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Relationship between noise-induced hearing-loss, persistent tinnitus and GAP-43 expression in the rat cochlear nucleus: Does synaptic plasticity in ventral cochlear nucleus suppress tinnitus?

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Abstract

Aberrant, lesion-induced neuroplastic changes in the auditory pathway are believed to give rise to the phantom sound of tinnitus. Noise-induced cochlear damage can induce extensive fiber growth and synaptogenesis in the cochlear nucleus, but it is currently unclear if these changes are linked to tinnitus. To address this issue, we unilaterally exposed nine rats to narrowband noise centered at 12 kHz at 126 dB SPL for two hours and sacrificed them 10 weeks later for evaluation of synaptic plasticity (GAP-43 expression) in the cochlear nucleus. Noise-exposed rats along with three age-matched controls were screened for tinnitus-like behavior with gap prepulse inhibition of the acoustic startle (GPIAS) before, 1–10 days after and 8–10 weeks after the noise exposure. All nine noise-exposed rats showed similar patterns of severe hair cell loss at high- and mid-frequency regions in the exposed ear. Eight of the 9 showed strong up-regulation of GAP-43 in auditory nerve fibers and pronounced shrinkage of the ventral cochlear nucleus (VCN) on the noise-exposed side, and strong up-regulation of GAP-43 in the medial ventral VCN, but not in the lateral VCN or the dorsal cochlear nucleus. GAP-43 up-regulation in VCN was significantly greater in Noise-No-Tinnitus rats than in Noise-Tinnitus rats. One Noise-No-Tinnitus rat showed no up-regulation of GAP-43 in auditory nerve fibers and only little VCN shrinkage, suggesting that auditory nerve degeneration plays a role in tinnitus generation. Our results suggest that noise-induced tinnitus is suppressed by strong up-regulation of GAP-43 in the medial VCN. GAP-43 up-regulation most likely originates from medial olivocochlear neurons. Their increased excitatory input on inhibitory neurons in VCN may possibly reduce central hyperactivity and tinnitus.

Keywords

noise; tinnitus; hair cell loss; growth associated protein-43; ventral cochlear nucleus; dorsal cochlear nucleus

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Subjective tinnitus, the perception of a phantom ringing, buzzing or hissing sensation, affects 5–15% of adults. Approximately 1–3% suffers from moderate to severe chronic tinnitus that negatively affects the quality of daily life by causing stress, depression, anxiety, sleep disturbance or work impairment (Halford and Anderson, 1991; Dobie 2003; Heller 2003). Tinnitus is very often associated with hearing loss arising from various forms of cochlear pathology (Eggermont and Roberts, 2004; Weisz et al., 2006) or central plasticity induced by the lesion (Kaltenbach et al., 2000; Salvi et al., 2000; Bartels et al., 2007; Schaette and Kempster, 2009). Despite the fact that cochlear damage triggers tinnitus, there is strong evidence that the tinnitus generator itself resides centrally as evidenced by clinical studies showing that transection of the auditory nerve fails to eliminate tinnitus in the majority of patients (House and Brackman, 1981). Moreover, human brain imaging studies as well as physiological studies in animals show that tinnitus may be related to hyperactivity in central auditory nuclei such as the dorsal cochlear nucleus (DCN) (Kaltenbach et al., 1998; Brozoski et al., 2002), the inferior colliculus (IC) (Jastreboff and Sasaki 1986; Melcher et al., 2000; Argence et al., 2008; Bauer et al., 2008; Lanting et al., 2008) or the auditory cortex (AC) (Lockwood et al., 1998, 2001; Reyes et al., 2002; Norena and Eggermont 2003; Smits et al., 2007; Yang et al., 2007; Knipper et al., 2010). Cochlear pathologies that commonly induce tinnitus, such as acoustic trauma and hair cell degeneration caused by ototoxic drugs, typically decrease spontaneous firing rates in the auditory nerve thereby reducing the spontaneous neural input to the central auditory pathway (Liberman and Kiang, 1978; Kiang et al., 1970; Wang et al., 1997). The loss of excitatory drive from the periphery is thought to reduce inhibition in the central auditory system thereby increasing the excitability of the central auditory system resulting in hyperactivity and enhanced synchrony in higher auditory brain regions (Gerken, 1996; Abbott et al., 1999; Qiu et al., 2000; Salvi et al., 2000; Eggermont and Roberts, 2004; Wang et al., 2006).

Hearing loss through cochlear ablation (Benson et al., 1997; Illing et al., 1997), noise exposure (Bilak et al., 1997; Michler and Illing., 2002; Muly et al., 2002) or ototoxic drugs (Kraus et al., 2009a) stimulates fiber outgrowth and synaptogenesis in the ventral cochlear nucleus (VCN). Many of these new synapses appear to be excitatory (Kim et al., 2004). In addition, cholinergic activity increases (Jin et al., 2005, 2006; Jin and Godfrey, 2006). Cochlear ablation studies suggest that all or many of these new synapses originate from axon collaterals of medial olivocochlear neurons (MOC) (Kraus and Illing, 2004; Meidinger et al., 2006) which are cholinergic and excitatory (Happe and Morley, 1998; Yao and Godfrey, 1999; Fujino and Oertel, 2001). All together, these observations show a delayed increase of excitatory inputs to the VCN after cochlear hearing loss. While these changes are intriguing, their functional correlates are largely unknown. These changes may be beneficial for rebalancing the excitatory-inhibitory drive to neurons in the VCN thereby normalizing spontaneous activity and suppressing tinnitus. Alternatively, increased excitatory activity may contribute to consolidation of chronic tinnitus.

To examine the functional correlates of these anatomical changes we examined the relationship between noise-induced tinnitus and synaptic plasticity in VCN using a rat behavioral model and immunolabeling with GAP-43, a well-established marker for axonal outgrowth and synaptic sprouting (Benowitz and Routtenberg, 1997). Part of this study has been previously published in abstract form (Kraus et al., 2009b, 2011).

Experimental Procedures

1. Animals

Twelve adult male Sprague-Dawley rats (Sasco, Charles River Laboratories International, Inc., Wilmington, MA) were used for this study. Nine experimental rats were unilaterally

exposed to high intensity noise in order to induce tinnitus-like behavior in some rats. Rats were screened for tinnitus before and after the noise exposure using gap prepulse inhibition of acoustic startle (GPIAS) as described below and subsequently separated into two groups: 'Noise-No-Tinnitus' and 'Noise-Tinnitus'. Three age-matched controls underwent the same schedule of screening, but were not noise exposed. Ten weeks after the noise-exposure the animals were sacrificed and their cochleae and brains harvested for histological analysis. All animal procedures were approved by the University of Buffalo Institutional Animal Care and Use Committee (Protocol #HER05080Y).

2. Noise exposure

Under isoflurane anesthesia (Webster Veterinary Supply Inc., Sterling, MA, USA; 4% induction, 1.5–2% maintenance), rats were unilaterally exposed to narrowband noise (bandwidth 100 Hz) centered at 12 kHz and presented at 126 dB SPL for 2 h. Sound stimuli were generated by Tucker-Davis Technology (TDT; Tucker-Davis Technologies, Alachua, FL, USA) hardware and presented by a super compression driver (D-59; GMI Sound Corp, Brooklyn, NY, USA) positioned 10 mm directly in front 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, Depew, NY, USA). The contralateral ear was plugged in order to maintain normal hearing in the right ear. This provided an in-animal control ear and in addition allowed the animals to respond to the acoustic stimuli used for the tinnitus behavioral tests.

3. Behavioral evidence for tinnitus

Rats were tested for behavioral evidence of tinnitus using the gap-prepulse inhibition of the acoustic startle (GPIAS) paradigm as described in our previous report (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 (Radio Shack, Fort Worth, TX, USA). The output of the piezoelectric transducer was sent to a low-pass filter (1000 Hz, LPF-300, World Precision Instruments, Sarasota, FL, USA) and then to an A/D converter on a digital signal processing module (RP2.1, TDT). Using MATLAB software (The MathWorks Inc., Natick, MA, USA) and custom software (generously provided by Dr. P. Allen, University of Rochester), the root mean square (RMS, i.e., the square root of the mean of the squared instantaneous voltages over the 100 ms measurement window) amplitude of the startle response was calculated over a 100 ms interval following the onset of the startle stimulus. The sound level within each cage was measured and calibrated (Model 824 sound level meter, one-half inch microphone, Larson Davis). 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, Foster Electric (U.S.A.), Inc., Schaumburg, IL, USA) 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).

4. GPIAS

The amplitude of the acoustic startle response was measured in the noise exposed rats and controls. The startle stimulus was embedded in a continuous narrow band background noise (60 dB SPL); the center frequency of the noise was varied to test for the presence of tinnitus in different frequency regions. The RMS amplitude of the acoustic startle response was measured in the presence of continuous noise with "no gap" (Fig. 1A) or with a 50 ms silent "gap" (Fig. 1B). Half the trials contained a gap and the other half did not. The onset of the gap preceded the onset of the startle stimulus by 100 ms.

As described previously, in normal rats the silent gap reduces the amplitude of the startle response to the startle stimulus (Fig. 1A vs. 1B), i.e. the gap serves as a pre-pulse and inhibits the startle response (Ison et al., 2002, Turner et al., 2006; Yang et al., 2007). The presence of significant gap prepulse inhibition of acoustic startle (GPIAS) compared to the no-gap condition indicated that the rat was able to detect the silent gap. In contrast, GPIAS was 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 response in the “gap” condition (Fig. 1C) would no longer be significantly different from the “no-gap” condition. GPIAS tests were run with continuous 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 s.

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 on day 1–10 post-exposure (“Short-term”, 7 test days in total) and then at week 8–10 post-exposure (“Long-term”, 7 test days in total). Age-matched controls were tested along with the noise exposed rats. For each day of testing, amplitudes were normalized to the average amplitudes at the “no-gap” condition. The mean amplitude of each “no-gap” condition was set to 1, and the average amplitudes were presented as mean $\pm$ standard error of the mean (SEM). For each testing period (“baseline”, “short-term” and “long-term”), 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 ($P < 0.05$). Calculations were done with Microsoft Excel (Version 2003) and statistical evaluations were done with GraphPad Prism (Version 5.01; GraphPad Software Inc, La Jolla, CA, USA).

5. Fixation

As described previously (Kraus et al, 2009a), on the day of sacrifice, rats received a lethal dose of sodium pentobarbital and were transcardially perfused with phosphate buffered saline (PBS, 0.1 M, pH 7.4; Sigma-Aldrich, St. Louis, MO, USA) for 5 minutes and then with 10% phosphate buffered formalin (Fisher Scientific, Pittsburgh, PA, USA) 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.

6. 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; Carl Zeiss MicroImaging, Inc., Thornwood, NY, USA). 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.

7. Immunohistochemistry

As described in detail previously (Kraus et al, 2009a, 2010), brain tissue containing the left and right cochlear nuclei was immersed in 30% sucrose (Sigma-Aldrich) 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 . Care was taken to obtain symmetric sections in order to keep the VCN of the two sides comparable. Brainstems were cut from caudal to rostral, and prior to collecting sections were examined for symmetry: when the cochlear nucleus appeared on one side of the brainstem but not yet on the other side, the cutting angle was modified until the nucleus appeared equally on both sides. Free floating sections were collected and washed in 0.1 M PBS, pH 7.4, pretreated with H_2O_2 (Fisher Scientific) for peroxidase deactivation for 30 min, and then pre-incubated in blocking buffer containing 10% normal horse serum (NHS; Vector Laboratories, Burlingame, CA, USA) and 0.05% Triton X-100 (Fisher Scientific) 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 GAP-43 (MAB347; made in mouse; clone 9-1E12; Millipore), which has been shown to specifically detect and bind GAP-43 (Goslin et al., 1991; Schreyer and Skene, 1991). GAP-43 antibody was added at a concentration of 0.2 $\mu\text{g}/\text{ml}$ in 0.1 M PBS pH 7.4 with 1.0% NHS and 0.05% Triton. GAP-43 was visualized through the indirect staining method utilizing a secondary antibody (rat-adsorbed anti-mouse IgG, Vector Laboratories, Burlingame, CA; 7 $\mu\text{l}/\text{ml}$) in PBS with 0.05% Triton X-100 and 1.5% NHS, the avidin-biotin-peroxidase complex (Elite-ABC, Vector Laboratories; 20 μl of Reagent A and B respectively/ml) in PBS with 0.05% Triton X-100, and finally diaminobenzidine tetrahydrochloride (DAB, 0.05%, 2-5 minutes, Sigma-Aldrich) with nickel ammonium sulfate (0.3%; Fisher Scientific) and H_2O_2 (0.0015%) in 0.1 M Tris buffer, pH 7.2 (Trizma-base, Sigma-Aldrich). 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. Before DAB staining sections were washed in 0.1 M Tris buffer, pH 7.2. After staining, sections were washed in Tris buffer once, then in PBS several times, 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 (Fisher Scientific).

8. Photomicrographs, quantification and statistical analysis

Images visualized under bright field illumination (Axioskop, Carl Zeiss MicroImaging, Inc) were photographed using a digital camera (SPOT Insight, Diagnostic Instruments, Inc., Sterling Heights, MI, USA) and processed with imaging software (SPOT Software, version 4.6) into 8-bit grey scale. Evaluation and assembly of images were done with Adobe Photoshop 5.5 (Adobe Systems Inc., San Jose, CA, USA). Calculations were done with Microsoft Excel (Version 2003) and statistical evaluations were accomplished with GraphPad Prism (Version 5.01; GraphPad Software Inc.).

For evaluation of GAP-43 expression in the three subregions of the cochlear nucleus, grey scale images (100X magnification) of left and right DCN, PVCN or AVCN sections were taken under identical illumination conditions. Left and right DCN, PVCN or AVCN were matched with respect to location on the rostral-caudal axis. Number of sections analyzed per animal and side was 5 for the DCN, 4 for the PVCN, and 5 for the AVCN. The most caudal and rostral sections were not used for analysis due to their small size and because the medial region did not fully extend to the very caudal or rostral end of the nucleus. Sections at the border between PVCN and AVCN were not used because of heavy abundance of auditory nerve fiber bundles and because the border between PVCN and AVCN cannot be exactly determined. For each of the subnuclei, measurements were done in six distinct regions located within the (1) lateral and (2) medial part of the (A) dorsal region encoding high frequencies, the (B) mid-region, and the (C) ventral region encoding low frequencies. Separation into a lateral and medial part was done due to different degrees of immunoreactivity laterally and medially. From each region, mean grey scale values were obtained from three representative square fields and the average grey tone value was

calculated. The size of the field was adjusted to fit within the area in question (Fig. 2; DCN lateral fusiform layer, lateral PVCN and lateral AVCN: $100 \times 100 \mu\text{m}$; DCN medial fusiform layer: $150 \times 150 \mu\text{m}$; medial PVCN and medial AVCN: $100 \times 100 \mu\text{m}$ dorsally, $200 \times 200 \mu\text{m}$ in mid-region, $150 \times 150 \mu\text{m}$ ventrally). Regions at the margin of VCN as well as areas containing axon bundles or larger blood vessels were excluded from measurement. Mean grey scale values of unstained tissue plus background grey scale were subtracted from GAP-43 immunolabeled sections in order to obtain net GAP-43 immunostaining values. Ipsilateral changes were expressed as ipsilateral-to-contralateral (i:c) ratios reflecting the change in net immunostaining.

To correct for potential cochlear nuclei shrinkage due to tissue degeneration, the size of each subnucleus within the cochlear nuclei was measured and the ipsilateral-to-contralateral (i:c) ratio calculated. For each individual animal, the sizes of DCN, PVCN or AVCN from grey-scale images were obtained as pixel counts of every 6th section along the caudal-rostral axis using Adobe Photoshop. Total pixel count ipsilaterally was divided by total pixel count contralaterally. Value for net increase of GAP-43 expression for each rat was obtained by multiplying the i:c ratio of size with the i:c ratio of staining density. Statistical comparisons were made to determine significant changes of size and GAP-43 expression. The i:c ratios of size as well as the size corrected i:c ratios of GAP-43 expression were compared in controls, Noise-No-Tinnitus rats and Noise-Tinnitus rats (One-way ANOVA). All average values were presented as means $\pm$ standard error of the mean (SEM). Significance level was set to $P < 0.05$ in all tests.

Results

1. Tinnitus screening

All experimental rats and age-matched controls were screened for tinnitus-like behavior with the GPIAS paradigm before noise exposure ("Baseline"), days 1–10 post-exposure ("Short-term") and weeks 8–10 post-exposure ("Long-term"; Fig. 3). Startle-amplitudes at the "no-gap" condition were present in all rats at baseline as well as after noise-exposure showing that all rats were capable of a significant startle response. At baseline, all rats showed significant inhibition of startle amplitude (GPIAS) indicating that they could detect the silent gap, results consistent with the absence of tinnitus. The extent of GPIAS varied among individual rats (30%–60%) but the differences of startle amplitudes in the "no-gap" and "gap" conditions were significant for all rats and at all frequencies ($P < 0.05$).

In both post-noise testing periods, four rats (B1, D3, E and F) showed significant GPIAS at all test frequencies similar to controls, and thus no signs of tinnitus ('Noise-No-Tinnitus' group) (Figure 3). The other five experimental animals (C1, D1, C3, A4 and B4) showed a lack of GPIAS after the noise exposure at one or more frequencies, i.e., after the noise exposure there was no significant difference in startle amplitudes in "no-gap" versus "gap" conditions at one or more frequencies ($P > 0.05$). Therefore rats C1, D1, C3, A4 and B4 were considered to have behavioral evidence of tinnitus ('Noise-Tinnitus' group) (Turner et al., 2006, Yang et al., 2007). Additionally, these five rats showed one other change after the noise exposure. At the lower frequencies, particularly 12 kHz (i.e., the exposure frequency) the average startle amplitude in the "gap condition was larger than in the 'no-gap' condition; in rats C1, C3 and D1 this increase was significant. Thus, the gap signal enhanced the response to the startle stimulus (facilitation) instead of reducing it as has been noted previously (Kellogg et al., 1991; Ison et al., 1997). 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). Facilitation-like behavior was only observed in rats with behavioral evidence of tinnitus.

2. Hair cell loss

Figure 4, 5 and 6 show hair cell loss in controls and experimental rats 10 weeks after noise exposure. Controls had only a few hair cells missing in both ears. Experimental rats showed severe hair cell loss in the left exposed ear, and the pattern was similar in all experimental rats: In basal and middle turn, all or most hair cells were gone. OHC loss extended further to the apex than IHC loss. Region of hair cell loss roughly corresponded to a frequency range of 2 kHz to > 50 kHz (Müller, 1991, Greenwood, 1996). Partial hair cell loss was seen between 2 kHz and 10 kHz, and 100% loss was seen at frequencies above 10 kHz. The slope of the lesion between 100% hair cell death and hair cell survival was similar with only slight variation among rats with hair cell loss going from 0% to 100% within approximately 10% to 30% of cochlear length. There was no detectable difference in hair cell loss patterns between the Noise-No-Tinnitus group and the Noise-Tinnitus group. In most (7/9) of the experimental rats the right-protected, contralateral ears resembled controls with 5% or less hair cells missing. Two rats showed some hair cell loss at the extreme high-frequency base of the cochlea well above the highest GPIAS test frequency of 24 kHz (F, C1).

3. Changes in VCN and auditory nerve fibers

3.1. Expression of GAP-43, qualitative description—In the normal hearing control animals (N=3), GAP-43 was expressed in the cochlear nucleus (Fig. 7A, 8A, D), but not in auditory nerve fibers (Fig. 9A). In the cochlear nucleus, GAP-43 was located in puncta previously identified as presynaptic endings (Meidinger et al., 2006) and in fine fibers (Fig. 10A). These structures morphologically differed from auditory nerve fiber fascicles and endings which were thicker and had larger synaptic endings (Illing et al., 1997; 2005). In the DCN, GAP-43 was strongly expressed in the regions occupied by fusiform cells (Fig 7A); the lateral portion was darker stained than the medial portion. In PVCN as well as in AVCN (Fig 8A, D), GAP-43 was expressed at a moderate level throughout the entire nucleus. Rats unilaterally exposed to noise (N=9) showed changes of GAP-43 expression on the treated side in some but not all regions. Importantly, no changes in GAP-43 could be seen contralaterally. Thus, the contralateral side was used as a within-animal reference for quantification of ipsilateral changes.

In the DCN, there was little or no difference of GAP-43 expression between sides, and little or no difference between controls and noise-exposed rats (Fig. 7A vs. B). In contrast, noise-exposed rats showed increased expression of GAP-43 in the VCN ipsilaterally to noise exposure. Pattern and extent of GAP-43 labeling varied strongly among individual animals. One rat (Rat E) showed only a modest increase of GAP-43 staining throughout the VCN (Fig 8B, E) and like controls no GAP-43 expression in auditory nerve fibers (Fig 9B vs. A). This rat showed no behavioral evidence of tinnitus. The eight other rats showed a strong up-regulation of GAP-43 in the medial portion of VCN (Fig 8C, F) as well as a strong up-regulation in auditory nerve fibers (Fig 9C). Interestingly, the pattern of the GAP-43 increase in the VCN did not topographically match the frequency region of hair cell loss: While hair cell loss was predominantly at high- and mid-frequencies (Fig. 6), the GAP-43 increase was predominantly in regions encoding mid- and low-frequencies. Of these eight rats, three showed no evidence of tinnitus ('Noise-No-Tinnitus') while five showed evidence of severe tinnitus ('Noise-Tinnitus'). The extent of GAP-43 expression in the medial PVCN and the medial AVCN appeared stronger in the Noise-No-Tinnitus group (Fig. 11A, C) than in the Noise-Tinnitus group (Fig 11B, D).

3.2. Size reduction of cochlear nucleus—In order to determine the extent of GAP-43 up-regulation, staining densities had to be corrected for potential size reduction of the ipsilateral cochlear nucleus. To accomplish this, we measured the size of the ipsilateral (i = exposed) and contralateral (c = non-exposed) DCN, PVCN and AVCN and then computed

the i:c size ratio for the DCN, PVCN and AVCN (Figure 12); this correction factor was used later for quantification of GAP-43 immunoreactivity. In the DCN, no significant change of size could be detected (Fig. 12A). The i:c size ratio was close to 1.0 in all rats, and there were no significant differences between controls, Noise-No-Tinnitus and Noise-Tinnitus rats (one way-ANOVA). In contrast, the size of the ipsilateral VCN was only 70–75% of the contralateral VCN (Fig. 12B, C). For the PVCN, the average i:c size ratio for controls was 1.051 ± 0.057 (N=3), for Noise-No-Tinnitus rats it was 0.743 ± 0.040 (N=3), and for Noise-Tinnitus rats it was 0.741 ± 0.025 (N=5). The difference between controls and both of the noise exposed groups was significant (one way-ANOVA with Tukey's Multiple Comparison Test; $P = 0.0008$), but there was no significant difference between Noise-No-Tinnitus rats and Noise-Tinnitus rats. For AVCN, the average i:c size ratio for controls was 1.038 ± 0.02511 (N=3), for Noise-No-Tinnitus rats the i:c ratio was 0.7248 ± 0.06515 (N=3), and for Noise-Tinnitus rats the i:c ratio was 0.7152 ± 0.03662 (N=5). The difference between controls and both of the exposed groups was significant (one way-ANOVA with Tukey's Multiple Comparison Test; $P = 0.0017$), but there was no significant difference between Noise-No-Tinnitus rats and Noise-Tinnitus rats. The i:c size ratio of PVCN and AVCN showed no significant difference, neither in the Noise-No-Tinnitus group nor in the Noise-Tinnitus group. Rat E with only weak GAP-43 up-regulation showed a i:c size ratio of 0.90, indicating an ipsilateral size reduction but it was considerably smaller than in the other noise-exposed rats.

3.3. Quantification of GAP-43—The noise-induced increase of ipsilateral GAP-43 staining was expressed as the i:c ratio corrected for nucleus size reduction; this was accomplished by multiplying the uncorrected i:c staining intensity ratio by the i:c size ratio. Figure 13 shows the corrected i:c ratios for GAP-43 immunoreactivity in the DCN, PVCN and AVCN of controls and the two noise exposed groups. In DCN, the corrected i:c staining intensity ratio was close to 1.0 in all six regions and in all groups of rats (Fig. 13 A,D,G,J,M,P). There was no significant difference between controls, Noise-No-Tinnitus and Noise-Tinnitus rats (one way-ANOVA). In contrast, there was a significant increase of GAP-43 expression in the medial PVCN as well as in the medial AVCN. In the medial PVCN, GAP-43 labeling in Noise-No-Tinnitus rats was significantly greater than in controls in the dorsal, mid and ventral regions (Fig. 13 K,N,Q; One-way ANOVA; Tukey's Multiple Comparison Test, $P < 0.05$). There was a trend towards higher GAP-43 labeling in Noise-Tinnitus rats versus Controls; however, the difference was not significant. Additionally, in the mid-region of the medial PVCN, GAP-43 labeling was significantly higher in the Noise-No-Tinnitus rats than Noise-Tinnitus rats (Fig. 13 N). The dorsal as well as the ventral medial PVCN showed a similar trend, but it was not significant (Fig. 13 K,Q).

In the medial AVCN, GAP-43 labeling was significantly higher in the ventral and mid-regions of Noise-No-Tinnitus rats than in Control rats (Fig. 13 O, R; One-way ANOVA; $P < 0.01$, Tukey's Multiple Comparison Test). GAP-43 labeling in the mid-region of the medial AVCN was also significantly higher in Noise-Tinnitus rats than in Controls (Fig. 13 O; One-way ANOVA; $P < 0.01$, Tukey's Multiple Comparison Test). In the ventral region of the medial AVCN, GAP-43 labeling was significantly higher in the Noise-No-Tinnitus rats than in the Noise-Tinnitus rats (Fig. 13 R; One-way ANOVA; $P < 0.01$, Tukey's Multiple Comparison Test). The medial mid-region showed a similar trend, but it was not significant.

Discussion

Unilateral exposure to narrow-band noise centered at 12 kHz and presented at 126 dB SPL for two hours caused nearly complete loss of IHC and OHC in the exposed ear except in the extreme apex. In contrast, little or no hair cell loss was observed in the opposite plugged ear. Ten weeks after exposure, eight rats showed strong expression of GAP-43 in the auditory

nerve and medial VCN on the noise-exposed side, but not in lateral VCN or in DCN. Up-regulation of GAP-43 in medial VCN was significantly stronger in Noise-No-Tinnitus rats than in Noise-Tinnitus rats despite the fact that there was no difference in amount of hair cell loss. One Noise-No-Tinnitus rat showed no changes in the auditory nerve and only a mild up-regulation in the VCN even though hair cell loss was severe and not different from other experimental rats.

1. Hair cell loss, auditory nerve changes and cochlear nucleus shrinkage

Persistent noise-induced tinnitus is typically associated with hearing loss and cochlear damage. The pattern is often characterized by a noise-notch or high-frequency hearing loss with a steep edge (Gerken, 1996; König et al., 2006), and greater loss of OHC than IHC loss (Salvi et al., 1983; Hamernik et al. 1993; Jastreboff and Hazell, 1993). All rats in our study showed this characteristic pattern but still only some developed tinnitus. Thus, additional factors besides cochlear damage must determine whether tinnitus develops after noise exposure.

In eight of nine rats, GAP-43 was up-regulated in the auditory nerve on the deafened side. GAP-43 in fibers is typically associated with regeneration, but it is also expressed in injured neurons that attempt to regenerate but eventually die (Schmitt et al., 1999; Kraus and Illing, 2005). Noise-induced auditory nerve degeneration has been previously reported (Ylikoski et al., 1998; Michler and Illing, 2003). These rats also showed severe VCN shrinkage (25–30%) which may be related to fiber degeneration as well as loss of cell bodies. Transsynaptic degeneration in the VCN after hearing loss has been reported earlier (Morest et al., 1997; Willott et al., 2008; Sekiya et al., 2009; Gröschel et al., 2010). One rat did not show GAP-43 up-regulation in the auditory nerve and only little cochlear nucleus shrinkage, and no evidence of tinnitus despite severe hair cell loss comparable to all other rats. Thus, auditory nerve fiber changes or VCN shrinkage may represent an additional trigger for persistent tinnitus besides hair cell loss. Given that tinnitus is associated with reduced spontaneous activity in the auditory nerve, it is conceivable that auditory fiber loss which results in further reduction of spontaneous activity would promote tinnitus. Auditory nerve fiber degeneration in the absence of hair cell loss has been proposed as a cause of persistent tinnitus (Bauer 2007; Kujawa and Liberman, 2009). Moreover, sectioning the auditory nerve to eliminate vertigo or to remove an acoustic neuroma can induce tinnitus or fail to suppress tinnitus (House and Brackman, 1981, Biggs and Ramsden, 2002). Furthermore, cell death in the VCN may disturb the balance between excitatory and inhibitory activity. However, since Noise-No-Tinnitus and Noise-Tinnitus rats both showed significant increases in GAP-43 in auditory nerve as well as significant shrinkage in the cochlear nuclei of the same extent (Fig. 12), these anatomical changes alone cannot distinguish between these No-Tinnitus and Tinnitus conditions.

2. VCN plasticity

2.1. Origin—All animals with increased GAP-43 expression in auditory fibers and severe VCN shrinkage showed a pronounced increase in GAP-43 in the VCN, while the one case without GAP-43 in the auditory nerve and little VCN shrinkage did not show GAP-43 up-regulation in the VCN. Since auditory nerve fibers containing GAP-43 project to the cochlear nucleus, the increase of GAP-43 in the cochlear nuclei would not be surprising. However, the GAP-43 staining pattern and morphology suggests that the auditory nerve cannot be the major source regulating GAP-43 expression in the cochlear nuclei because 1) the DCN and lateral VCN showed no increase in GAP-43 expression despite the fact that auditory nerve fibers project heavily to these regions, and 2) GAP-43 staining in the VCN was found in thin fibers and presynaptic endings dissimilar from those originating from auditory nerve as noted in earlier studies (Illing et al., 1997; Michler and Illing 2002).

Cochlear ablation studies revealed that MOC neurons with their axonal collaterals to VCN (White and Warr, 1983) are a major source of GAP-43 expression in VCN (Kraus and Illing, 2004). Given that GAP-43 is a marker for synaptic plasticity (Benowitz and Routtenberg, 1997), we interpret the strong GAP-43 up-regulation in VCN as evidence of massive synaptic growth induced by noise.

2.2. Pattern—Several models of hearing loss such as cochlear ablation, noise trauma and ototoxicity induce transient GAP-43 up-regulation in the VCN. However, the long-term up-regulation of GAP-43 we observed in the medial PVCN and AVCN 10 weeks after noise exposure represents a major difference from the patterns seen after other damaging paradigms. For example, cochlear ablation (Illing et al., 1997; 2005) and drug-induced cochlear lesions (Kraus et al., 2009a) increased GAP-43 expression over the entire width (lateral and medial) of the VCN. In addition, the spatial distribution of GAP-43 in the VCN mirrored the tonotopic profile of the cochlear lesion. In contrast, we found GAP-43 up-regulation predominantly in mid- and low-frequency regions of the VCN despite the fact that the hair cell lesion was predominantly in the mid- and high-frequency regions of the cochlea. Michler and Illing (2002) reported a similar ventral preference after noise-exposure, also topographically mismatching the location of cochlear damage which was predominantly in the high- and mid-frequency regions. Additionally, in our study GAP-43 was increased only medially, not laterally as previously seen after cochlear ablation or drug damage. Thus, the topography of GAP-43 expression in the VCN 10 week post-noise exposure does not mirror the location of the hair cell lesion, but must be related to secondary effects as discussed below.

3. Tinnitus

All rats that demonstrated strong up-regulation of GAP-43 in the auditory nerve and a large VCN size reduction also showed pronounced GAP-43 in the medial VCN, but the extent of the response differed among individual rats in a tinnitus dependent manner. Noise-No-Tinnitus rats showed significantly stronger up-regulation of GAP-43 than Noise-Tinnitus rats. One interpretation of these results is that strong synaptic growth suppresses the development of tinnitus, i.e., synaptic plasticity alters neuronal connectivity such that it no longer generates tinnitus. The resulting changes of neuronal connectivity or synaptic efficiency may suppress tinnitus-associated hyperactivity in cochlear nucleus or other central auditory regions (details in sections 3.1–4 below). An alternative interpretation is that tinnitus suppresses auditory plasticity and GAP-43 expression. Chronic stress (Chao et al 1993; Kuroda and McEwen, 1998; Park et al, 2008; Kavushansky et al 2009) or stress hormones (Federoff et al., 1988; Chao et al., 1998) are capable of reducing GAP-43 expression in the limbic system and in cell culture. Since stress or stress hormones are also capable of affecting the auditory system (Tahera et al., 2006; Bose et al., 2010), we cannot rule out a possibility that tinnitus may affect synaptic plasticity in VCN. These two interpretations may even co-exist as a self-reinforcing circle: reduced VCN plasticity causes tinnitus and stress, which in turn suppresses VCN plasticity and thus stabilizes or further increases tinnitus. Finally, reduced VCN plasticity/GAP-43 expression and persistent tinnitus may simply be correlated with one another, but not causally related. For instance, both may be related to susceptibility to stress, but may not be directly connected to each other causally.

3.1. Does VCN plasticity protect against chronic tinnitus?—Cochlear lesion studies have shown that sprouting synapses in the VCN arise from MOC neurons (Kraus and Illing, 2004). MOC neurons, while inhibitory at the level of cochlea, have collateral projections to VCN which are excitatory (Happe and Morley, 1998; Yao and Godfrey, 1999; Fujino and Oertel, 2001); the increase in these excitatory synapses in the VCN may thus

compensate for reduced input from the auditory nerve. Fávero et al. (2006) found a relationship between the presence of tinnitus and the absence of DPOAE amplitude suppression; these results suggest that lack of activity in the MOC neurons may be involved in the generation of tinnitus. Conversely, stimulation of olivocochlear fibers at the floor of the IVth ventricle reduced noise-induced spontaneous hyperactivity in the IC (Mulders et al., 2010). Since spontaneous hyperactivity in the IC has been proposed as a neural correlate of tinnitus, increased excitatory activity of MOC neurons may reduce tinnitus. Synaptic endings positive for GAP-43 are located on glutamatergic and glycinergic, but not GABAergic cell bodies (Illing et al., 2005), matching earlier findings showing that postsynaptic targets of MOC neurons are multipolar cells (Benson et al., 1996) which are glutamatergic (Fujino and Oertel, 2001) or glycinergic (Mulders et al., 2002). Multipolar glycinergic cells in the VCN project to the DCN and are strong candidates for mediating broadband inhibition (Arnott et al., 2004), thereby suppressing DCN hyperactivity. Additionally, there is a glycinergic projection from VCN to ipsilateral IC (Adams, 1979; Oliver, 1987; Alibardi, 1998) and to the contralateral VCN (Wenthold, 1987; Babalian et al., 2002). Glutamatergic multipolar cells project to the contralateral IC (Fujino and Oertel, 2001) where their increased activity may compensate for reduced auditory nerve activity and augment inhibitory activity at higher levels.

3.2. Non-topographic GAP-43 up-regulation and tinnitus frequency—One inductor of tinnitus may be a steep edge of hair cell loss (Gerken, 1996; König et al., 2006). Neurons in frequency regions not affected by hearing loss themselves become hyperactive because they receive reduced inhibition from neurons encoding neighboring frequencies which are damaged and inactivated. Additionally, homeostatic plasticity may shift hyperactivity from frequencies at the edge to higher frequencies (Schaette and Kempter, 2009), an effect which matches with observations that tinnitus occurs at frequencies above the edge rather than at the edge itself (Norena et al., 2003; Eggermont and Roberts, 2004). A further proposed inductor of tinnitus is a mismatch between OHC and IHC loss at the same frequency (Jastreboff and Hazell, 1993). The DCN receives excitatory inputs originating from the IHC and it has been proposed that inhibitory inputs originate from OHC (Kaltenbach et al., 2005; Kaltenbach and Godfrey, 2008): thus a greater loss of OHC than IHC at the edge of the cochlear lesion would reduce inhibition more than excitation and cause hyperactivity. In this study, all noise-exposed rats had an audiometric edge as well as OHC loss exceeding IHC loss at lower frequencies between 2 kHz and 10 kHz and 100% hair cell loss at frequencies above. When considering the edge effect, homeostatic plasticity and the pattern of OHC versus IHC loss together, we would expect the tinnitus pitch to be located at low and mid-frequencies, and synaptic plasticity which suppresses tinnitus would be required particularly at these frequencies. Noise-No-Tinnitus rats showed a high degree of synaptic plasticity in the VCN at low and mid-frequencies but little or no plasticity at the highest frequencies most dorsally. Noise-Tinnitus rats showed behavioral evidence of tinnitus at one or more of the tested low and mid-frequencies from 6 kHz to 24 kHz. Tests at higher frequencies were not possible due to equipment limitations. The presence of some synaptic plasticity in the VCN of Noise-Tinnitus rats may provide partial tinnitus suppression and may explain why Noise-Tinnitus rats did not show evidence of tinnitus at all frequencies.

3.3. Low-SR fibers—Type I auditory nerve fiber spontaneous rates (SR) and the location of their synaptic contact on IHC are thought to play a role in tinnitus generation. Low-SR and medium-SR fibers, which are located along the scala vestibuli side of Rosenthal's canal project to the medial portion of VCN while high-SR fibers which are located along the scala tympani side of Rosenthal's canal project to the lateral VCN (Leake and Snyder, 1989; Kawase and Liberman, 1992). Thus, increased GAP-43 expression in the medial VCN may be related to low-SR or medium-SR fiber circuitry. Low-SR and medium-SR fibers appears

to be more vulnerable to noise exposure than high-SR fibers (Lin et al., 2011), and may therefore play an important role in noise-induced tinnitus. In the VCN, low-SR fibers target multipolar cells in PVCN (Friauf and Ostwald, 1988) and AVCN (Liberman, 1991) which may be part of the circuitry involved in tinnitus suppression as discussed above. They also likely provide input to the crossed olivocochlear reflex which consists of MOC-neurons (Schmiedt et al., 1996).

3.4. Pathway of tinnitus—Based on our findings, we propose a pathway for the development of noise-induced tinnitus. Noise-induced hair cell loss leads to injury or degeneration of auditory nerve fibers resulting in the loss of spontaneous activity; this reduces activity in central inhibitory circuits, which in turn cause hyperactivity or synchronized firing in the auditory brainstem consistent with earlier proposals. However, unlike earlier models, we propose that a high degree of GAP-43 synaptic plasticity in the VCN suppresses the hyperactivity and prevents the development of persistent tinnitus. In contrast, when GAP-43 synaptic plasticity is low or absent, it fails to suppress the hyperactivity resulting in chronic tinnitus.

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Abbreviations

AC	Auditory cortex
AVCN	Anterior ventral cochlear nucleus
DCN	dorsal cochlear nucleus
DPOAE	distortion product otoacoustic emissions
GAP-43	growth associated protein 43
GPIAS	gap prepulse inhibition of the acoustic startle
IC	inferior colliculus
IHC	inner hair cells
MOC neurons	medial olivocochlear neurons
OHC	outer hair cells
PVCN	Posterior ventral cochlear nucleus
RMS	root mean square
SPL	Sound pressure level
SR	Spontaneous rate
VCN	ventral cochlear nucleus

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Highlights

- Adult rats were unilaterally exposed to narrow band noise centered at 12 kHz at 126 dB for 2 hours.
- Noise exposure causes hair cell loss, auditory nerve degeneration and synaptic growth in the VCN.
- Noise causes tinnitus in some but not all rats despite same pattern of hair cell loss.
- No-tinnitus rats show more GAP-43 expression in VCN than Tinnitus rats 10 weeks after noise.
- Long-term synaptic plasticity in ventral cochlear nucleus may suppress noise-induced tinnitus.

Figure 1.

Gap-prepulse inhibition of the acoustic startle (GPIAS): Each rat was put in a sound transparent mesh cage inside a sound attenuated booth. A speaker inside the booth presented a background narrowband noise floor of 60 dB centered at 6, 12, 16, 20 or 24 kHz as well as short broadband noise bursts at 100 dB, which elicits an acoustic startle response (body twitch) of the rat (A). When a silent gap in the background noise floor preceded the startle eliciting stimulus (B), the rat showed a reduced startle response, i.e. gap-prepulse inhibition. Rats with tinnitus could not detect the silent gap as the perceived phantom noise “filled the gap” (C). Thus, tinnitus rats did not show any gap-prepulse inhibition, meaning that there was no difference in startle responses at the ‘no-gap’ and ‘gap’ condition.

Figure 2.

Line drawing of the rat cochlear nucleus with areas of measurement (grey squares) for GAP-43 staining density: Six regions were measured in the PVCN, AVCN and in the fusiform cell layer of the DCN: dorsal, mid-region and ventral portion within each subnucleus corresponding to high, middle and low frequencies, and laterally and medially separately as these regions showed different staining patterns. AVCN: anterior ventral cochlear nucleus; DCN: dorsal cochlear nucleus; l: lateral; PVCN: posterior ventral cochlear nucleus; v: ventral. Scalebar: 250 μ m

Figure 3.

Gap-prepulse inhibition of the acoustic startle (GPIAS) in controls (A, BB and CC) and noise exposed rats (E, B1, D3, F, C1, D1, C3, A4, B4): Noise exposed rats were measured during three test periods, before noise (“Baseline”, round symbols), during day 1–10 after noise exposure (“Short-term”, triangles) and again from week 8 to 10 (“Long-term”, diamonds) along with age-matched controls. All control animals as well as four noise exposed rats showed significant inhibition of the acoustic startle at all frequencies tested and at all test periods, and thus no signs of persistent tinnitus (‘Noise-No-Tinnitus’: E, B1, D3, F). Five noise exposed rats showed lack of inhibition at one or more frequencies tested, which is interpreted as behavioral evidence of tinnitus (‘Noise-Tinnitus’: C1, D1, C3, A4, B4) at “Short-term” (small T) as well as “Long-term” (bolded T). Increase of the startle-amplitude, often seen at 12 kHz or surrounding frequencies, may be related to loudness

recruitment. These results have been previously published in a similar figure (Kraus et al., 2010).

Figure 4.

Basilar membranes with organ of Corti from the rat ear stained with Harris' hematoxylin solution: Arrows point to hair cells, large arrow heads point to empty spaces where hair cells are missing, and small arrow heads point to support cells. Asterisks indicate tunnel of Corti. In the control ear (A) the three rows OHC and one row IHC can be clearly recognized (arrows). Occasionally, one hair cell is missing (large arrow head). In the noise-exposed ear (B, C, D, E) there was heavy hair cell damage and more OHC than IHC were missing. Hair cell loss was greatest near the base and decreased toward the apex; (B) many OHC were missing while most IHC were still present. (C) Most OHC and many IHC were missing. (D) All OHC and most IHC were missing. (E) All hair cells missing. Scalebar: 50 μ m

Figure 5. Outer hair cells (OHC) and inner hair cells (IHC) from a noise-exposed ear, apical turn: Arrows point to examples of intact hair cells and asterisks indicate regions where hair cells are missing. Scale bar: 25 μ m.

Figure 6.

Cochleograms of left and right ear from three control animals (A, BB and CC) and from 9 animals unilaterally exposed to 126 dB narrowband noise centered at 12kHz (E, B1, D3, F, C1, D1, C3, B4, A4): Frequency map on the top of the diagrams is based on a study done by Müller (1991). All noise exposed rats showed severe damage in the exposed ear (left), where the pattern of damage was similar in all rats with all and almost all hair cells missing in the basal and middle turn, and more outer hair cells (OHC) missing than inner hair cells (IHC). Seven of the noise exposed rats (E, B1, D3, D1, C3, A4 and B4) showed no noise induced damage on the protected contralateral ear as compared to Controls. The pattern of missing hair cells contralaterally resembled controls with no loss exceeding 5% in any area. The last

two rats (F, C1) showed some hair cell loss contralaterally as compared with controls. These results have been previously published in a similar figure (Kraus et al., 2010).

Figure 7.

GAP-43 expression in dorsal cochlear nucleus (DCN) in a control animal (left) and 10 weeks after noise exposure (right): GAP-43 was present in fibers and presynaptic endings throughout the fusiform cell layer, with the lateral region showing a darker staining than the medial region. There was no or only little difference of staining intensity between controls (A) and noise exposed animals (B). Dashed line indicates border of DCN. ICP: inferior cerebellar penduncle; VCN: ventral cochlear nucleus. Scalebar: 400 μ m.

Figure 8.

GAP-43 expression in the ventral cochlear nucleus in a control animal and 10 weeks after noise exposure: In PVCN (upper row) as well as in AVCN (lower row) GAP-43 was present in fibers and presynaptic endings throughout the nucleus. In controls (left panels, A, D), there was only a moderate expression of GAP-43. Animals exposed to noise showed varying patterns 10 weeks post-noise. A rare case, one of nine animals ("Rat E", panels B, E) showed little or no increase of GAP-43 expression. In most cases (eight of nine animals; panels C, F) there was a strong increase of GAP-43 expression in the medial portion of the nucleus. Dashed lines in the panels A and B indicate borders of the PVCN and AVCN. Dashed lines in panels C and F indicate borders between lateral and medial regions which showed different extent of GAP-43 expression. AVCN: anterior ventral cochlear nucleus; n8: eighth nerve, PVCN: posterior ventral cochlear nucleus. Scalebar: 200 μ m.

Figure 9.

GAP-43 expression in auditory nerve fibers at the root of cochlear nucleus in a control animal (A) and 10 weeks after noise exposure (B, C): Squares indicate region of auditory nerve fiber bundles shown at higher magnification in the insets. In controls (A), the fibers are unstained. One of the noise exposed animals (B), which showed only a modest GAP-43 response had no fiber staining as well (“Rat E”, see figure 8). In contrast, the other eight noise-exposed rats with a strong GAP-43 response in VCN showed pronounced fiber staining in the auditory nerve (C). Arrow heads point to auditory nerve fibers. Arrows point to stained presynaptic endings. n8: eighth nerve, VCN: ventral cochlear nucleus. Scalebar: 200 μm ; Scalebar in inset: 50 μm .

Figure 10.

GAP-43 immunoreactivity in ventral cochlear nucleus showed at high magnification in a control (left, panel A) and in a noise exposed animal (right, panel B): GAP-43 is expressed in presynaptic endings (arrowheads) and some fibers. After noise exposure, GAP-43 expression was increased, indicating growth of fibers and synaptic endings. The medial part of VCN contained considerably more staining than laterally. Dashed lines indicate border between lateral and medial regions. Scalebar: 100 μ m.

Figure 11.

Expression of GAP-43 in posterior ventral cochlear nucleus (PVCN, upper panels) and anterior ventral cochlear nucleus (AVCN, lower panels) in a Noise-No-Tinnitus rat (left, panels A, C) and a Noise-Tinnitus rat (right, panel B, D): Up-regulation of GAP-43 in the medial regions was more pronounced in Noise-No-Tinnitus rats than in Noise-Tinnitus rats. Arrows point to medial part with increased GAP-43 expression. Scalebar: 200 μ m.

Figure 12.

Noise-induced size reduction of the the ipsilateral cochlear nucleus, measured for DCN (A), PVCN (B) and AVCN (C) separately: Noise-induced ipsilateral size change is presented as ipsi-to-contralateral (i:c) ratio. Asterisks indicate significant difference between groups. DCN showed no significant size reduction with a i:c ratio close to 1, while the PVCN as well as AVCN showed a significant reduction of 25–30%. There was no difference between Noise-No-Tinnitus and Noise-Tinnitus rats. There was also no difference in size reduction between PVCN and AVCN. Since nucleus shrinkage itself increases staining density, the size reduction from each individual rat was used as a correction factor for the quantified GAP-43 up-regulation.

Figure 13.

GAP-43 expression in six areas of the ipsilateral DCN, PVCN and AVCN as compared to corresponding contralateral areas in Control rats, in Noise-No-Tinnitus rats and Noise-Tinnitus rats. GAP-43 expression is presented as i:c ratio of staining density multiplied by i:c size ratio. Asterisks and horizontal bars indicate significant difference from controls. Dashed lines indicate a significant difference between the Noise-Tinnitus group and the Noise-No-Tinnitus group. No significant changes could be seen in the DCN (A, D, G, J, M, P), lateral PVCN (B, E, H), lateral AVCN (C, F, I) or the dorsal part of medial AVCN (L). Medial PVCN (K, N, Q) as well as medial AVCN (O, R) showed significant increase of GAP-43 expression compared to controls at 10 weeks after noise exposure; a significantly stronger increase was seen in the No-Tinnitus rats versus Tinnitus rats (N, R).