etymotics Research) acoustic system consisting of two sound sources and one microphone. The ear-canal sound pressure was digitally sampled at 4-μs intervals (16-bit DAQ boards, NI 6052E; National Instruments), amplified and averaged (20 consecutive waveform traces), and spectrum was computed by fast Fourier transform (FFT). The process was repeated four times, the resultant spectra averaged, and $2f1–f2$ DPOAE amplitude and surrounding noise floor (six bins on each side of the $2f1–f2$ DPOAE) were extracted. The frequency resolution of the FFT is 20 Hz. Threshold was computed by interpolation as the primary level ($f2$) required to produce a DPOAE of 0 dB SPL.

2.5.2. Cochlear histological preparation

After assessing the cochlear function, tissues were recovered from representative animals to characterize the histopathology.

2.5.2.1. Plastic sections. Animals were perfused with 2.5% glutaraldehyde and 4% paraformaldehyde in 0.1 M PBS, pH 7.4. Cochleas were osmicated (1% OsO_4 in dH_2O), decalcified (0.1 M EDTA), dehydrated in ethanol and propylene oxide, embedded using EMBed-812 kit, and serially sectioned at 25 μm in a quasi-horizontal plane. To analyze the afferent innervation density, axonal counts were made in crossections through the osseous spiral lamina near the habenula perforata in tangential sections through the upper basal turn. In each selected section, several fascicles of the peripheral axons of auditory nerve fibers were cut in cross section. All neuronal profiles in all fascicles in each section were counted using an oil-immersion lens (100×) with DIC optics.

2.5.2.2. Immunostaining. Whole cochleae were dissected and immediately perfused through the round window with 4% paraformaldehyde (PFA) in PBS, pH 7.4. Cochleae were fixed in 4% PFA overnight at 4 °C before rinsing with PBS. Apical turns of the organs of Corti were excised from the cochleae and blocked in blocking buffer (PBS with 5% normal goat serum and 0.3% Triton X-100) for 2 hr at room temperature. Cochlear apical turns were incubated with the primary antibody diluted in blocking buffer overnight at 4 °C, rinsed three times for 20 min in PBT (PBS with 0.3% Triton X-100), incubated with the secondary antibody diluted in blocking buffer overnight at 4 °C, rinsed three times for 20 min in PBT, and rinsed in PBS before mounting on glass slides in Vectashield mounting medium (Vector Laboratories, CA, USA). A laser scanning confocal microscope (Olympus Fluoroview, Tokyo, Japan) was used to acquire images of the whole mounted organs of Corti. Cochleas were immunostained with antibodies to (1) C-terminal binding protein 2 (mouse anti-CtBP2 from BD Biosciences used at 1:200), and (2) calbindin (from Millipore used at 1:2000).

2.6. Statistical analysis

Significant differences between groups were analyzed through one or two way ANOVA statistical (SigmaStat, v. 3.5). For post hoc comparisons, Tukey or Bonferroni tests were used. The correlation analysis was performed through linear regression analysis and the correlation Pearson r was calculated. Levene' test of equal variances was applied for all tests. Results are expressed as mean values ± SEM. A probability <0.05 was accepted as significant, even when lower p values were found.

3. Results

3.1. Time course of biochemical changes

To analyze if noise-induced hippocampal biochemical changes were triggered at early stages, ROS, Cat and SOD levels were measured 1 and 2 h after exposure. To evaluate if the observed changes were long-lasting, biochemical parameters were assessed at PND 30 and PND 90. Fig. 1(a) shows that hippocampal ROS levels remained unaltered at early intervals after noise exposure; in contrast, a significant decrease was observed 15 days after exposure (at PND 30) when compared to control values ($F(2,22) = 110.5$, $p < 0.05$; Ct vs ANE, $p < 0.05$, Ct vs SANE, $p < 0.05$, Fig. 1(b)), being similar to control 75 days after exposure (at PND 90, $F(2,21) = 0.38$, NS). Conversely, hippocampal Cat levels show a fivefold increase with respect to control as early as 1 h after exposure ($F(2,22) = 80.55$, $p < 0.05$, Ct. vs noise, $p < 0.05$, Fig. 1(c)), returning to basal levels after 2 h; at PND 30, Cat levels were increased only in ANE rats ($F(2,21) = 16.29$, $p < 0.05$; Ct vs ANE, $p < 0.05$; Ct vs SANE, NS) and decreased after 75 days only in SANE rats when compared to controls ($F(2,17) = 4.37$, $p < 0.05$, Ct vs SANE, $p < 0.05$, Fig. 1(d)). Finally, Fig. 1(e) shows that whereas SOD levels dropped significantly 2 h after exposure ($F(2,23) = 27.3$, $p < 0.05$, Ct vs noise, $p < 0.05$), a further increase was observed at PND 30 in ANE rats ($F(2.31) = 5.09$, $p < 0.05$, Ct vs ANE, $p < 0.05$, Ct vs SANE, NS), which was followed by a decrease at 90 days in ANE rats when compared to controls ($F(2,17) = 7.8$, $p < 0.05$, Ct vs ANE, $p < 0.05$, Ct vs SANE, NS, Fig. 1(f)).

3.2. Time course of behavioral changes

To evaluate if behavioral changes induced by noise exposure were modified over time, different tasks were carried out at PND 30 and 90.

Fig. 2(a) and (b) shows that a decrease in the number of lines crossed in the second session in an open field task, was produced at ST in Ct, ANE and SANE animals when compared to the first session, both at PND 30 ($F(1,36) = 28.17$, $p < 0.05$; first session vs second session: Ct: $p < 0.05$; ANE: 0.05; SANE: 0.05) and PND 90 ($F(1,42) = 49.8$, $p < 0.05$; first session vs second session: Ct: $p < 0.05$; ANE: 0.05; SANE: 0.05), results which are comparable to controls. In contrast, no difference between the number of crossed lines in the first and second sessions was found in exposed PND 30 rats at LT ($F(2,34) = 3.77$, $p < 0.05$; Ct: $p < 0.05$; ANE, NS; SANE, NS, Fig. 2(c), whereas a decrease in the number of lines crossed in the second session was observed in these animals at 90 days ($F(1,42) = 52.09$, $p < 0.05$, Ct: $p < 0.05$, ANE: $p < 0.05$; SANE, $p < 0.05$, Fig. 2(d)).

On the other hand, Fig. 3(a) shows that SANE impaired the capability of the PND 30 animals to distinguish between novel and familiar object at ST in an object recognition task ($F(1,34) = 14.6$, $p < 0.05$; novel vs familiar: Ct: $p < 0.05$; ANE: $p < 0.05$; SANE, NS). However, this alteration was not observed at PND 90 ($F(1,36) = 114.79$, $p < 0.05$, novel vs familiar: Ct: $p < 0.05$; ANE: $p < 0.05$; SANE: $p < 0.05$, Fig. 3(b)). At LT, both ANE and SANE animals undergo a deficit in recognizing the novel object at PND 30 ($F(1,38) = 8.43$, $p < 0.05$, novel vs familiar: Ct: $p < 0.05$; ANE: NS; SANE, NS Fig. 3(c)), whereas this trouble was not found at PND 90 ($F(1,36) = 166.8$, $p < 0.05$, novel vs familiar: Ct: $p < 0.05$; ANE: $p < 0.05$; SANE, $p < 0.05$, Fig. 3(d)).

Inhibitory avoidance task performance was found to be significantly impaired in SANE rats, both after ST ($F(2,21) = 8.3$, $p < 0.05$; Ct vs ANE, NS; Ct vs SANE, $p < 0.05$, Fig. 4(a)) and LT ($F(2,23) = 7.3$, $p < 0.05$; Ct vs ANE, NS; Ct vs SANE, $p < 0.05$, Fig. 4(c)) at PND 30. Nevertheless, at PND 90 values of $T2/T1$ ratio were similar to control values (Fig. 4(b) and (d) for ST ($F(2,19) = 0.72$, NS) and LT ($F(2,16) = 0.85$, NS), respectively).

Finally, although an increase in the entries to the open arms in the elevated plus maze task was observed at PND 30 in SANE rats ($F(2,22) = 4.3$, $p < 0.05$, Ct vs ANE, NS; Ct vs SANE, $p < 0.05$, Fig. 5(a)), no changes were found at PND 90 ($F(2,15) = 1.28$, NS, Fig. 5(b)).

3.3. Time course of auditory changes

To examine if auditory function was affected by noise exposure, we evaluated ABR and DPOAEs at PND 30 and 90. At PND 30, exposure to SANE induced a 30–50 dB elevation in ABRs thresholds at all frequencies tested (Fig. 6(a)), coupled with bigger threshold elevations in DPOAEs (Fig. 6(b)), indicative of neuronal damage and OHCs dysfunction. Similarly, ANE produced threshold elevations in both ABRs and DPOAEs, but with a lesser magnitude. As shown in Fig. 6(a), there is a 10–30 dB elevation in ABRs threshold starting at frequencies higher than 8 kHz. In addition, after ANE we observed DPOAEs threshold elevations at all the frequencies tested (Fig. 6(b))

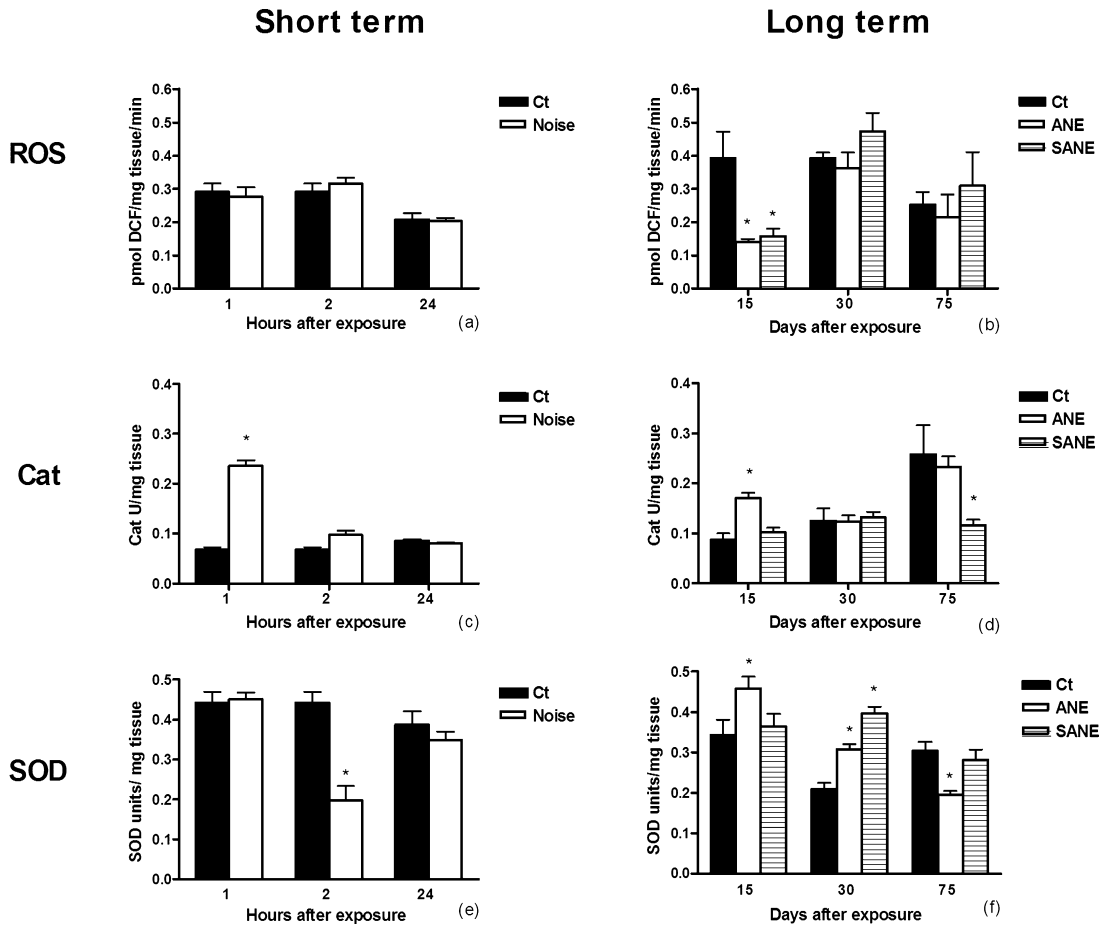

Fig. 1. Hippocampal biochemical markers levels in control and noise-exposed animals. Filled bars: Ct; open bars: in (a) noise, in (b) ANE; dashed bars: SANE. Ct: control rats; noise: ANE or SANE exposed rats; ANE: acute noise exposed rats; SANE: sub-acute noise exposed rats. Reactive oxygen species (ROS): (a) no significant changes in hippocampal ROS levels were observed shortly after noise-exposure; (b) a significant decrease in hippocampal ROS levels was found 15 days after the first noise exposure (PND 30) in ANE and SANE animals and was restored by PND 90. Catalase (Cat): (c) noise induced a significant increase in hippocampal Cat 1 h after exposure, that return to control at 2 h; (d) a significant increase in hippocampal Cat levels of ANE animals was found 15 days after the first noise exposure (at PND 30) and was restored by PND 90, while a decrease in hippocampal Cat levels was found in SANE animals. Superoxide dismutase (SOD): (e) noise induced a significant decrease in hippocampal SOD levels 2 h after exposure, that return to control at 24 h; (f) hippocampal SOD levels in ANE rats were increased at 15 days after exposure (PND 30) that remained increased at PND 45, returning to control values at PND 90. A significant decrease in hippocampal SOD levels was found at PND 90 in ANE animals. $^{*}p < 0.05$ respect to Ct. Data are mean of the concentration of pmol DCF/mg tissue/min ± SEM (ROS), Cat units/mg tissue ± SEM (Cat) and SOD units/mg tissue ± SEM (SOD).
$n = 7$ rats per group.

($F(2,55) = 92.22$, $p < 0.05$ and $F(2,49) = 75.92$, $p < 0.05$, for ABRs and DPOAEs, respectively).

We next analyzed the amplitudes and latencies of the ABRs wave 1, which reflects the summed activity of the auditory nerve at the best frequency (in our case, 22.65 kHz). As it is shown in Fig. 6(c), the mean amplitude of the ABR wave 1 was reduced after both ANE and SANE, being more pronounced after SANE, suggesting a loss of cochlear nerve synapses (at 80 dB, $p < 0.05$). In contrast, there was no difference in response latency (1.23 ± 0.06 μs for control; 1.42 ± 0.07 μs after ANE and 1.54 ± 0.11 μs at 80 dB after SANE. $p = 0.11$), indicating that conduction velocity in the auditory nerve was unchanged.

At PND 90, namely 60 days after the end of noise exposure in SANE animals and 75 days after the end of noise exposure in ANE rats, response threshold of both ABRs and DPOAEs returned to normal pre-exposure values (Fig. 6(d) and (e), respectively) ($F(2,63) = 2.07$, $p = 0.13$ and $F(2,47) = 9.62$, $p = 0.09$, for ABRs and DPOAEs, respectively). Although threshold sensitivity recovered, supra-threshold response did not (Fig. 6(c)). The reduction in peak 1 amplitude was similar to the one observed at PND 30 (Fig. 6(f)), indicating a loss of cochlear nerve synapses on surviving hair cells (at 80 dB, $p < 0.05$). Similarly, there was no difference in response latency (1.27 ± 0.05 μs for control; 1.25 ± 0.03 μs after ANE and 1.56 ± 0.06 μs at 80 dB after SANE $p = 0.49$), indicating that conduction velocity in the auditory nerve was unchanged.

To analyze the degree of inner ear damage after acoustic trauma at PND 30, we used confocal immunohistochemistry and light microscopy of the sensory epithelium. In noise-exposed ears, as shown by whole mount calbindin immunostaining and osmium stained plastic sections, there was no loss of hair cells, either IHCs or OHCs (Ct: Fig. 7(a); ANE: Fig. 7(b); SANE: Fig. 7(c)). Other cochlear structures, including supporting cells, the tectorial membrane, spiral limbus and stria vascularis did not appear affected. To evaluate the presence of primary neural degeneration, ribbon synapses were rendered visible by immunostaining for CtBP2, a component of the presynaptic machinery. As described in Kujawa and Liberman (2009) and Liberman et al. (2011), ribbon counts provide an accurate metric of the IHC afferent innervation. Likewise, as previously shown by these authors, the numbers of synapses were reduced after ANE and SANE (Ct: Fig. 7(d); ANE: Fig. 7(e); SANE: Fig. 7(f)). Ribbons were counted at the mid-basal half region of the cochlea (approximately 22.65 kHz) in controls and noise-exposed ears. In this region of noise-exposed ears, where acute threshold shifts were significant and where ABR amplitude

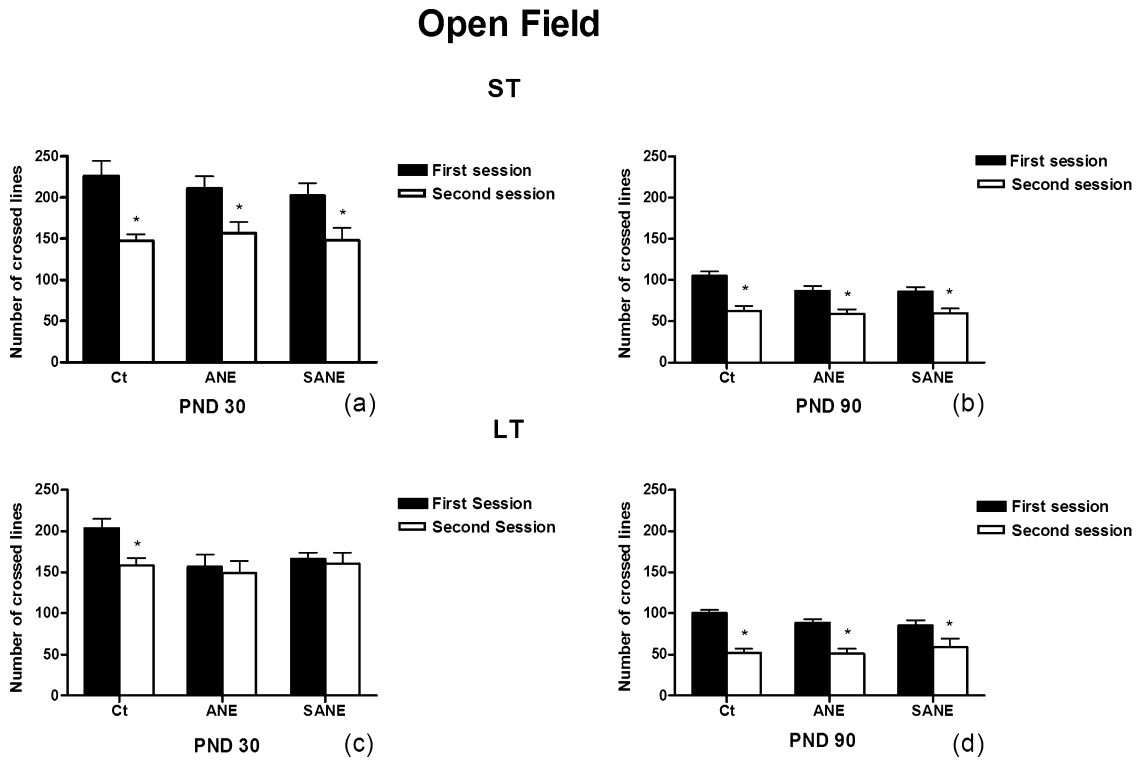

Fig. 2. Number of lines crossed in the open field task by control and noise-exposed animals. Filled bars: first session; open bars: second session. Ct: control rats; ANE: acute noise exposed rats; SANE: sub-acute noise exposed rats. ST: short term (1 h intertrial interval). LT: long term (24 h intertrial interval). (a) A decrease in the number of lines crossed was observed at PND 30 in the second session in all groups at ST. (b) Similar decreases in the number of lines crossed in the second session was observed at PND 90 in all groups at ST. (c) No change in the number of lines crossed was observed at postnatal day PND 30 in the second session in exposed animals when compared with control at LT. (d) At PND 90, the number of crossed lines was decreased again in the second session at LT in noise-exposed groups, similar to control. $^{*}p < 0.05$ when compared with the first session. Data are mean of the number of lines crossed ± SEM. $n = 7$ for each group.

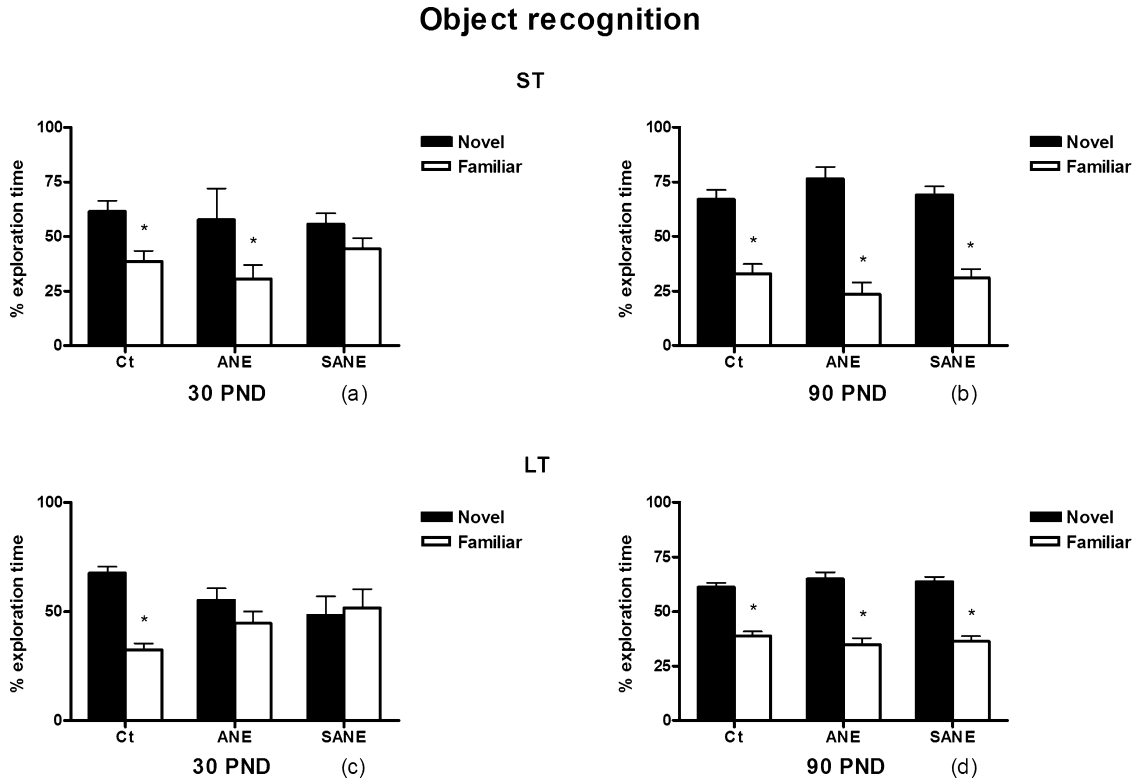

Fig. 3. Percentage of exploration time in the object recognition task of control and noise-exposed animals.
Filled bars: novel object; open bars: familiar object. Ct: control rats; ANE: acute noise exposed rats; SANE: sub-acute noise exposed rats. ST: short term (1 h intertrial interval). LT: long term (24 h intertrial interval). (a) At ST, Ct and ANE groups explored more time the novel than the familiar object at PND 30, whereas SANE rats explored the same time both objects. (b) All groups explored more the novel than the familiar object at PND 90 at ST. (c) At LT, only Ct group explored more the novel object at PND 30. (d) All groups explored more the novel object at PND 90 at LT. $^{*}p < 0.05$ when compared with the familiar object. Data are mean of the percent of time exploring objects ± SEM. $n = 7$ for each group.

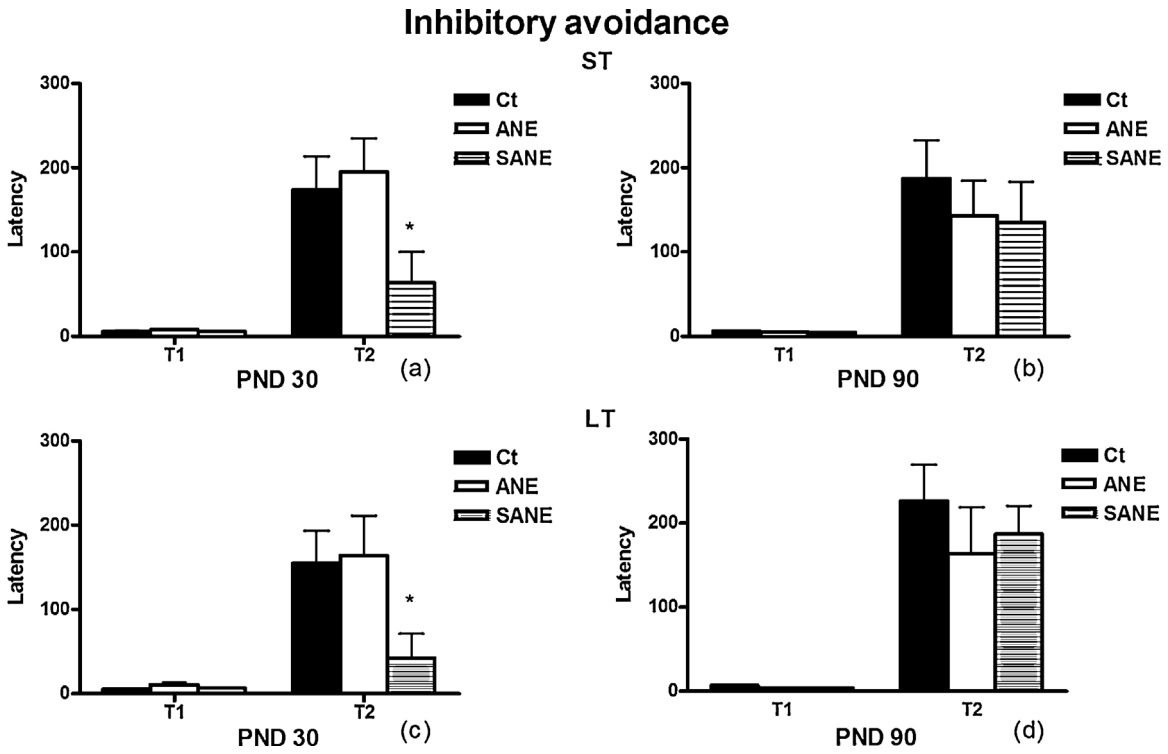

Fig. 4. Latency to enter the dark compartment of control and noise-exposed animals in the IA task. Filled bars: first session; open bars: second session. Ct: control rats; ANE: acute noise exposed rats; SANE: sub-acute noise exposed rats. ST: short term (1 h intertrial interval). LT: long term (24 h intertrial interval). (a) A significant decrease in the latency to enter the dark compartment in the retention session was observed only in PND 30 SANE animals when compared with control rats at ST. (b) At PND 90, the latency to enter the dark compartment in the retention session equaled to control value at ST. (c) A significant decrease in the latency to enter the dark compartment in the retention session was observed only in PND 30 SANE animals when compared with control rats at LT. (d) At PND 90, the latency to enter the dark compartment in the retention session equaled to control at LT.*$p < 0.05$ when compared with control animals. Data are mean ± SEM of the latency to enter the dark compartment (in minutes) of control and noise-exposed animals in the IA task. $n = 7$ for each group.

decrements suggested significant neuronal loss, ribbon counts were reduced from 20.50 ± 0.64 to 9.25 ± 0.48 and 8.00 ± 0.41 per IHC after ANE and SANE, respectively ($F(2,12) = 175.0$, $p < 0.05$).

Plastic sections were also used to examine the spiral ganglion neurons, which make synaptic contact only with IHCs. As schematized in Fig. 7(a)–(c), the unmyelinated terminals of type I afferent neurons contact the IHC at the base, the periheral axons (myelinated) are in the osseous spiral lamina, and their cell bodies are in the spiral ganglion. The pictures in Fig. 7(b) and (c) show no obvious loss of spiral ganglion neurons after acoustic trauma. Moreover, close examination of the spiral ganglion showed no signs of neurodegeneration, such as nuclear condensation or demyelination. Peripheral axons can be clearly resolved and easily counted in osmium-stained plastic sections oriented tangentially to the cochlear spiral (Stankovic et al., 2004). After both ANE and SANE, the neuronal counts were not significantly different (data not shown).

3.4. Correlation coefficients between biochemical, behavioral and auditory parameters

Although r coefficient of correlations between ROS levels and LT habituation, recognition and associative memories, as well as

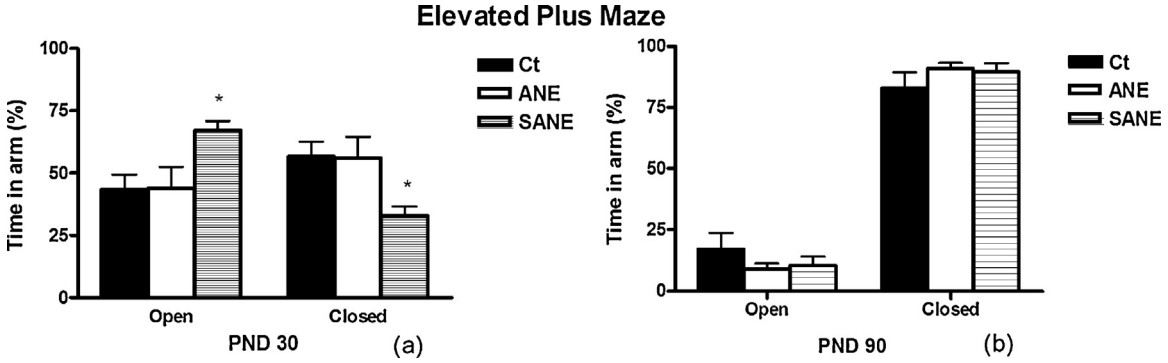

Fig. 5. Percent of time spent in arms in EPM task by control and noise-exposed rats. Filled bars: first session; open bars: second session. Ct: control rats; ANE: acute noise exposed rats; SANE: sub-acute noise exposed rats. (a) A significant increase in the % of time spent in open arms and a decrease in the % of time spent in closed arms was observed in SANE rats when tested in EPM task at PND 30. (b) The percent of time spent in arms in EPM task return to control values at PND 90. *$p < 0.05$ when compared with control animals. Data are mean ± SEM of the percent of time spent in arms in EPM task by control and noise-exposed animals. $n = 7$ for each group.

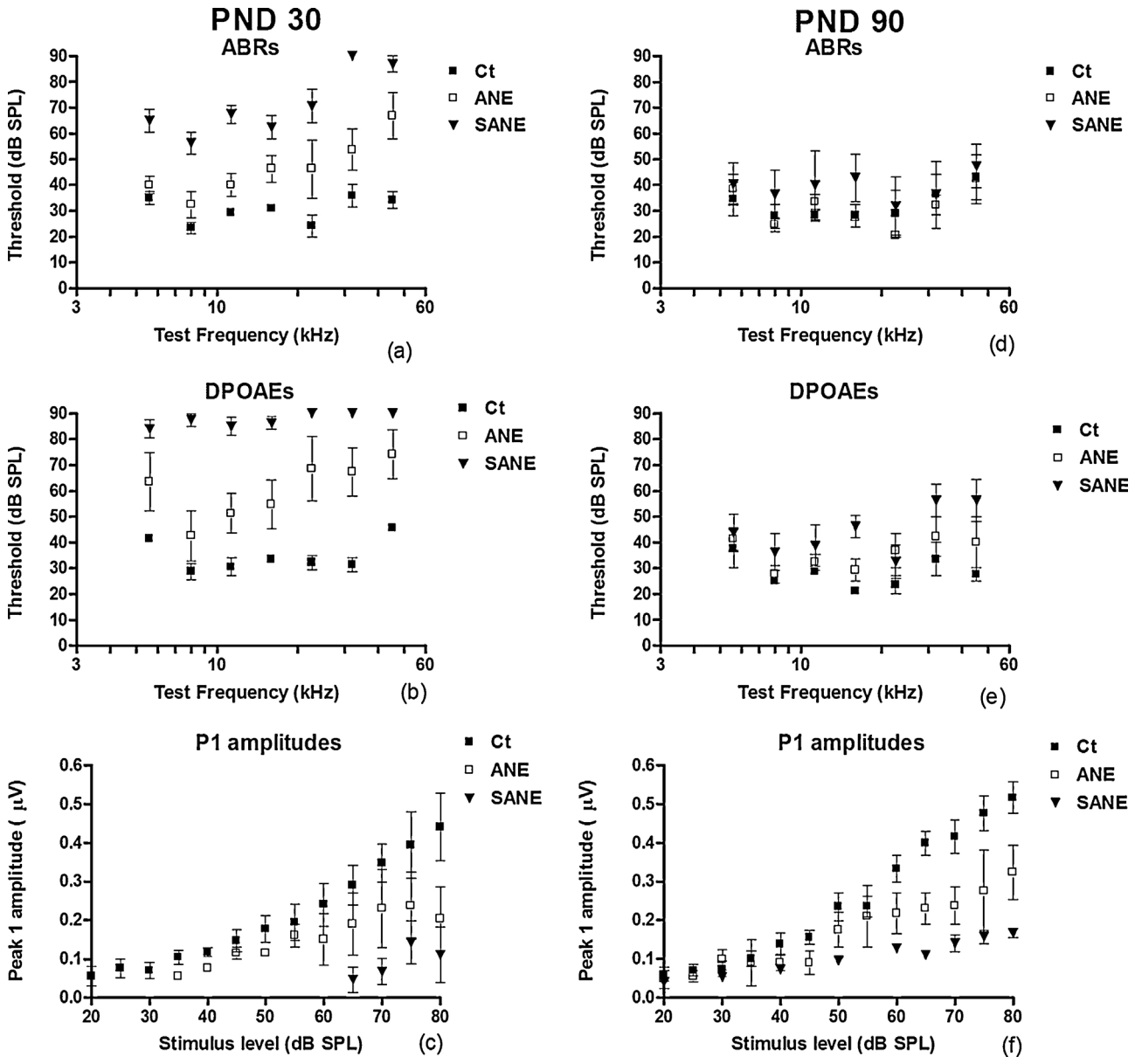

Fig. 6. ABR thresholds in control and noise-exposed rats at PND 30 and 90. Ct: control rats; ANE: acute noise exposed rats; SANE: sub-acute noise exposed rats. Cochlear thresholds were elevated and ABR response amplitudes were reduced after both ANE and SANE in PND rats, as measured by either ABRs (PND 30: a, c; PND 90: d, f) or DPOAEs (PND 30: b; PND 90: e). (a, b, d, e) Mean thresholds vs test frequencies. (c, f) Mean amplitude vs level functions. Data are mean ± SEM of thresholds vs test frequencies for control and noise-exposed animals or mean amplitude vs level functions (±SEM) for responses evoked by 22.65 kHz tone pips. ABRs amplitudes are for wave 1 (i.e., cochlear nerve response). $n = 4$ for each group.

anxiety-like behavior were found to be not significant, their values almost reach 0.9 (habituation:0.93, $p = 0.07$; recognition: 0.89, $p = 0.1$; associative: 0.87, $p = 0.12$; anxiety: 0.6, $p = 0.4$).

In contrast, when SOD or Cat activities were analyzed vs behavioral parameters, r values were much lower (for SOD: habituation: 0.34, $p = 0.66$; recognition: 0.76, $p = 0.23$; associative: 0.58, $p = 0.41$; anxiety: 0.35, $p = 0.65$. for Cat: habituation: 0.71, $p = 0.29$; recognition: 0.72, $p = 0.27$; associative: 0.62, $p = 0.37$; anxiety: 0.24, $p = 0.76$).

On the other hand, when auditory parameters were compared with hippocampal-related behavioral changes, we found a non significant correlation between ABR as well as DPOAEs and noise-induced behavioral changes (ABR: habituation: 0.67, $p = 0.33$; recognition: 0.89, $p = 0.1$; associative: 0.93, $p = 0.07$; anxiety: 0.97, $p = 0.03$. DPOAEs: habituation: 0.59, p = 0.4; recognition: 0.85, $p = 0.15$; associative: 0.88, $p = 0.11$; anxiety: 0.97, $p = 0.03$).

4. Discussion

Our results show that exposure to moderate levels of white noise (95–97 dB SPL, 2 h daily), either through acute or a sub-acute exposure, had the ability to trigger an early onset of different alterations in hippocampal oxidative markers that oscillated over time, some of which are long-lasting. On the other hand, an impairment of hippocampal-related behaviors at PND 30 was induced after noise exposure, being similar to control values 75 days after the first exposure (at PND 90). Finally, whereas an auditory threshold shift was observed in ANE and SANE animals at PND 30 when compared with controls, normal values were observed at PND 90. However, the decrease in peak I observed at PND 30, which would be correlated with the observed loss of cochlear ribbon synapses, remained unchanged at least until PND 90 in both exposure schemes.

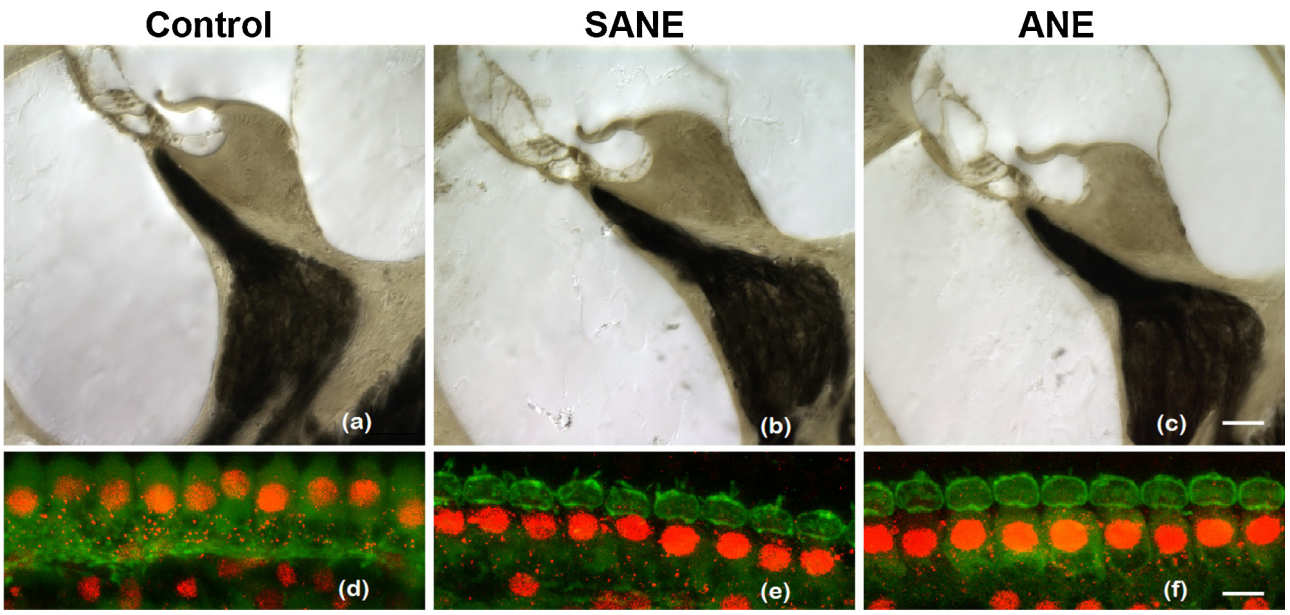

Fig. 7. Cochlear histology in control and noise exposed animals. Noise exposed ears showed a rapid degeneration of cochlear synaptic terminals with no loss of hair cells, supporting cells or cochlear neurons. Ct: control rats; ANE: acute noise exposed rats; SANE: sub-acute noise exposed rats. Sections through the upper basal turn of a control (a), SANE (b) and ANE (c) cochlea at PND 30 (~22 kHz region, approximately the region of maximum normal sensitivity). Photomicrographs reveal neither loss of hair cells nor an obvious decrease in density of spiral ganglion neurons after acoustic trauma. Scale bar 50 μm. Immunostaining reveals synaptic ribbons (red, anti-CtBP2) and inner hair cells (green, anti-calbindin) in control (d), SANE (e) and ANE (f) whole mounts organ of Corti. Confocal analysis shows noise-induced loss of inner hair cells afferent synapses. Scale bar 5 μm.

The decrease in hippocampal ROS levels observed in exposed animals at PND 30 when compared with control animals may have arisen as a compensatory response of the former increase in Cat and SOD activities. Furthermore, it could be suggested that the persistent increase in hippocampal Cat levels might induce a normalization of hippocampal ROS values, as observed at PND 90. However, the finding of a subsequent decrease in hippocampal Cat and SOD levels at PND 90 when compared with control values might suggest that an ongoing oxidative imbalance aimed to stabilize oxidative state could be taking place (Valko et al., 2007; Pourova et al., 2010; Popa-Wagner et al., 2013; Pietraforte and Malorni 2014).

Data show that an analogous time course of hippocampal and auditory-related alterations was produced, that seems to be transient. Considering the interconnections between auditory pathway and hippocampus, it could be proposed that the effects of noise exposure on the hippocampus might be, at least in part, mediated by the damage on the auditory pathway. It should be highlighted that a correlation between auditory and behavioral changes can be observed, even though they were not statistically significant. It is known that the HC receives sensory auditory input from auditory associative cortices and from HC via parahippocampal cortex or perirhinal cortex (Munoz-Lopez et al., 2010; Kraus and Canlon, 2012; Goble et al., 2009). Based on this knowledge, it could be suggested that the HC-related behavioral alterations reported in the present study would be triggered by an abnormal auditory input to the brain (Kraus et al., 2010). However, it should not be discarded that noise could be affecting the HC through a different, more direct mechanism, independent from the auditory pathway. Further experiments should be done to address this point.

The present study shows that early exposure to acute and subacute noise produced transient changes in both ABRs and DPOAEs thresholds, without loss of hair cells. Other cochlear structures, including supporting cells, the tectorial membrane, spiral limbus and stria vascularis did not appear to be affected by the acoustic overexposure. Moreover, our results show that spiral ganglion neurons survive, and even maintain their peripheral axon projections into the organ of Corti after noise exposure. However, we found an irreversible loss of hair cells synapses, which is supported by the corresponding observations of a reduction in presynaptic ribbons and a decrease in neural response amplitudes (ABRs peak I amplitudes) at PND 30, and a similar decrease in peak 1 amplitudes at PND 90. Recent work (Kujawa and Liberman, 2009; Maison et al., 2013; Lin et al., 2011) has shown that moderate acoustic overexposures can produce significant loss of cochlear nerve fibers without auditory threshold elevations. In addition, it has been suggested lately by Furman et al. (2013) that auditory thresholds can recover because the neuropathy is selective for a subset of cochlear nerve fibers with high threshold activation. It has been proposed that these high-threshold auditory nerve fibers are essential for normal hearing in noisy environments due to their resistance to masking by continuous background noise (Costalupes et al., 1984).

An area of potential public concern is sound exposure during early infancy. The most vulnerable group could be the premature infants who spend much time in noisy neonatal intensive care units (Pienkowski and Eggermont, 2012). As reported by Kujawa and Liberman (2006), young mice are more vulnerable to noise damage than older animals (Zheng and Knudsen, 2001; Chang and Merzenich, 2003). Interestingly, developing animals have the ability of recover from various injuries. Since almost complete restoration of hippocampal-related noise-induced changes as well as auditory threshold shifts were observed in our developing animals, it could be suggested that HC together with auditory pathway could counteract noise impact some time after the end of noise exposure, probably due to their early developmental age. In the present work, the recovery of most hippocampal-related behavioral and biochemical alterations 75 days following the first noise exposure support data of Chengzhi et al. (2011), which exposed 21-days-old pups to 80 or 100 dB SPL for 30 days and they were recovered after 30 days from cessation of exposure.

Results from different authors show that noise levels similar to those used in the present work (95–97 dB) did not induce permanent hearing loss. Chen et al. (2012) reported that exposure to 92 dB SPL of white noise induced only temporary threshold shifts whereas

with 106 dB SPL permanent threshold shifts were found. Moreover, Pienkowski and Eggermont (2012) reported that long-term occupational or recreational sound exposure at moderate levels can induce different problems with hearing ability, despite normal cochlear and lower brainstem function. In addition, Maison et al. (2013) showed that although a transitory threshold shift was observed after moderate noise exposure, a 20% loss of inner hair cells was found in some cochlear areas. Therefore, it could be postulated that moderate noise exposure can lead to cochlear structure damage without necessarily producing permanent absolute threshold shifts as observed in the present work.

From the correlation analysis, it could be suggested that noise-induced hippocampal oxidative imbalance produced as a consequence of a decrease in hippocampal ROS levels, might be sufficient for inducing different hippocampal-related behavioral changes, disregarding the antioxidant enzymes levels. Therefore, even a slight change in hippocampal oxidative status (e.g., a decrease in ROS levels) plays a crucial role for the development of behavioral alterations, suggesting that these parameters might be correlated: when ROS levels reach control value, the value of a given behavioral parameter is also close to control. In contrast, it appears to be no correlations between the antioxidant enzymes and the behavioral parameters, since an increase in antioxidant enzymes activity might compromise many different hippocampal-dependent behaviors, as observed at PND 30, while a decrease in their levels seems to have little influence on the modification of behavioral parameters. For this reason, although antioxidant enzymes activities are still altered at PND 90, no behavioral alterations were found at this age. On the other hand, functional auditory changes might also be responsible for the generation of hippocampal-related behavioral alterations. As hippocampal function was completely restored at PND 90, in parallel with auditory parameters, it could be postulated that recovery of auditory function might drive, at least in part, recovery of hippocampal alterations. Nevertheless, their values are close to 0.9 and normalization of behavioral changes matched with normalization of auditory alterations. The lack of recovery of ABR P1 amplitude correlates with the permanent loss of ribbon synapses observed in noise-exposed cochlea.

Therefore, it could be concluded that noise might affect auditory pathway that, in turn, can change hippocampal function. In addition, a direct effect of noise on hippocampus might be produced, that is not linked with auditory function.

5. Conclusions

In conclusion, hippocampal and auditory-related alterations seem to be mostly transient and the effects of noise exposure on the hippocampus might be, at least in part, mediated by the damage to the auditory pathway.

The moderate nature of the acoustic overexposure used in the present manuscript suggests that auditory and extra-auditory tissues are at risk even in everyday acoustic environments. Moreover, since continuous high level exposures (100–120 dB for 1–4 h) are rare in the absence of man-made devices, the biological significance of using these noise intensities could be questioned. For this reason, present experiments were designed using moderate noise levels (95–97 dB, 2 h) that are more common in natural world.

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References

Barros, D., Amaral, O.B., Izquierdo, I., Geracitano, L., do Carmo Bassols Raseira, M., Henriques, A.T., Ramirez, M.R., 2006. Behavioral and genoprotective effects of Vaccinium berries intake in mice. Pharmacol. Biochem. Behav. 84 (2), 229–234.

Beers Jr., R.F., Sizer, I.W., 1952. A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. J. Biol. Chem. 195 (1), 133–140.

Bevins, R.A., Besheer, J., 2006. Object recognition in rats and mice: a one-trial non-matching-to-sample learning task to study 'recognition memory'. Nat. Protoc. 1 (3), 1306–1311.

Bohne, B.A., 1977. Growth of cochlear damage with increasing severity of exposure. Trans. Sect. Otolaryngol. Am. Acad. Ophthalmol. Otolaryngol. 84 (2), 420–421.

Brenes, J.C., Padilla, M., Fornaguera, J., 2009. A detailed analysis of open-field habituation and behavioral and neurochemical antidepressant-like effects in postweaning enriched rats. Behav. Brain Res. 197 (1), 125–137.

Campeau, S., Dolan, D., Akil, H., Watson, S.J., 2002. c-fos mRNA induction in acute and chronic audiogenic stress: possible role of the orbitofrontal cortex in habituation. Stress 5 (2), 121–130.

Chang, E.F., Merzenich, M.M., 2003. Environmental noise retards auditory cortical development. Science 300, 498–502.

Chen, F.Q., Zheng, H.W., Hill, K., Sha, S.H., 2012. Traumatic noise activates Rho-family GTPases through transient cellular energy depletion. J. Neurosci. 32 (36), 12421–12430.

Chengzhi, C., Yan, T., Xuejun, J., Xiang, L., Youbin, Q., Baijie, T., 2011. Recovery of chronic noise exposure induced spatial learning and memory deficits in young male Sprague-Dawley rats. J. Occup. Health 53 (3), 157–163.

Clark, R.E., Zola, S.M., Squire, L.R., 2000. Impaired recognition memory in rats after damage to the hippocampus. J. Neurosci. 20 (23), 8853–8860.

Costalupes, J.A., Young, E.D., Gibson, D.J., 1984. Effects of continuous noise backgrounds on rate response of auditory nerve fibers in cat. J. Neurophysiol. 51 (6), 1326–1344.

Cui, B., Wu, M., She, X., 2009. Effects of chronic noise exposure on spatial learning and memory of rats in relation to neurotransmitters and NMDAR2B alteration in the hippocampus? J. Occup. Health 51 (2), 152–158.

Cui, B., Wu, M.Q., Zhu, L.X., She, X.J., Ma, Q., Liu, H.T., 2013. Effect of chronic noise exposure on expression of N-methyl-D-aspartic acid receptor 2B and Tau phosphorylation in hippocampus of rats? Biomed. Environ. Sci. 26 (3), 163–168.

Driver, A.S., Kodavanti, P.R., Mundy, W.R., 2000. Age-related changes in reactive oxygen species production in rat brain homogenates. Neurotoxicol. Teratol. 22 (2), 175–181.

Ennaceur, A., Delacour, J., 1988. A new one-trial test for neurobiological studies of memory in rats. 1. Behavioral data. Behav. Brain Res. 31 (1), 47–59.

Fonseca, J., Martins dos Santos, J., Oliveira, P., Laranjeira, N., Castelo Branco, N.A., 2012. Noise-induced duodenal lesions: a light and electron microscopy study of the lesions of the rat duodenal mucosa exposed to low frequency noise. Clin. Res. Hepatol. Gastroenterol. 36 (1), 72–77.

Furman, A.C., Kujawa, S.G., Liberman, M.C., 2013. Noise-induced cochlear neuropathy is selective for fibers with low spontaneous rates. J. Neurophysiol. 110 (3), 577–586.

Goble, T.J., Møller, A.R., Thompson, L.T., 2009. Acute high-intensity sound exposure alters responses of place cells in hippocampus. Hear. Res. 253 (1–2), 52–59.

Hamernik, R.P., Turrentine, G., Roberto, M., Salvi, R., Henderson, D., 1984. Anatomical correlates of impulse noise-induced mechanical damage in the cochlea. Hear. Res. 13 (3), 229–247.

Heldt, S.A., Stanek, L., Chhatwal, J.P., Ressler, K.J., 2007. Hippocampus-specific deletion of BDNF in adult mice impairs spatial memory and extinction of aversive memories. Mol. Psychiatry 12 (7), 656–670.

Hu, B.H., Zheng, G.L., 2008. Membrane disruption: an early event of hair cell apoptosis induced by exposure to intense noise. Brain Res. 239, 107–118.

Izquierdo, I., Medina, J.H., 1997. Memory formation: the sequence of biochemical events in the hippocampus and its connection to activity in other brain structures. Neurobiol. Learn. Mem. 68 (3), 285–316.

Jáuregui-Huerta, F., García-Estrada, J., Ruvalcaba-Delgadillo, Y., Trujillo, X., Huerta, M., Feria-Velasco, A., Gonzalez-Perez, O., Luquín, S., 2011. Chronic exposure of juvenile rats to environmental noise impairs hippocampal cell proliferation in adulthood. Noise Health 13 (53), 286–291.

Kraus, K.S., Canlon, B., 2012. Neuronal connectivity and interactions between the auditory and limbic systems. Effects of noise and tinnitus. Hear. Res. 2288 (1-2), 34–46.

Kraus, K.S., Mitra, S., Jimenez, Z., Hinduja, S., Ding, D., Jiang, H., Gray, L., Lobarinas, E., Sun, W., Salvi, R.J., 2010. Noise trauma impairs neurogenesis in the rat hippocampus. Neuroscience 167 (4), 1216–1226.

Krebs, H., Macht, M., Weyers, P., Weijers, H.G., Janke, W., 1996. Effects of stressful noise on eating and non-eating behavior in rats. Appetite 26 (2), 193–202.

Kujawa, S.G., Liberman, M.C., 2006. Acceleration of age-related hearing loss by early noise exposure: evidence of a misspent youth. J. Neurosci. 26 (7), 2115–2123.
Kujawa, S.G., Liberman, M.C., 2009. Adding insult to injury: cochlear nerve degeneration after temporary noise-induced hearing loss. J. Neurosci. 29 (45), 14077–14085.
Liberman, L.D., Wang, H., Liberman, M.C., 2011. Opposing gradients of ribbon size and AMPA receptor expression underlie sensitivity differences among cochlear-nerve/hair-cell synapses. J. Neurosci. 31 (3), 801–808.
Lin, H.W., Furman, A.C., Kujawa, S.G., Liberman, M.C., 2011. Primary neural degeneration in the Guinea pig cochlea after reversible noise-induced threshold shift. J. Assoc. Res. Otolaryngol. 12, 605–616.
Lukashkin, A.N., Lukashkina, V.A., Russell, I.J., 2002. One source for distortion product otoacoustic emissions generated by low- and high-level primaries. J. Acoust. Soc. Am. 111 (6), 2740–2748.
Maison, S.F., Usubuchi, H., Liberman, M.C., 2013. Efferent feedback minimizes cochlear neuropathy from moderate noise exposure. J. Neurosci. 33 (13), 5542–5552.
Manikandan, S., Padmab, M.K., Srikumar, R., Parthasarathy, N.J., Muthuvel, A., Sheela Devi, R., 2006. Effects of chronic noise stress on spatial memory of rats in relation to neuronal dendritic alteration and free radical-imbalance in hippocampus and medial prefrontal cortex. Neurosci. Lett. 399, 17–22.
McCord, J.M., Fridovich, I., 1969. Superoxide dismutase: an enzymic function for erythrocuprein (hemocuprein). J. Biol. Chem. 244, 6049–6055.
Melcher, J.R., Kiang, N.Y., 1996. Generators of the brainstem auditory evoked potential in cat. III. Identified cell populations. Hear. Res. 93 (1–2), 52–71.
Munoz-Lopez, M.M., Mohedano-Moriano, A., Insausti, R., 2010. Anatomical pathways for auditory memory in primates. Front. Neuroanat. 4, 129.
National Institute for Occupational Safety and Health (NIOSH), 1998. Criteria for a Recommended Standard: Occupational Noise Exposure, NIOSH Publication No. 98-126.
Pereira, M., Dombrowski, P.A., Losso, E.M., Chioca, L.R., Da Cunha, C., Andreatini, R., 2011. Memory impairment induced by sodium fluoride is associated with changes in brain monoamine levels. Neurotox. Res. 9 (1), 55–62.
Pienkowski, M., Eggermont, J.J., 2012. Reversible long-term changes in auditory processing in mature auditory cortex in the absence of hearing loss induced by passive, moderate-level sound exposure. Ear Hear. 33 (3), 305–314.
Pietraforte, D., Malorni, W., 2014. Focusing at the double-edged sword of redox imbalance: signals for cell survival or for cell death? Antioxid. Redox Signal. 21 (1), 52–55.
Popa-Wagner, A., Mitran, S., Sivanesan, S., Chang, E., Buga, A.M., 2013. ROS and brain diseases: the good, the bad, and the ugly. Oxid. Med. Cell. Longev. 2013, 963520.
Pourova, J., Kottova, M., Voprsalova, M., Pour, M., 2010. ROS and RNS in physiological processes. Acta Physiol. 198, 15–35.
Rabat, A., Bouyer, J.J., Aran, J.M., Courtiere, A., Mayo, W., Le Moal, M., 2004. Deleterious effects of an environmental noise on sleep and contribution of its physical components in a rat model. Brain Res. 1009 (1–2), 88–97.
Rabat, A., 2007. Extra-auditory effects of noise in laboratory animals: the relationship between noise and sleep. J. Am. Assoc. Lab. Anim. Sci. 46 (1), 35–41.
Roozendaal, B., 2002. Stress and memory: opposing effects of glucocorticoids on memory consolidation and memory retrieval. Neurobiol. Learn. Mem. 78 (3), 578–595.
Sakurai, Y., 2002. Coding of auditory temporal and pitch information by hippocampal individual cells and cell assemblies in the rat. Neuroscience 115 (4), 1153–1163.
Säljö, A., Bao, F., Jingshan, S., Hamberger, A., Hansson, H.A., Haglid, K.G., 2002. Exposure to short-lasting impulse noise causes neuronal c-Jun expression and induction of apoptosis in the adult rat brain. J. Neurotrauma. 19 (8), 985–991.
Säljö, A., Mayorga, M., Bolouri, H., Svensson, B., Hamberger, A., 2011. Mechanisms and pathophysiology of the low-level blast brain injury in animal models. Neuroimage 54 (Suppl. 1), S83–S88.
Sasse, S.K., Greenwood, B.N., Masini, C.V., Nyhuis, T.J., Fleshner, M., Day, H.E., Campeau, S., 2008. Chronic voluntary wheel running facilitates corticosterone response habituation to repeated audiogenic stress exposure in male rats. Stress 11 (6), 425–437.
Saunders, J.C., Dear, S.P., Schneider, M.E., 1985. The anatomical consequences of acoustic injury: a review and tutorial. J. Acoust. Soc. Am. 78 (3), 833–860.
Shera, C.A., Guinan Jr., J.J., 1999. Evoked otoacoustic emissions arise by two fundamentally different mechanisms: a taxonomy for mammalian OAEs. J. Acoust. Soc. Am. 105 (2 Pt 1), 782–798.
Stankovic, K., Rio, C., Xia, A., Sugawara, M., Adams, J.C., Liberman, M.C., Corfas, G., 2004. Survival of adult spiral ganglion neurons requires erbB receptor signaling in the inner ear. J. Neurosci. 24 (40), 8561–8651.
Stansfeld, S.A., Matheson, M.P., 2003. Noise pollution: non-auditory effects on health. Br. Med. Bull. 68, 243–257.
Trapanotto, M., Benini, F., Farina, M., Gobber, D., Magnavita, V., Zacchello, F., 2004. Behavioural and physiological reactivity to noise in the newborn. J. Paediatr. Child Health 40, 275–281.
Uran, S.L., Aon-Bertolino, L., Caceres, L.G., Capani, F., Guelman, L.R., 2012. Rat hippocampal alterations could underlie behavioral abnormalities induced by exposure to moderate noise levels. Brain Res. 1471, 1–12.
Uran, S.L., Caceres, L.G., Guelman, L.R., 2010. Effects of loud noise on hippocampal and cerebellar-related behaviors. Role of oxidative state. Brain Res. 1361, 102–114.
Valko, M., Leibfritz, D., Moncol, J., Cronin, M.T.D., Mazur, M., Telser, J., 2007. Free radicals and antioxidants in normal physiological functions and human disease. Int. J. Biochem. Cell Biol. 39, 44–84.
Vianna, M.R., Alonso, M., Viola, H., Quevedo, J., De-Paris, F., Furman, M., de Stein, M.L., Medina, J.H., Izquierdo, I., 2000. Role of hippocampal signaling pathways in long-term memory formation of nonassociative learning task in the rat. Learn. Mem. 7, 333–340.
de Villers-Sidani, E., Simpson, K.L., Lu, Y.F., Lin, R.C.S., Merzenich, M.M., 2008. Manipulating critical period closure across different sectors of the primary auditory cortex. Nat. Neurosci. 11 (8), 957–965.
Winer, J.A., Lee, C.C., 2007. The distributed auditory cortex. Hear. Res. 229 (1–2), 3–13.
Xi, M.C., Woody, C.D., Gruen, E., 1994. Identification of short latency auditory responsive neurons in the cat dentate nucleus. Neuroreport 5 (13), 1567–1570.
Zheng, W., Knudsen, E.I., 2001. Gabaergic inhibition antagonizes adaptive adjustment of the owl's auditory space map during the initial phase of plasticity. J. Neurosci. 21 (12), 4356–4365.