Time course of cell death due to acoustic overstimulation in the mouse medial geniculate body and primary auditory cortex

Felix Fröhlich, Dietmar Basta, Ira Strübing, Arne Ernst, Moritz Gröschel

Department of Otolaryngology, Unfallkrankenhaus Berlin, Charité Medical School, Warrener Straße 7, Berlin, Germany

Abstract

It has previously been shown that acoustic overstimulation induces cell death and extensive cell loss in key structures of the central auditory pathway. A correlation between noise-induced apoptosis and cell loss was hypothesized for the cochlear nucleus and colliculus inferior. To determine the role of cell death in noise-induced cell loss in thalamic and cortical structures, the present mouse study (NMRI strain) describes the time course following noise exposure of cell death mechanisms for the ventral medial geniculate body (vMGB), medial MGB (mMGB), and dorsal MGB (dMGB) and the six histological layers of the primary auditory cortex (AI 1–6). Therefore, a terminal deoxynucleotidyl transferase dioxouridine triphosphate nick-end labeling assay (TUNEL) was performed in these structures 24 h, 7 days, and 14 days after noise exposure (3 h, 115 dB sound pressure level, 5–20 kHz), as well as in unexposed controls. In the dMGB, TUNEL was statistically significant elevated 24 h postexposure. AI-1 showed a decrease in TUNEL after 14 days. There was no statistically significant difference between groups for the other brain areas investigated. dMGB’s widespread connection within the central auditory pathway and its nontotopical organization might explain its prominent increase in TUNEL compared to the other MGB subdivisions and the AI. It is assumed that the onset and peak of noise-induced cell death is delayed in higher areas of the central auditory pathway and takes place between 24 h and 7 days postexposure in thalamic and cortical structures.

Keywords: Central hearing loss, noise-induced apoptosis, noise-induced hearing loss, TUNEL-staining

INTRODUCTION

Noise-induced hearing loss has been a topic of research for decades, whereby most attention has been paid to inner ear pathologies.[1-6] Noise leads to oxidative stress and modified blood flow in the cochlea, with altered expression of proapoptotic and antiapoptotic genes, cell death and hair cell loss along the basilar membrane.[7-12] Changes in the central auditory pathway have been proposed to result from deafferentation due to cochlear pathologies after noise. Degeneration in the cochlear nucleus (CN) after cochlear ablation was already described more than 40 years ago.[13] Alterations within the central auditory system contribute to a shift in hearing thresholds (central hearing loss)[14] and might be triggered by noise trauma[15] or deafferentation.[13,16] However, it was also found that acoustic overstimulation appears to have a direct impact on the central auditory pathway. It was shown that noise leads to a significant decrease in cell densities in the central auditory system during the first days after acoustic overexposure. The CN seems to be affected immediately, while 7 days after a noise trauma, a reduction in cell density was found in the colliculus inferior (IC), medial geniculate body (MGB), and primary auditory cortex (AI).[17,18] Lately, the time course of apoptosis after acoustic overstimulation was described for the CN and IC.[19] Supporting the results described above. Significantly, neuronal loss in the CN varies between its subdivisions, depending on whether the trauma was induced by noise exposure or mechanical compression of the auditory nerve.[19,20] A correlation between noise exposure and cell death

Access this article online

Quick Response Code:

Website: www.noiseandhealth.org

DOI: 10.4103/nah.NAH_10_17

Address for correspondence: Moritz Gröschel, Department of Otolaryngology, Unfallkrankenhaus Berlin, Charité Medical School, Warrener Straße 7, 12683 Berlin, Germany.
E-mail: moritz.groeschel@biologie.hu-berlin.de

This is an open access article distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 3.0 License, which allows others to remix, tweak, and build upon the work noncommercially, as long as the author is credited and the new creations are licensed under the identical terms.

How to cite this article: Fröhlich F, Basta D, Strübing I, Ernst A, Gröschel M. Time course of cell death due to acoustic overstimulation in the mouse medial geniculate body and primary auditory cortex. Noise Health 2017;19:133-9.
in the central auditory pathway has been reported by several studies. Following exposure to impulse noise, cell death mechanisms have been found in neurons of auditory cortex layers 2 to 6 from 6 h up to 7 days.\textsuperscript{[21]} The expression of C-Jun, a gene playing a key role in neuronal cell death, is also elevated in the central auditory system after a noise trauma.\textsuperscript{[21, 22]} Free radicals, reactive oxygen species or lipid peroxidation are stimulated by noise exposure and promote cell death mechanisms such as apoptosis and necrosis.\textsuperscript{[23-27]} With regard to these results, noise-induced cell death mechanisms are understood to induce the observed neuronal loss in several auditory brain structures, including the MGB and A1.\textsuperscript{[17, 18]}

Therefore, the aim of the present study was to investigate the time course of noise-induced cell death in the three subdivisions of the MGB [ventral MGB (vMGB), medial MGB (mMGB), and dorsal MGB (dMGB)], as well as in the six histological layers of the AI (AI 1–6) so as to contribute to the understanding of different pathological findings in the central auditory system following acoustic overstimulation.

**EXPERIMENTAL PROCEDURES**

Nineteen young adult (30–40 days of age) female normal hearing mice (NMRI strain) were used in this study. The experimental protocol was approved by the governmental commission for animal studies (LaGeSo, Berlin, Germany; PI: Dr. Dietmar Basta, approval number: G0416/10). Experiments were carried out in accordance with the EU Directive 2010/63/EU on the protection of animals used for scientific purposes. All efforts were made to minimize the number of animals used and their suffering.

**Noise Exposure**

The noise exposure paradigm is described in detail elsewhere.\textsuperscript{[18]} Briefly, a broadband flat spectrum noise (5–20 kHz) was applied to the animals for 3 h at 115 dB sound pressure level (SPL) under anesthesia (6 mg/kg xylazine and 60 mg/kg ketamin) in a soundproof chamber (80 cm × 80 cm × 80 cm, minimal attenuation 60 dB). An amplifier (Tangent AMP-50, Aulum, Denmark) and a DVD player were connected to loudspeakers (HTC 11.19; Visaton, Haan, Germany) placed above the animal’s head. A sound level meter (Volcraft 329; Conrad Electronic, Hirschau, Germany) was located next to the animal’s ear to calibrate SPL. A heating pad (Thermolux CM 15W; Acculux, Murrhardt, Germany) was placed under the mice to keep body temperature constant at 37°C during video-camera-controlled anesthesia. Different groups of animals were investigated at various points in time following noise exposure [Figure 1]. Four animals were investigated 24 h postexposure (24-h group), five animals were investigated after 7 days postexposure (7-day group), and another five animals were investigated 14 days postexposure (14-day group). Five mice were kept as unexposed controls (control group).

**Terminal Deoxynucleotidyl Transferase Dideoxynucleotide Triphosphate Nick-End Labeling Assay Staining**

On the day of investigation, following anesthesia, animals were perfused via the left heart chamber with 4% paraformaldehyde to reach a good fixation. The skull was opened carefully to remove the brain. After embedding in paraffin, 10-μm thick slices in the frontal plane were made using a rotation microtome (Euromex Präzisisionen Minot Rotationsmikrotom MT.5505; Arnhem, The Netherlands). The slices were stained by using the terminal deoxynucleotidyl transferase (TdT) dideoxynucleotide triphosphate (dUTP) nick-end labeling assay (TUNEL) method (In Situ Cell Death Detection Kit POD; Roche, Mannheim, Germany) to visualize cell death mechanisms.\textsuperscript{[28]} After removing the paraffin [2× Rotihistol\textsuperscript{©} (Carl Roth, Karlsruhe, Germany) for 10 min], rehydrating in a descending ethanol series (90 and 70%, 5 min each) and distilled water (5 min), a 10-min pretreatment with 5% proteinase K (20.5 μg/ml in 10-mM Tris–hydrogen chloride (HCL), pH 7.5, 100 μl per slice) was performed.\textsuperscript{[29]} DNA strain breaks were provoked via deoxyribonuclease (DNase) I recombinant (100 U/ml; Roche) used as a positive control as proposed by the manufacturer (Roche). A 3% H\textsubscript{2}O\textsubscript{2} solution (dissolved in methanol) was applied for 5 min to block endogenous nucleases and avoid false-positive results. Each slice was incubated for 60 min with 50 μl TUNEL reaction mixture (diluted 1:2 with phosphate buffered saline (PBS)) at 37°C in a humidified chamber. To obtain a light microscopic analysis, each slice was incubated for 30 min with 50 μl converter-POD, with diaminobenzidine as substrate (50 μl per slice for 10 min). After washing in PBS and distilled water, a dehydration in an ascending alcohol series (70% ethanol, 90% ethanol, and 100% isopropanol for 1 min each) was performed. Slices were stored in Rotihistol until mounting with Roti Histokit\textsuperscript{©} (Carl Roth).

The stained slices were microscopically magnified (250x, Carl Zeiss; Axiовert 25C, Göttingen, Germany) and colored photomicrographs were taken using a digital camera (Canon Eos 1000D, Tokyo, Japan). Pictures were standardized (“autocontrast” function, Adobe Photoshop CS3 Extended, Version 10.0, 2007, San José, California, USA) and

**Figure 1:** Paradigm of investigation. At day 0, mice were noise-exposed (115 dB SPL, 5–20 kHz, 3 h) under anesthesia. The central auditory pathway was histologically analyzed at different points in time after the noise exposure. The MGB and AI of the “24-h group” were TUNEL-stained 24 h postexposure. Other TUNEL stainings were performed for different animals one and 2 weeks after noise exposure for the “7-day group” and “14-day group”, respectively.
TUNEL-positive cells were counted manually in grids within the vMGB (0.21 mm × 0.39 mm), mMGB (0.1 mm × 0.39 mm), dMGB (0.21 mm × 0.2 mm), and six layers of the AI (0.05 mm × 0.39 mm). The brain areas were defined in accordance with the mouse brain atlas of Paxinos and Franklin. Equivalent regions of interest were investigated in earlier studies for determination of cell densities in the MGB and AI.

Statistical Analysis

Data from the 24-h and 7-day groups were tested for significant differences against unexposed controls, according to earlier publications investigating cell death and loss of neurons in central auditory structures. Data from the 14-day group were tested against the 7-day group in accordance with earlier studies of calcium-related neuronal activity.

With regard to the data, Pearson's Chi-square test (SPSS® Statistics, Version 20; IBM®, Armonk, New York, USA) was used to calculate P values. Therefore, grids with TUNEL-positive cells were distinguished from grids without TUNEL-positive cells. Results are visualized as ratio of TUNEL-positive grids to all analyzed grids [Figures 2 and 3].

The significance level was set at P < 0.05 for all statistical analysis. Bonferroni alpha adjustment was applied to account for multiple comparisons (P < 0.025 for two tests, P < 0.017 for three tests). Results with statistical significance at the level of P < 0.05 were marked with an asterisk, whereas differences that lost statistical significance due to multiple comparison were marked with asterisk in brackets (').

RESULTS

Terminal Deoxynucleotidyl Transferase Dioxyuridine Triphosphate Nick-End Labeling Assay-Labeling in the Primary Auditory Cortex

Between 28 and 46 grids were analyzed in the AI. In layer 1, the ratio of TUNEL-positive grids to all grids decreased in the 14-day group compared to 7-day group (14 vs 38%; P = 0.035). No other statistically significant differences were found at any time point for any of the six histological layers of the AI [Figure 3].

A total of 32% of the grids counted showed TUNEL-positive cells in layer 1 in unexposed controls (layer 2: 20%, layer 3: 33%, layer 4: 36%, layer 5: 43%, and layer 6: 29%). These values did not differ significantly from the ratio of TUNEL-positive grids in the 24-h group (ratio of TUNEL-positive grids and P values in layer 1: 32%, P = 0.972; layer 2: 30%, P = 0.343; layer 3: 34%, P = 0.948; layer 4: 27%, P = 0.448; layer 5: 24%, P = 0.100; and layer 6: 24%, P = 0.656) nor from 7-day group (layer 1: 38%, P = 0.621; layer 2: 24%, P = 0.731; layer 3: 33%, P = 0.949; layer 4: 33%, P = 0.835; layer 5: 39%, P = 0.722; and layer 6: 33%, P = 0.730). Fourteen days after the noise exposure, ratio of TUNEL-positive cells was not significant compared to the 7-day group (P = 0.229). A similar time course was found in the ventral subdivision of the MGB, whereby the differences in TUNEL-positive cells per grid were not statistically significant at any of the three investigation times. In the vMGB of unexposed controls, a ratio of 35% TUNEL-positive grids was present. TUNEL-positive grids were at 49% 24 h after the noise exposure and increased to 53% in the 7-day group. 33% TUNEL-positive grids were found in the 14-day group. However, the observed changes did not reach statistical significance. In the mMGB subdivision, 35% TUNEL-positive grids were counted in unexposed controls, 37% in the 24-h group (P = 0.923) and 32% in the 7-day group (P = 0.791). Two weeks after the noise exposure, 39% TUNEL-positive grids were found. This increase did not show statistically significant difference from TUNEL-positive cells counted in the 7-day group (P = 0.544).

Figure 2: Results from the medial geniculate body. Ratio of TUNEL-positive grids to all grids in the ventral, medial, and dorsal subdivisions of the MGB (vMGB, mMGB, and dMGB). In the dMGB, an elevation in TUNEL-positive grids was found at any time point for any of the six histological layers of the AI [Figure 3].

Noise & Health | Volume 19 | Issue 88 | Month 2017
DISCUSSION

The present study investigated the influence of traumatizing noise on cell death mechanisms such as apoptosis, necrosis, and autolytic cell death\(^{[33]}\) over time in the thalamic and cortical structures of the auditory system, which might reflect one of the underlying pathophysiological mechanisms in noise-induced neuronal loss in the MGB and AI determined in earlier studies.\(^{[17,18]}\)

**Medial Geniculate Body**

The TUNEL labeling in the MGB was analyzed for its three subdivisions separately. The ventral subdivision of the MGB is tonotopically organized as part of the classical auditory pathway and receives its main input from the central nucleus of the inferior colliculus (ICC).\(^{[34-37]}\) In the present study, the increase in TUNEL in the 24-h and 7-day groups in vMGB was more than 40% compared to unexposed controls, but not statistically significant [Figure 2]. Protection of the vMGB might be due to large GABAergic feed-forward inhibition from ICC to vMGB\(^{[38,39]}\) or inhibitory feedback from the reticular thalamic nucleus, which receives excitatory inputs from AI-6 and multimodal cortical areas as well as from the thalamus.\(^{[39]}\) Previous investigations in the lower auditory pathway showed increased TUNEL labeling immediately after noise trauma that lasted for more than 1 week.\(^{[15]}\) Effects in CN and IC were significant and the time course was similar for both structures. However, the total number of apoptotic cells was lower in IC, possibly caused by inhibitory projections from CN to higher structures suppressing cellular hyperexcitation, resulting in neuronal protection.\(^{[40]}\) The time course of cell death in vMGB was comparable to its preconnected IC and CN but again less prominent and statistically not significant. However, as neuronal loss was similar 1 week postexposure in CN, IC, and vMGB (30, 31, and 31%, respectively),\(^{[18]}\) peak of cell death might be delayed along the ascending auditory pathway rather than being diminished.

Similar conclusions were drawn from another study measuring the accumulation of calcium by using a manganese technique at different times following exposure to the present noise paradigm. No differences were found between unexposed controls, an acutely investigated group (due to the timespan of manganese accumulation comparable to the 24-h group in the present study) or after 14 days, but was enhanced 1 week after exposure in the MGB.\(^{[32]}\) Elevated intracellular calcium has been proposed to serve as a key factor in necrosis and apoptosis, both being stained by TUNEL.\(^{[33,41-43]}\)

Noise-induced hyperactivity has been described for several structures in the central auditory pathway including the MGB\(^{[44-49]}\) and might contribute to the present TUNEL findings in vMGB, as upregulation of excitatory neurotransmission plays an important role during induction of cell death.\(^{[50,51]}\) Similar results have been shown in the central olfactory system, where neuronal plasticity and reorganization accompany cell death.\(^{[52]}\) Supporting these ideas, a 2-deoxyglucose increase, representing a marker for neuronal activity and neuroplasticity, was also found after noise exposure in MGB.\(^{[53]}\)

Medial and dorsal subdivisions of the MGB show different characteristics compared to vMGB. They are smaller in size in mice, they are not part of the classical auditory pathway, they receive different input (e.g., other IC substructures and somatosensory system) and they are not tonotopically organized.\(^{[35,37,54]}\) Both, mMGB and dMGB, show different neuronal responses to acoustic stimulation compared to vMGB.\(^{[54]}\) In any case, a massive neuronal loss 1 week postexposure was found in all subdivisions of the MGB, which was shown to be slightly less prominent in the mMGB (~28%) compared to dMGB (~31%), and vMGB (~31%).\(^{[18]}\) Therefore, an increase in cell death mechanisms should occur in mMGB and dMGB during the week following noise exposure. Results in mMGB led to the assumption that apoptosis starts later (>24 h postexposure) and ends earlier (<7 days postexposure), possibly due to slightly weaker degeneration. In contrast, dMGB data showed a significant increase in TUNEL staining in the 24-h group (P = 0.012) and a
slight elevation in the 7-day group compared to controls. As discussed for the vMGB, neuronal degeneration is quite prominent 7 days postexposure.[18] Inhibitory and excitatory inputs originating from the central nucleus and other substructures of the IC reach the MGB,[55] but feed-forward inhibition from the ICC mentioned before might have a stronger protective function in the vMGB[38,39] and could explain the earlier on-set of cell death after noise exposure in the dMGB data.

Significantly, only the mMGB subdivision showed a slight, although not significant, increase in TUNEL-positive grids in 14-day group (39%) compared to 7-day group (32%), and might be a first indication of degeneration due to deafferentation and reorganization in higher central auditory structures. Similar effects occur in the visual cortex after enucleation.[56] In contrast to vMGB and dMGB, the mMGB appears more vulnerable to deafferentation, perhaps due to its extralemniscal innervation and multisensory integration, whereas ascending connections to ICC might explain the massive neuronal loss at day 7 postexposure.[17,18,57] Comparable consequences were described for the DCN, which receives multisensory projections and changes its responsiveness to trigeminal stimulation after noise damage.[58] With regard to reduced neuronal density 1 week postexposure, another important finding is the ratio of TUNEL-positive grids being similar or higher in the 7- and 14-day groups compared to unexposed controls, suggesting ongoing degeneration. An upregulation of plasticity markers was found in the CN between 15 and 30 days but not within the first week postlesion[59] and axonal sprouting was described in the DCN from weeks up to months after trauma.[60,61] This might compensate for reduced connectivity accompanying cell death.[62] An increase of calcium activity in the MGB[32] is proposed to act as a neuroprotective,[42,43] possibly responsible for the results seen in mMGB. On the other hand, calcium accumulation could be related to apoptosis in each subdivision of the MGB due to different concentrations occurring over time.[41]

Primary Auditory Cortex

The thalamus is the primary source of sensory information to the cerebral cortex.[63] The AI receives its main input from the ventral subdivision of the MGB that terminates in the middle layers of the AI, but also extralemniscal connections including dMGB and mMGB exist.[64] In addition, inputs arise from several multisensory thalamic and cortical areas.[63] Former studies showed a reduced cell loss in AI 1 week postexposure compared to lower auditory structures (layer-dependent between 10 and 18%)[18] indicating that the magnitude of cell death should also be less. In the present study, no statistically significant increase in TUNEL-positive staining could be found within 1 week postexposure for any of the six layers of the AI, suggesting that maximum apoptotic levels are present between the points in time when measurements were performed.

Since the increase in TUNEL-positive grids in the mMGB in the 14-day group was interpreted as deafferentation-induced degeneration, a similar, but reduced effect may be present in AI-2, the only layer showing even a slight increase in TUNEL-positive grids in the present study after 14 days. This hypothesis is underpinned by earlier investigations that did not find a significant cell loss 1 week after noise exposure in AI-2 (in contrast to all other AI layers).[18] As AI-2 receives no direct input from the MGB but rather from higher auditory structures, such as the contra and ipsilateral AI or secondary auditory cortex,[37,63,65-70] auditory information arises partially from intracortical inhibitory interneurons, representing up to 20 to 30% of cortical neurons. This may provide short-term neuroprotective effects.[71,72] Inhibitory interneurons could be one possible source of long-term neurodegeneration, leading to increased activity due to upregulation of excitatory and downregulation of inhibitory synaptic transmission.[72-75] Upregulation of neuronal firing induced by layer-dependent changes in inhibition and excitation could provoke excitotoxicity and might contribute to layer-specific cell death in AI.[76]

In layer 1 of AI, decrease in TUNEL-positive grids in the 14-day group compared to 7-day group lost statistical significance on the level $P < 0.05$ due to Bonferroni’s correction to counteract the problem of multiple comparison. The TUNEL result is remarkable with regard to the strong decrease of neuronal density in AI-1 (−18%), the strongest decrease among all AI layers 1 week postnoise-exposure.[18] This might be related to the MGB results, as the AI-1 receives input directly from dMGB.[68] All AI-1 neurons are inhibitory including large neurons with horizontal processes, named the Cajal–Retzius cell, that are unique to layer I.[71] As neuronal loss in AI-1 postexposure might affect mainly inhibitory interneurons,[18] loss of inhibition could induce an exacerbation and ongoing cell loss. This process might still take place 1 week postexposure but seems to be diminished at day 14. As a consequence, the resulting lower number of cells in AI-1, and the reduced cell death might be responsible for the decrease in TUNEL after 2 weeks.

The ascending input from the ventral subdivision of the MGB terminates mainly in layers 3 and 4 of the AI.[63] These areas, as well as layers 5 and 6, which also receive direct MGB input,[77] show a weaker impact on neuronal density 1 week after noise exposure,[18] probably due to protection from both the MGB and intracortical interneurons.[71] In layers 3 to 6, the ratio of TUNEL-positive grids in the 7-day group remained unchanged compared to unexposed controls, which might also be an effect of a delayed but long-lasting neurodegeneration. A study on calcium-related activity showed a decrease in AI 2 weeks after noise exposure and supports this idea, as it may be related to reduced cell density in the auditory cortex.[132] Therefore, noise-induced apoptosis in AI might be a consequence of multiple complex interactions within the auditory pathway and not simply...
caused by diminished input from the noise-damaged cochlea alone.

Acknowledgements
We would like to thank Dr. Patrick Boyle for his helpful comments and for the correction of English language spelling and grammar.

Financial support and sponsorship
Funding: The work was supported by the Deutsche Forschungsgemeinschaft DFG (grant number: GR 3519/3-1).

Conflicts of interest
There are no conflicts of interest.

References
1. Borg E. Loss of hair cells and threshold sensitivity during prolonged noise exposure in normotensive albino rats. Hear Res 1987;30:119-26.
2. Hamernik RP, Patterson JH, Turrentine GA, Ahroon WA. The quantitative relation between sensory cell loss and hearing thresholds. Hear Res 1989;38:199-211.
3. Chen G-D, Fechter LD. The relationship between noise-induced hearing loss and hair cell loss in rats. Hear Res 2003;177:81-90.
4. Chen GD, McWilliams ML, Fechter LD. Succinate dehydrogenase (SDH) activity in hair cells: a correlate for permanent threshold elevations. Hear Res 2000;145:91-100.
5. Pourbakht A, Yamasoba T. Cochlear damage caused by continuous and intermittent noise exposure. Hear Res 2003;178:70-8.
6. Hong O, Kerr MJ, Poling GL, Dhar S. Understanding and preventing noise-induced hearing loss. Dis Mon 2013;59:110-8.
7. Jacono AA, Hu B, Kopke RD, Henderson D, Van De Water TR, Steinman HM. Changes in cochlear antioxidant enzyme activity after sound conditioning and noise exposure in the chinchilla. Hear Res 1998;117:31-8.
8. Hu BH, Henderson D, Nicotera TM. Involvement of apoptosis in progression of cochlear lesion following exposure to intense noise. Hear Res 2002;166:62-71.
9. Hu BH, Henderson D, Nicotera TM. Extremely rapid induction of outer hair cell apoptosis in the chinchilla cochlea following exposure to impulse noise. Hear Res 2006;211:16-25.
10. Huang T, Cheng AG, Stupak H, Liu W, Kim A, Staeker H, et al. Oxidative stress-induced apoptosis of cochlear sensory cells: otoprotective strategies. Int J Dev Neurosci 2000;18:259-70.
11. Michiels C. Physiological and pathological responses to hypoxia. Am J Pathol 2004;164:1875-82.
12. Yu CH, Moon CT, Sur JH, Chun YI, Choi WH, Yhee JY. Serial expression of hypoxia inducible factor-Lalpha and neuronal apoptosis in hippocampus of rats with chronic ischemic brain. J Korean Neurosurg Soc 2011;50:481-5.
13. Kane EC. Patterns of degeneration in the caudal cochlear nucleus of the cat after cochlear ablation. Anat Rec 1974;179:67-91.
14. Popelar J, Grecova J, Rybalko N, Syka J. Comparison of noise-induced changes of auditory brainstem and middle latency response amplitudes in rats. Hear Res 2008;245:82-91.
15. Coore J, Görschel M, Ernst A, Basta D. Apoptotic cascades in the central auditory pathway after noise exposure. J Neurotrauma 2012;29:1249-54.
16. Mostafapour SP, Cochran SL, Del Puerto NM, Rubel EW. Patterns of cell death in mouse anteroventral cochlear nucleus neurons after unilateral cochlea removal. J Comp Neurol 2000;426:561-71.
17. Basta D, Tzschentke B, Ernst A. Noise-induced cell death in the mouse medial geniculate body and primary auditory cortex. Neurosci Lett 2005;381:199-204.
18. Gröschel M, Götzte R, Ernst A, Basta D. Differential impact of temporary and permanent noise-induced hearing loss on neuronal cell density in the mouse central auditory pathway. J Neurotrauma 2010;27:1499-507.
19. Sekiya T, Canlon B, Viberg A, Matsumoto M, Kojima K, Ono K, et al. Selective vulnerability of adult cochlear nucleus neurons to de-afferentation by mechanical compression. Exp Neurol 2009;218:117-23.
20. Sekiya T, Viberg A, Kojima K, Sakamoto T, Nakagawa T, Ito J, et al. Trauma-specific insults to the cochlear nucleus in the rat. J Neurosci Res 2012;90:1924-31.
21. Saljo A, Bao F, Jingshan S, Hamberger A, Hansson HA, Haglid KG. Exposure to short-lasting impulse noise causes neuronal c-Jun expression and induction of apoptosis in the adult rat brain. J Neurotrauma 2002;19:985-91.
22. Oo TF, Henchcliffe C, James D, Burke RE. Expression of c-fos, c-jun, and c-jun N-terminal kinase (JNK) in a developmental model of induced apoptotic death in neurons of the substantia nigra. J Neurochem 1999;72:557-64.
23. Yamane H, Nakai Y, Takayama M, Iguchi H, Nakagawa T, Kojima A. Appearance of free radicals in the guinea pig inner ear after noise-induced acoustic trauma. Eur Arch Otorhinolaryngol 1995;252:504-8.
24. Ohlemiller KK, Wright JS, Dugan LL. Early elevation of cochlear reactive oxygen species following noise exposure. Audiol Neurotol 1999;4:229-36.
25. Henderson D, Bielefeld EC, Harris KC, Hu BH. The role of oxidative stress in noise-induced hearing loss. Ear Hear 2006;27:1-19.
26. Raimundo N, Song L, Shutt TE, McKay SE, Cotney J, Guan MX, et al. Mitochondrial stress engages E2F1 apoptotic signaling to cause deafness. Cell 2012;148:716-26.
27. Maulucci G, Troiani D, Eramo SL, Paciello F, Podda MV, Paladetti G, et al. Time evolution of noise induced oxidation in outer hair cells: role of NAD(P)H and plasma membrane fluidity. Biochim Biophys Acta 2014;1840:2192-202.
28. Gavriel Y, Sherman Y, Ben-Sasson SA. Identification of programmed cell death in situ via specific labeling of nuclear DNA fragmentation. J Cell Biol 1992;119:493-501.
29. Negoescu A, Lorimier P, Labat-Moleur F, Drouet C, Robert C, Guillermet C, et al. In situ apoptotic cell labeling by the TUNEL method: improvement and evaluation on cell preparations. J Histochem Cytochem 1996;44:959-68.
30. Kroener G, Galluzzi L, Vandenabeele P, Abrams J, Almenri ES, Baecherek EH, et al. Classification of cell death: recommendations of the Nomenclature Committee on Cell Death 2009. Cell Death Differ 2009;16:3-11.
31. Paxinos G, Franklin KB. The Mouse Brain in Stereotaxic Coordinates. Oxford, UK: Elsevier Science & Technology; 2001.
32. Gröschel M, Müller S, Götzte R, Ernst A, Basta D. The possible impact of noise-induced Ca2+-dependent activity in the central auditory pathway: a manganese-enhanced MRI study. NeuroImage 2011;57:190-7.
33. Grasl-Kraupp B, Ruttka-Nedeycki B, Koudelka H, Bukowska K, Bursch W, Schulte-Hermann R. In situ detection of fragmented DNA (TUNEL assay) fails to discriminate among apoptosis, necrosis, and autolytic cell death: a cautionary note. Hapatology 1995;21:1465-8.
34. Mostek DK. The neuronal architecture of the medial geniculate body of the cat. J Anat 1964;98:611-30.
35. Calford MB. The parcellation of the medial geniculate body of the cat defined by the auditory response properties of single units. J Neurosci 1983;3:2350-64.
36. Calford MB, Aitkin LM. Ascending projections to the medial geniculate body of the cat: evidence for multiple, parallel auditory pathways through thalamus. J Neurosci 1983;3:2365-80.
37. Møller AR. Hearing—Anatomy, Physiology and Disorders of the Auditory System. 2nd ed; 2000.
38. Winer JA, Saint Marie RL, Larue DT, Oliver DL. GABAergic feedforward projections from the inferior colliculus to the medial geniculate body. Proc Natl Acad Sci U S A 1996;93:8005-10.
39. Ito T, Bishop DC, Oliver DL. Functional organization of the local circuit in the inferior colliculus. Anat Sci Int 2016;91:22-34.
40. Ryan AF, Axelsson GA, Woolf NK. Central auditory metabolic activity induced by intense noise exposure. Hear Res 1992;61:24-30.
41. Kim JJ, Gross J, Morest DK, Potter GB, Kim C, Sohal VS. Thalamocortical projections to layer I of the primary auditory cortex in the cat: a horseradish peroxidase study. Brain Res 1984;310:347-50.
42. Markram H, Toledo-Rodriguez M, Wang Y, Gupta A, Silberberg G, Wu C. Interneurons of the neocortical inhibitory system. Nat Rev Neurosci 2004;5:793-807.
43. Fröhlich, et al.: noise-induced cell death in the mouse brain
58. Shore SE, Kohler S, Oldakowski M, Hughes LF, Syed S. Dorsal cochlear nucleus responses to somatosensory stimulation are enhanced after noise-induced hearing loss. Eur J Neurosci 2008;27:155-68.
59. Kraus KS, Ding D, Zhou Y, Salvi RJ. Central auditory plasticity after carboplatin-induced unilateral inner ear damage in the chinchilla: up-regulation of GAP-43 in the ventral cochlear nucleus. Hear Res 2009;255:33-43.
60. Bilak M, Kim J, Potashner SJ, Bohne BA, Morest DK. New growth of axons in the cochlear nucleus of adult chinchillas after acoustic trauma. Exp Neurol 1997;147:256-68.
61. Kim JJ, Gross J, Morest DK, Potter GB, Sanes DH. Quantitative study of degeneration and new growth of axons and synaptic endings in the chinchilla cochlear nucleus after acoustic overstimulation. J Neurosci 2004;24:829-42.
62. Mitani A, Itoh K, Nomura S, Kudo M, Kaneko T, Mizuno N. Thalamocortical projections to layer I of the primary auditory cortex in the cat: a horseradish peroxidase study. Brain Res 1984;310:347-50.
63. Mitani A, Shimokouchi M. Neuronal connections in the primary auditory cortex: an electrophysiological study in the cat. J Comp Neurol 1985;235:417-29.
64. Cruikshank SJ, Rose HJ, Metherate R. Auditory thalamocortical synaptic transmission in vitro. J Neurophysiol 2002;87:361-84.
65. Niimi K, Naito F. Cortical projections of the medial geniculate body in the cat. Exp Brain Res 1974;19:326-42.
66. Kelly JP, Wong D. Laminar connections of the cat’s auditory cortex. Brain Res 1981;212:1-15.
67. Mitani A, Itoh K, Nomura S, Kudo M, Kaneko T, Mizuno N. Thalamocortical connections to layer I of the primary auditory cortex in the cat: a horseradish peroxidase study. Brain Res 1984;310:347-50.
68. Markram H, Toledo-Rodriguez M, Wang Y, Gupta A, Silberberg G, Wu C. Interneurons of the neocortical inhibitory system. Nat Rev Neurosci 2004;5:793-807.
69. Seybold BA, Stancs A, Cho KK, Potter GB, Kim C, Sohal VS, et al. Chronic reduction in inhibition reduces receptive field size in mouse auditory cortex. Proc Natl Acad Sci U S A 2012;109:13829-34.
70. Seki S, Eggermont JJ. Changes in spontaneous firing rate and neural synchrony in cat primary auditory cortex after localized tone-induced hearing loss. Hear Res 2003;180:28-38.
71. Takeuchi T, Naito F. Thalamocortical projections to layer I of the primary auditory cortex in the cat: a horseradish peroxidase study. Brain Res 1984;310:347-50.
72. Seybold BA, Stancs A, Cho KK, Potter GB, Kim C, Sohal VS, et al. Chronic reduction in inhibition reduces receptive field size in mouse auditory cortex. Proc Natl Acad Sci U S A 2012;109:13829-34.
73. Seki S, Eggermont JJ. Changes in spontaneous firing rate and neural synchrony in cat primary auditory cortex after localized tone-induced hearing loss. Hear Res 2003;180:28-38.
74. Tokat VC, Fujisawa S, Lee FA, Karthikeyan O, Aoki C. Sanes DH. Hearing loss raises excitability in the auditory cortex. J Neurosci 2005;25:3908-18.
75. Sanes DH, Tokat VC. Developmental plasticity of auditory cortical inhibitory synapses. Hear Res 2011;279:140-8.
76. Novak O, Zelenka O, Hromadka T, Syka J. Immediate manifestation of acoustic trauma in the auditory cortex is layer specific and cell type dependent. J Neurophysiol 2016;115:1860-74.
77. Read HL, Winer JA, Schreiner CE. Functional architecture of auditory cortex. Curr Opin Neurobiol 2002;12:433-40.