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Noise Trauma Impairs Neurogenesis in the Rat Hippocampus

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Abstract

The hippocampus, a major site of neurogenesis in the adult brain, plays an important role in memory. Based on earlier observations where exposure to high-intensity noise not only caused hearing loss but also impaired memory function, it is conceivably that noise exposure may suppress hippocampal neurogenesis. To evaluate this possibility, nine rats were unilaterally exposed for 2 h to a high-intensity, narrow band of noise centered at 12 kHz at 126 dB SPL. The rats were also screened for noise-induced tinnitus, a potential stressor which may suppress neurogenesis. Five rats developed persistent tinnitus-like behavior while the other four rats showed no signs of tinnitus. Age-matched sham controls showed no signs of hearing loss or tinnitus. The inner ear and hippocampus were evaluated for sensory hair cell loss and neurogenesis 10 weeks post-exposure. All noise exposed rats showed severe loss of sensory hair cells in the noise-exposed ear, but essentially no damage in the unexposed ear. Frontal sections from the hippocampus were immunolabeled for doublecortin to identify neuronal precursor cells, or Ki67 to label proliferating cells. Noise-exposed rats showed a significant reduction of neuronal precursors and fewer dividing cells as compared to sham controls. However, we could not detect any difference between rats with behavioral evidence of tinnitus versus rats without tinnitus. These results show for the first time that high intensity noise exposure not only damages the cochlea but also causes a significant and persistent decrease in hippocampal neurogenesis that may contribute to functional deficits in memory.

Keywords

doublecortin; Ki67; hearing loss; tinnitus; memory; subgranular zone

The hippocampus is a major site of neurogenesis in the adult brain (Altman and Das, 1965, Kaplan and Hinds, 1977, Kuhn et al., 1996). Approximately 9000 new cells are born each day in the rat hippocampus; most cells differentiate into neurons, form synapses and generate electrical responses (Kaplan and Bell, 1984, Cameron et al., 1993, Hastings and Gould, 1999, Cameron and McKay, 2001, Song et al., 2002, van Praag et al., 2002). The hippocampus is known to play important roles in learning, memory, mood, and spatial navigation (Squire, 1982, Gould et al., 1999, Moscovitch et al., 2005, Becker and Wojtowicz, 2007). Recent studies have shown a link between hippocampal neurogenesis and memory (Kempermann, 2002b, Aimone et al., 2006, Leuner et al., 2006; Ehninger and Kempermann, 2008), particularly the consolidation and retention of long-term memory and trace-fear conditioning (Shors et al., 2002, Snyder et al., 2005, Winocur et al., 2006).

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Neurogenesis is upregulated by environmental enrichment, learning, exercise and brain injury, and may play a role in central plasticity and recovery of function (Kempermann et al., 1997, Jin et al., 2001, Brown et al., 2003a, Prickaerts et al., 2004, Ernst et al., 2006, Olson et al., 2006, Taupin, 2006, Uda et al., 2006, Brene et al., 2007, Ploughman, 2008). In contrast, anti-cancer drugs, irradiation and alcohol suppress neurogenesis (Crews et al., 2006, Dietrich et al., 2006, Duffner, 2006, Nixon, 2006). In animal models of depression, neurogenesis is decreased and conversely, treatment with anti-depressant drugs increases neurogenesis (Kempermann 2002a, Campbell and Macqueen, 2004, Duman, 2004, Chen et al., 2006, Paizanis et al., 2007, Sahay et al., 2007, Vollmayr et al., 2007, Pittenger and Duman, 2008). Chronic exposure to stressors such as isolation, crowding, or unpredictable aversive conditions such as immobilization or forced swimming depress neurogenesis (Joels et al., 2004, Lucassen et al., 2006, Warner-Schmidt and Duman, 2006).

Rats exposed to high intensity blast waves showed cognitive deficits in behavioral testing (Cernak et al., 2001, Saljo et al., 2009). Recent data from combat personnel exposed to excessive noise levels, explosions and blast waves show not only severe hearing loss and tinnitus (Cave et al., 2007) but also cognitive and memory impairment (Myers et al., 2008, Belanger et al., 2009). The hippocampus responds to auditory stimuli (Bickford-Wimer et al., 1990, Ehlers et al., 1994, Xi et al., 1994, Sakurai, 2002) and is thus directly affected by noise. Intense noise levels have been shown to cause cell death of granule and pyramidal neurons (Saljo et al., 2002), and alter the firing patterns of hippocampal place cells (Goble et al., 2009). In this study, we wanted to determine if intense noise may impair hippocampal neurogenesis.

Subjective tinnitus, a phantom auditory sensation (e.g., ringing, buzzing) is strongly correlated with noise-induced hearing loss, stress and depression (Halford and Anderson, 1991, Dobie et al., 1992, Henry and Wilson, 1995, Folmer et al., 1999, Nicolas-Puel et al., 2002, Holgers, 2003) and may therefore be able to suppress neurogenesis. The emotions and memories evoked by severe tinnitus have been postulated to involve the hippocampus and the limbic system. Human brain-imaging studies showing changes in hippocampal activity by tinnitus or tinnitus-like acoustic stimuli provide support for this hypothesis (Jastreboff and Hazell, 1993, Lockwood et al., 1998, Andersson et al., 2000, Mirz et al., 2000, Jastreboff and Jastreboff, 2007). Moreover, a decrease in hippocampal grey matter has been observed in tinnitus patients (Landgrebe et al., 2009), possibly related to impaired neurogenesis. Thus, noise exposure could affect hippocampal neurogenesis indirectly through noise-induced tinnitus and stress.

To evaluate the hypothesis that noise exposure or noise-induced tinnitus may affect hippocampal neurogenesis, we unilaterally exposed rats to a high intensity noise designed to damage the inner ear and induce tinnitus-like behavior in some but not all animals. The microtubule-associated protein doublecortin (DCX) and the mitosis marker Ki67 were used as tools for visualization of neurogenesis. DCX is expressed in neuronal precursor cells, but not in glial cells nor in neural stem cells from which the precursors develop. Upon migration and maturation into functional neurons, DCX is down-regulated (Brown et al., 2003b, Couillard-Despres et al., 2005). Ki67, a marker for cell proliferation, is expressed during all active phases of the cell cycle but is absent in resting cells (Scholzen and Gerdes 2000).

Experimental Procedures

Animals

Twelve adult male Sprague-Dawley rats (Sasco, Charles River Laboratories International, Inc., Wilmington, MA) were used for this study. Rats used for DCX and Ki67 immunolabeling were divided into two groups, Sham Controls (N=3) and Noise Trauma rats (N=9 for DCX staining and N=5 for Ki67 staining). To determine if noise trauma caused persistent changes in

neurogenesis, Noise Trauma rats were unilaterally exposed to noise, screened for tinnitus, and sacrificed 10 weeks after noise exposure. Sham Controls underwent the same kind of treatment (i.e. tinnitus screening procedures and anesthetic exposure) except for noise trauma. The age of controls and experimental rats was matched: On day of sacrifice the average age was 174.3 ± 2.7 days for Sham Controls, 189.4 ± 7.8 days for Noise Trauma rats used for DCX immunolabeling, and 187.4 ± 10.9 days for Noise Trauma rats used for Ki67 labeling.

All animal procedures were carried out in accordance with the NIH guidelines for use and care of laboratory animals and approved by the University of Buffalo Institutional Animal Care and Use Committee (protocol HER05080Y).

Noise exposure

Under isoflurane anesthesia (4% induction, 1.5-2% maintenance), rats were unilaterally exposed to narrowband noise (bandwidth 100 Hz) centered at 12 kHz, 126 dB SPL for 2 hours. Sound stimuli were generated by Tucker-Davis Technology (TDT) hardware and presented by a super compression driver (D-59; GMI Sound Corp) positioned 10 mm from the opening of the rat's left ear. The SPL was calibrated with a sound level meter coupled to a half-inch condenser microphone (Model 824 Audiometer, Larson Davis). The contralateral ear was plugged in order to maintain normal hearing in the right ear. The normal hearing ear allowed the animals to respond to the acoustic stimuli used for the tinnitus behavioral tests.

Tinnitus Assessment

Gap-prepulse inhibition of the acoustic startle (GPIAS) was utilized to obtain behavioral evidence of tinnitus as described in our previous publication (Yang et al., 2007). Each rat was positioned in an acoustically transparent wire mesh cage mounted on an acrylic base attached to a sensitive piezoelectric transducer. The sound level within each cage was measured and calibrated (Model 824 sound level meter, one-half inch microphone, Larson Davis). The output of the piezoelectric transducer was sent to a low-pass filter (1000 Hz, LPF-300, World Precision Instruments) and then to an A/D converter on a digital signal processing module (RP2.1, TDT). Using MATLAB software (The MathWorks Inc.) and custom software (generously provided by J. Ison and P. Allen, University of Rochester), the root mean square (RMS) amplitude of the startle response was calculated over a 100 ms interval following the onset of the startle stimulus. Sound stimuli were generated by two digital signal processors (RP2.1, TDT) controlled by custom software, amplified (SA1; TDT), and presented by a high frequency dome tweeter (FT28D, Fostex) located 25 cm above the rat. The startle eliciting stimulus consisted of a broadband noise burst presented at 100 dB SPL (1 to 30 kHz bandwidth, 20 ms duration, 0.1 ms rise/fall time, 100 kHz sampling rate).

GPIAS

The amplitude of the acoustic startle response was measured in the Noise Trauma rats and Sham Controls under two different test conditions. For GPIAS testing, the startle stimulus was embedded in a continuous background noise (60 dB SPL); the center frequency of the narrow band was varied across GPIAS test conditions. On half the trials, the continuous noise contained a silent gap (50 ms). The onset of the gap preceded the onset of the startle stimulus by 100 ms. The RMS amplitude of the acoustic startle response was measured in the presence of continuous noise with “no gap” (Fig. 1A) or with a “gap” (Fig. 1B). When the gap preceded the startle stimulus, there was a reduction in the amplitude of the startle response in normal hearing rats (Fig. 1A vs. 1B), i.e. the gap serves as a pre-pulse and inhibits the startle response (Ison et al., 2002, Yang et al., 2007). The presence of significant GPIAS indicated that the rat was able to detect the silent gap. In contrast, gap detection and GPIAS were expected to be greatly reduced in rats with severe tinnitus since the phantom sound of tinnitus presumably fills in the silent gap. Therefore in rats with tinnitus, the startle amplitude would no longer differ significantly

between the “gap” and “no-gap” conditions (Fig. 1C, D). To estimate the pitch of the tinnitus, GPIAS tests were run with narrow bands of noise (1000 Hz bandwidth) centered at 6, 12, 16, 20 or 24 kHz. For each frequency the test set contained 20 pairs of “no-gap” and “gap” trials, with the test order randomized. The inter-trial interval was randomly varied from 9 to 15 seconds.

Ten GPIAS tests were run during a 2 week period before the noise exposure (“baseline”). The results from the first three days were considered as acclimation tests and discarded from the analysis. After noise exposure, rats were again tested at 1-10 days post-exposure (“short-term”, 7 test days in total) and then at 8-10 weeks post-exposure (“long-term”, 7 test days in total). Age-matched Sham Controls were tested along with the Noise Trauma rats. For each day of testing, amplitudes were normalized to the average amplitudes at the “no-gap” condition, which were set to 1, and the average amplitudes were presented as mean $\pm$ standard error of mean (SEM). For each testing period, the average startle response amplitude in the “gap” condition was compared to the average amplitude in the “no-gap” condition using the two-tailed Student's t-test ($P < 0.05$).

Fixation

On the day of sacrifice, rats used for immunolabeling received a lethal dose of sodium pentobarbital and were transcardially perfused with phosphate buffered saline (PBS, 0.1M, pH 7.4) for 5 minutes and then with 10% phosphate buffered formalin (Fisher Scientific, Pittsburgh, PA) for 15 minutes at room temperature. The brain and the bulla containing the cochleae were removed from the skull. The brains were post-fixed in 10% phosphate buffered formalin for one week.

Cochleograms

The organ of Corti was carefully dissected out of the cochlea as a flat surface preparation and stained with Harris' hematoxylin solution as described in detail in our previous publications (Ding et al., 2001, Ding et al., 2002). The basal, middle and apical turns of the organ of Corti were carefully removed, mounted on glass slides in glycerin, coverslipped and examined from base to apex under a microscope (Zeiss Standard, 400X). Cochleograms were prepared showing percent missing outer hair cells (OHC) and inner hair cells (IHC) as a function of percent distance from apex of the cochlea using lab norms. Hair cells were counted in 0.24 mm intervals along the entire length of the cochlea. Hair cells were counted as present when the cell body and cuticular plate were intact.

Immunohistochemistry

Brain tissue containing the left or right hippocampus was immersed in 30% sucrose in 0.1 M PBS pH 7.4 (Sigma-Aldrich) overnight at 4° C. The following day the tissue was cut on a cryostat into 30 μ m frontal sections at -30 °C. Free floating sections were collected and washed in 0.1 M PBS, pH 7.4, pretreated with H₂O₂ for peroxidase deactivation, and then pre-incubated in blocking buffer containing 10% normal horse serum and 0.05% Triton X-100 in 0.1M PBS, pH 7.4 for 30 minutes at room temperature. Subsequently, sections were exposed for 2 h at room temperature to an antibody against DCX (polyclonal; made in goat; sc-8066; Santa Cruz Biotechnology, Inc; 2 μ g/ml) or against Ki67 (polyclonal, made in rabbit; Novocastra Laboratories Ltd, UK; 1 μ l/ml) in 0.1 M PBS, pH 7.4 with 1.0% normal horse serum and 0.05% Triton. DCX or Ki67 was visualized through the indirect staining method utilizing a biotinylated secondary antibody (anti-goat IgG or anti-rabbit IgG, Vector Laboratories, Burlingame, CA), the avidin-biotin-peroxidase complex technique (Elite-ABC, Vector Laboratories), and diaminobenzidine tetrahydrochloride (DAB, 0.05%, Sigma-Aldrich) with nickel ammonium sulfate (0.3%) and H₂O₂ (0.0015%) in 0.1 M Tris buffer, pH 7.2 (Trizma-base, Sigma). The secondary antibody and ABC incubation steps lasted for 1 h each at room

temperature. Between all incubation steps, sections were washed in 0.1 M PBS, pH 7.4. After staining, sections were washed in PBS and mounted on gelatin-coated slides (SuperFrost plus; Fisher Scientific) and dried overnight. Then sections were dehydrated in increasing concentrations of ethanol, cleared in xylene and sealed with DPX.

Photomicrographs, quantification and statistical analysis

Images visualized under bright field illumination (Axioskop, Carl Zeiss Inc) were photographed using a digital camera (SPOT Insight, Diagnostic Instruments Inc) and processed with imaging software (SPOT Software, version 4.6) into 8-bit grayscale. Assembly of images was done with Adobe Photoshop 5.5. Statistical evaluations were done with GraphPad Prism (Version 5.01; GraphPad Software Inc).

Cell bodies in the subgranular zone (SGZ) immunopositive for DCX or Ki67 were counted at high magnification (400X). A cell immunopositive for DCX was counted when the cell body was recognizable as such by morphology, either by a clearly visible nucleus or by size and shape characteristic for this type of cell. Specific-stained cells were clearly darker than its surroundings, and with cytoplasm homogenously stained. Cells immunopositive for Ki67 were recognized by a dark globular or oval structure with size comparable to the nucleus. Immunopositive cells in the SGZ in the dorsal dentate gyrus were counted in sections throughout the rostral-caudal axis of the hippocampus (-2.0 to -5.6 mm from bregma; Paxinos and Watson, 2004). For DCX quantification, cells were counted from 20 sections from the left or right side. For every section, the number of cell profiles was divided by the length of the SGZ to obtain cell density. Average cell densities were then normalized to the numbers obtained in Sham Controls, which were set to 100% (1.0 in figures). For Ki67 quantification, cells were counted in at least 25 sections from each animal and the number divided by the length of the SGZ. Average Ki67 cell densities were based on the median of 20 or 21 values in the samples. All numbers are presented as averages $\pm$ SEM and statistical comparisons were performed with the two-tailed Student's t-test ($P < 0.05$).

Results

Inner ear damage after unilateral noise trauma

All rats unilaterally exposed to the 126 dB SPL noise showed nearly complete loss of OHC and IHC in the exposed ear between 20% and 100% distance from the apex of the cochlea (Fig. 2). This region corresponds roughly from 2 to > 50 kHz on the rat cochlear frequency-place map (Müller, 1991, Greenwood, 1996). OHC loss extended further towards the apex than IHC. The pattern and extent of OHC and IHC damage showed relatively little variability across rats. Importantly, contralateral ears of all noise exposed rats showed no signs of damage (data not shown).

Tinnitus screening

Noise Trauma rats and Sham Controls showed significant GPIAS before noise exposure (Baseline; Fig. 3; Circles). These results indicated that the rats could detect the silent gap and were not experiencing tinnitus, consistent with previous research (Turner et al., 2006, Yang et al., 2007). The differences in startle amplitudes between the “no-gap” and “gap” conditions (Fig. 1) were significant at all frequencies ($P < 0.05$). However, the extent of GPIAS shown by startle amplitude reduction in percent varied among rats from approximately 30% to 60%.

After noise exposure, the Noise Trauma rats and Sham Controls were tested for a period from day 1 to day 10 post-exposure for “short term” effects (Fig. 3; Triangles) and re-tested from week 8 to week 10 for “long-term” effects (Fig. 3; Diamonds). The age-matched, normal hearing Sham Controls continued to show significant GPIAS at both testing periods and at all

frequencies. Rats in the Noise Trauma group showed varying outcomes. In both post-noise testing periods, four rats (B1, D3, E and F) continued showing significant GPIAS at all frequencies similar to the results from Sham Control rats, and thus no signs of tinnitus. Five animals (C1, D1, C3, A4 and B4) showed a lack of GPIAS at one or more frequencies. There was no significant difference in startle amplitudes in “no-gap” versus “gap” conditions at one or more frequencies in these rats ($P > 0.05$). Therefore rats C1, D1, C3, A4 and B4 were considered to have behavioral evidence of tinnitus (Turner et al., 2006, Yang et al., 2007). Additionally, there was one other change after the noise exposure. At the lower frequencies, particularly 12 kHz (i.e., the exposure frequency), rats C1, C3 and D1 showed a significant increase in startle amplitudes during the “gap” condition as compared to the “no-gap” condition, that is, the gap signal enhanced the response to the startle stimulus (facilitation) instead of reducing it. Rat A4 and B4 showed a similar tendency, but it was not significant. These changes may possibly be related to loudness recruitment or a collapse of sound tolerance, which sometimes accompanies tinnitus, and which may increase stimulus aversiveness (Sun et al. 2009). This facilitation-like behavior was only observed in rats with behavioral evidence of tinnitus.

Neuronal precursor cells and cell proliferation

Figure 4 shows DCX immunopositive neuronal precursor cells and Figure 5 shows Ki67 immunopositive nuclei of proliferating cells in the SGZ in the dorsal dentate gyrus. In the normal hearing Sham Controls, DCX positive cell bodies form a continuous or almost continuous band along the SGZ with vertically extending dendrites (Fig. 4A, C). After noise trauma, the number of DCX-positive cells was greatly reduced (Fig. 4B). There were only a few Ki67 positive cell nuclei in each section in Sham Controls (Fig. 5A) and Noise Trauma rats (Fig. 5B), requiring cell counts throughout a high number of sections to detect any difference between normal hearing Sham Controls and Noise Trauma rats. Ki67 immunolabeled nuclei in both Sham Controls (Fig. 5C, D) and Noise Trauma rats (Fig. 5E, F) tended to occur in pairs or small cluster consistent with previous hippocampal observations (Wang and Baraban, 2007).

Rats in the Noise Trauma group showed a reduction in DCX immunolabeling (Fig. 4A versus B). To determine whether unilateral noise trauma exerted an asymmetrical effect on hippocampal neurogenesis, DCX cell density in the left and right hippocampus were compared. As there was no difference between counts from the left and from the right hippocampus in any of the groups ($P > 0.05$) counts from the left and right hippocampus were pooled. To determine whether noise trauma significantly reduced hippocampal neurogenesis, DCX cell density was compared between the Noise Trauma rats and Sham Controls. In Noise Trauma rats, the DCX cell population was significantly reduced to $72.2\% \pm 6.3\%$ ($P = 0.036$) of Sham Control values (Fig. 6A). To determine if tinnitus was a factor regulating neurogenesis, DCX density was compared in noise trauma rats with and without behavioral evidence of tinnitus (Fig. 7A). The average DCX cell density in Noise Trauma rats with tinnitus ($71.1\% \pm 2.6\%$) was similar to the density in Noise Trauma rats without tinnitus ($73.6\% \pm 15.0\%$), and there was no significant difference ($P = 0.86$).

Rats in the Noise Trauma group showed a reduction in Ki67 immunolabeling compared to Sham Controls (Fig. 5A, B). To determine if cell proliferation was significantly reduced by the unilateral noise exposure, Ki67 cell density was compared in Noise Trauma rats versus Sham controls. In Noise Trauma rats the average Ki67 cell density was significantly reduced to $56.5\% \pm 9.0\%$ ($P = 0.025$) of Sham Control rats (Fig. 6B). To determine if tinnitus was a factor regulating cell proliferation, Ki67 cell density was compared in noise trauma rats with and without behavioral evidence of tinnitus (Fig. 7B). Again, there was no significant

difference in Ki67 cell density between Noise Trauma rats with tinnitus ($66.2\% \pm 19.3\%$) and Noise Trauma rats without tinnitus ($50.0\% \pm 9.6\%$; $P = 0.45$).

Discussion

High intensity noise exposure has long been known to cause hearing loss by damaging the sensory hair cells and supporting cells in the inner ear (Bohne, 1977, Hamernik et al., 1984). However, much is still unknown about its effects on the central nervous system, particularly in regions outside the classical auditory pathway. Our results show for the first time that acoustic overstimulation (126 dB SPL, 2 h, 12 kHz) causes a long lasting suppression of hippocampal neurogenesis in adult rats. On average, the number of DCX cells was reduced by almost 30% and the number of Ki67 cells by more than 40% 10 weeks after the noise trauma. This effect was seen in all noise exposed rats: we did not detect any difference in DCX or Ki67 cell density between rats with or without evidence of tinnitus-like behavior.

Noise exposure impairs neurogenesis

Soldiers and other personnel exposed to extremely high level noise (~170 dB SPL; such as blast waves or explosions) often suffer from cognitive and memory impairments that are often associated with traumatic brain injury (TBI) (Rimel et al., 1981, Cernak et al., 2001, Belanger et al., 2009). Similarly, rats exposed to shock waves at 10 kPa (174 dB SPL) also demonstrate poor cognitive function on the Morris water maze test, which is most likely the result of direct injury to the brain caused by the acoustic overpressure (Saljo et al., 2009). Our results demonstrate that continuous noise (126 dB SPL, 40 Pa), much less intense than the blast waves demonstrated to cause TBI, but of longer duration, causes significant, long-term suppression of neurogenesis. Given that hippocampal neurogenesis may be related to memory (Snyder et al., 2005, Aimone et al., 2006, Winocur et al., 2006; Becker and Wojtowicz, 2007), we speculate that our noise-exposed rats would show cognitive, mood or memory impairments, and that noise at intensities much lower than those causing TBI may impair memory function.

Mechanism for reduced neurogenesis after noise exposure

The decrease in hippocampal neurogenesis following acoustic overstimulation may arise through different mechanisms. Noise trauma could persistently suppress cell proliferation thereby reducing neurogenesis. Our results showed a reduced number of Ki67-labeled cells as well as DCX-labeled precursor cells in Noise Trauma rats. A similar connection between total number of neuronal precursors and rate of cell-proliferation has been observed in earlier studies (Couillard-Despres et al., 2005; Kim et al., 2009), suggesting that a reduced number of precursors is often directly linked to a reduced rate of cell proliferation. Alternatively, DCX cell numbers could be reduced directly by stimuli-induced cell death, as observed for granule and pyramidal neurons after exposure to extremely high levels of impulse noise (Saljo et al., 2002), or after treatment with anti-cancer drugs like cisplatin (Dietrich et al., 2006). However, the neuronal precursor cell population fully recovers a few weeks after termination of drug treatment; therefore it seems unlikely that reduced neurogenesis seen as late as 10 weeks post-exposure is related to noise-induced cell death alone. A reduced number of neuronal precursors may also be caused by a shift of differentiation from a neuronal lineage toward a glial lineage, thereby reducing the number of DCX cells, as seen after treatment with sedatives and anticonvulsant drugs (Stefovska et al. 2008). However, since also Ki67 immunolabeling showed a large reduction in cell proliferation, altered differentiation seems unlikely to play a major role after noise exposure.

Neurogenesis can be down-regulated through neuronal hyperactivity in the hippocampus. In the case of temporal lobe epilepsy, a condition of neuronal hyperactivity, neurogenesis increases during the acute phase, but decreases in the chronic phase (Hattiangady et al.,

2004, Kuruba et al., 2009). Chronic down-regulation of neurogenesis after noise exposure may be triggered by a similar mechanism. Noise exposure can cause acute (Puel et al., 1998) as well as long-term (Syka et al., 1994; Salvi et al., 2000) hyperactivity in the auditory system. Since the hippocampus responds to auditory stimuli (Bickford-Wimer et al., 1990, Ehlers et al., 1994, Xi et al., 1994, Sakurai, 2002), exposure to high intensity noise may be able to cause hyperactivity not only in the auditory system but also in the hippocampus, and cause a chronic decrease of neurogenesis. Alternatively, neurogenesis may be impaired by the unilateral noise-induced hearing loss which changes the afferent neuronal input to the brain. Intense noise-exposure was recently shown to alter the firing patterns of hippocampal place cells (Goble et al., 2009). Early onset age-related cochlear degeneration has been associated with a reduced number of hippocampal synapses and impaired spatial memory (Dai et al., 2009). In all cases, the reduced neurogenesis observed in this study would be related to abnormal auditory input to the brain, either hyperactivity during noise exposure or altered activity due to hearing loss.

Finally, neurogenesis may be reduced by stress related to hearing loss. Impaired hearing may cause aversive conditions for the animal and thus act as a chronic stressor, which in turn may down-regulate neurogenesis. Such aversive conditions may include impaired perception of the acoustic environment or increased stimulus aversiveness. We also considered tinnitus as a potential stressor, but our data obtained with the gap-startle method do not support any effect of tinnitus. Nevertheless, the results cannot rule out the possibility that tinnitus may be capable of suppressing neurogenesis under other conditions. One reason why tinnitus may not have caused a significant decrease in cell proliferation or neurogenesis in our study is that the tinnitus effect may have been masked by the stronger effects of the noise exposure itself. Alternatively, our behavioral test for tinnitus may not be sensitive enough to detect tinnitus in all of the rats with noise induced hearing loss.

Conclusions and outlook

The results presented here are the first to demonstrate that noise exposure not only damages the peripheral auditory system, but also causes a major, long-term reduction of hippocampal neurogenesis. Thus, hearing protection or noise avoidance may not only protect the ear, but also the hippocampus, which is linked to memory and emotion. Because noise-induced hearing loss is extremely prevalent in industrialized societies, an important question that needs to be answered is what level of noise or hearing loss is sufficient to suppress hippocampal neurogenesis. Moreover, understanding the mechanisms on how noise may impair hippocampal neurogenesis may be important for developing treatments for recovery of memory function after noise trauma.

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List of abbreviations

DCX	Doublecortin
GPIAS	Gap prepulse-induced inhibition of the acoustic startle
IHC	Inner hair cells
OHC	Outer hair cells
PBS	Phosphate buffered saline
RMS	Root mean square
SGZ	Subgranular zone
SPL	Sound pressure level

TBI Traumatic brain injury

Figure 1.

Gap prepulse-induced inhibition of the acoustic startle (GPIAS) paradigm used for tinnitus screening in rats after noise trauma: A noise burst at 100 dB SPL, embedded in a continuous noise floor of moderate SPL (60 dB), results in an acoustic startle response (A). When the background noise floor contains a short gap immediately before the startle-eliciting stimulus, the amplitude of the startle is reduced (B) showing that the rat is able to detect the silent gap by inhibiting its response to the subsequent startle stimulus. A rat with tinnitus however, shows no difference in startle amplitudes in the “no-gap” (C) and “gap” (D) condition, since the perceived phantom sound fills the gap and thus prevents the rat from detecting the silent gap thereby eliminating pre-pulse inhibition of the startle response.

Figure 2.

Cochleograms showing degree of outer hair cell (OHC, dashed line) and inner hair cell (IHC, solid line) loss as function of percent distance from the apex of the noise exposed cochlea (126 dB, 100 Hz narrowband noise centered at 12 kHz, 2 hours) in nine rats allowed to survive for 10 weeks. All nine Noise Trauma rats showed severe loss of both IHC and OHC throughout most of the cochlea except for the most apical, low-frequency region. OHC loss was greater than IHC loss.

Figure 3.

Gap pre-pulse induced inhibition of the acoustic startle (GPIAS) in Sham Controls (open symbols) and Noise Trauma rats (filled symbols): For each rat, frequency, and testing period, the diagrams show the average amplitude at the “gap” condition, relative to the average amplitude at the “no-gap” condition, which was normalized and set to 1 (horizontal dashed line). In case of gap detection, the average amplitude at the “gap” condition was significantly smaller than the amplitude at the “no gap” condition (inhibition). Circles: Before any treatment, all rats showed significant inhibition at the “gap” condition at all frequencies tested (“Baseline”). Average inhibition ranged from approximately 30% to 60% and varied among individual rats, but was significant at all frequencies tested for all rats. Triangles: At days 1 –

10 after noise exposure, five Noise Trauma rats (Rat C1, D1, C3, A4 and B4) showed changes in gap detection. At one or more frequencies tested, these rats now showed no significant difference in amplitudes at “no-gap” conditions and “gap” conditions (“T”), demonstrating no or only very poor gap-detection and thus signs of severe tinnitus. Some of these rats showed a significant increase of amplitudes (facilitation) which may be related to increased stimulus aversiveness. The other four Noise Trauma rats (B1, D3, E and F), along with age-matched Sham Controls (A, BB and CC), continued showing significant inhibition at all frequencies. Diamonds: When rats were re-tested at week 8-10 after the noise exposure, the five Noise Trauma rats with tinnitus-like behavior during days 1-10 again showed evidence of tinnitus (bold “T”) and facilitation. The other four Noise Trauma rats and Sham Controls continued showing significant inhibition at all frequencies.

Figure 4.

Neuronal precursor cells immunostained for DCX in the adult rat hippocampus in a normal hearing rat (A) and a rat with severe noise trauma 10 weeks after the noise exposure (B). A: In the normal rat, cell bodies of neuronal precursor cells (arrowheads) form a thin line along the subgranular cell zone (SGZ) in the dentate gyrus (DG). B: The noise exposed rat showed a strongly reduced number of neuronal precursor cells. Arrowheads point to some of the few remaining cells. C, D: Single DCX neuronal precursor cells shown at high magnification in the normal hearing rat (C) and in the rat with noise trauma (D). Scale bars: 200 μ m in Fig. 4B for Fig. 4A, B; 50 μ m in Fig. 4D for Fig. 4C, D.

Figure 5.

Dividing cells immunostained for Ki67 in the adult rat hippocampus in a normal hearing rat (A, C, D) and a rat with severe noise trauma 10 weeks after the noise exposure (B, E, F). A, B: In the normal rat as well as the noise-exposed rat, Ki67-immunopositive nuclei (arrowheads) are present in the subgranular cell zone (SGZ) in the dentate gyrus (DG). Ki67 immunopositive cells were typically clustered in pairs or small groups in normal hearing rats (5C, D) as well as in noise exposed rats (5E, F). Scale bars: 200 μ m in Fig. 5B for Fig. 5A, B; 50 μ m in Fig. 5C for Fig. 5C-F.

Figure 6. Reduction of density of DCX labeled cells (A) and Ki67 labeled cells (B) in the hippocampus after noise trauma. Numbers were normalized to average number of cells in Sham Controls (white bars), which was set to 1. Noise Exposed rats (black bars) showed a strong and significant reduction in numbers of DCX positive cells as well as Ki67 positive cells.

Figure 7.

DCX (A) and Ki67 (B) cell density in the SGZ after Noise Trauma in rats without signs of tinnitus and in rats with signs of tinnitus: We were not able to detect any difference in cell numbers between the two groups.