Published in final edited form as:

Neuroscience. 2019 February 10; 399: 184–198. doi:10.1016/j.neuroscience.2018.12.023.

Synaptic reorganization response in the cochlear nucleus following intense noise exposure

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Abstract

The cochlear nucleus, located in the brainstem, receives its afferent auditory input exclusively from the auditory nerve fibers of the ipsilateral cochlea. Noise-induced neurodegenerative changes occurring in the auditory nerve stimulate a cascade of neuroplastic changes in the cochlear nucleus resulting in major changes in synaptic structure and function. To identify some of the key molecular mechanisms mediating this synaptic reorganization, we unilaterally exposed rats to a high intensity noise that caused significant hearing loss and then measured the resulting changes in a synaptic plasticity gene array targeting neurogenesis and synaptic reorganization. We compared the gene expression patterns in the dorsal cochlear nucleus (DCN) and ventral cochlear nucleus (VCN) on the noise-exposed side versus the unexposed side using a PCR gene array at 2 d (early) and 28 d (late) post-exposure. We discovered a number of differentially-expressed genes, particularly those related to synaptogenesis and regeneration. Significant gene expression changes occurred more frequently in the VCN than the DCN and more changes were seen at 28 d versus 2 d post-exposure. We confirmed the PCR findings by in situ hybridization for Brain-derived neurotrophic factor (*Bdnf*), *Homer-1*, as well as the glutamate NMDA receptor *Grin1*, all involved in neurogenesis and plasticity. These results suggest that *Bdnf*, *Homer-1* and *Grin1* play important roles in synaptic remodeling and homeostasis in the cochlear nucleus following severe noise-induced afferent degeneration.

Keywords

noise-induced hearing loss; synaptic plasticity; gene expression; dorsal cochlear nucleus; ventral cochlear nucleus; long term depression; long term potentiation

Introduction

Acoustic information transduced by cochlear hair cells is transmitted through roughly 30,000-50,000 auditory nerve fibers which relay this information to the dorsal cochlear nucleus (DCN) and ventral cochlear nuclei (VCN) in the brainstem (Spoendlin and Schrott,

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1989). The cochlear nucleus plays an essential role in extracting key features from the acoustic signal and then relaying it on to other ipsilateral and contralateral auditory nuclei in the central auditory pathway (Doucet and Ryugo, 1997). Severe unilateral noise-induced hearing loss and cochlear damage greatly decreases neural input to the cochlear nuclei and disrupts the balance of excitatory and inhibitory synaptic activity throughout the auditory pathway, including the DCN and VCN. Damaging noise exposures (Bilak et al., 1997, Michler and Illing, 2002, Muly et al., 2002) and the resulting degeneration of auditory nerve fibers, stimulate complex neuroplastic changes and repair responses exemplified by fiber outgrowth and synaptogenesis in the cochlear nucleus, remodeling of excitatory synapses (Kim et al., 2004) and increased cholinergic activity (Jin et al., 2005, Jin and Godfrey, 2006). These changes contribute to a homeostatic rebalancing of excitatory-inhibitory neurotransmission and structural changes in the VCN and DCN, which may be relevant to spectral and temporal processing, loudness coding and hearing disorders such as impaired speech discrimination, tinnitus and hyperacusis (Kaltenbach and Afman, 2000, Auerbach et al., 2014, Jiang et al., 2017).

Previous studies have reported upregulation of GAP-43 in the VCN of rats after noise-induced hearing loss (Kraus et al., 2011), intense and prolonged microglia expression (Baizer et al., 2015), changes in neural tuning and firing rates (Boettcher and Salvi, 1993, Salvi et al., 2000) and altered expression of genes involved in excitation, inhibition, inflammation and pain (Manohar et al., 2016). However, little is known about the dynamic gene expression changes associated with synaptic remodeling in the cochlear nucleus after unilateral noise exposure, except for one study in guinea pigs (Dong, et al, 2010). In this study, we used a focused synaptic plasticity gene array targeting 84 genes to identify the acute (2 d post-exposure) and chronic (28 d post-exposure) changes in gene expression in the noise-affected cochlear nuclei of adult Sprague–Dawley rats in comparison to sham controls. We also profiled the relative abundance of synaptic plasticity genes in the VCN and DCN. We found many differentially-expressed genes in the cochlear nuclei innervated by the noise-damaged ear, particularly genes related to long term depression, long term potentiation, receptors, postsynaptic densities, synaptogenesis and regeneration. We confirmed the PCR findings by in situ hybridization for *Bdnf*, *Homer1*, and *Grin1*, all of which appear to be involved in the response to loss of afferent input to the cochlear nuclei.

Methods

Noise exposure:

All the procedures involving the use and care of animals were approved by the University at Buffalo Institutional Animal Care and Use Committee (IACUC) and were consistent with NIH guidelines. Fifteen adult Sprague-Dawley rats (age 3-4 months) were subjected to unilateral noise exposure. Rats were anesthetized with isoflurane, and then unilaterally exposed (right ear) to a narrow band noise (NBN, 100 Hz bandwidth) centered at 12 kHz for 2 h at 126 dB SPL. Details of the equipment and procedures used for this noise exposure are described in previous publications (Kraus et al., 2011, Baizer et al., 2015). The output port of the super compression acoustic driver was positioned in line with and approximately 10 mm from the opening of the rat's ear canal. A foam plug was inserted into the contralateral ear

canal and covered with petroleum jelly in order to prevent damage to the contralateral ear as described previously (Kraus et al., 2011, Manohar et al., 2016). Eight sham control rats were not subjected to the noise exposure, but received the same isoflurane anesthesia as the exposed rats.

Auditory Brainstem Response (ABR):

Auditory function was evaluated in the right ear of six noise-exposed rats and six sham controls using the ABR as described in detail in previous publications (Jamesdaniel et al., 2008, Chen et al., 2010, Liu et al., 2011). Briefly, rats were anesthetized with a ketamine (50 mg/kg)/xylazine (6 mg/kg) cocktail (i.p.) and placed on a regulated heating pad (FHC, model 40-90-2) set to maintain core body temperature at 37 °C. A foam earplug was inserted into the non-test, left ear and covered with petroleum jelly. Tone bursts (1 ms duration, 0.5 ms rise/fall time, cosine²-gating, 6, 12, 20, and 32 kHz) were digitally-generated (TDT system, SigGen, FL, USA) and presented at a rate of 21/s through a loudspeaker (FT28D, Fostex) located approximately 15 cm in front of the opening of the ear canal of the right, test ear. The sound level was decreased in 10 dB steps from 90 dB SPL to at least 10 dB below the intensity at which the ABR response disappeared. Needle electrodes (Grass Technologies) were placed at the vertex (active), posterior bulla (reference) and behind the shoulder blade (ground). The responses were amplified 5020 times by a TDT Headstage-4 bio-amplifier (10-3000 Hz, notch filter at 60 Hz) and averaged 400 times. Threshold was defined as the lowest intensity needed to obtain a just detectable peak in the largest wave in ABR response (wave III, peak-trough around 3 ms at high intensities) as described in our earlier publication (Jamesdaniel et al., 2008, Chen et al., 2014). To confirm that the noise exposure caused a severe, permanent hearing loss, ABR thresholds were measured 28 d post-treatment in the noise-exposed group and sham control group. ABR were not measured at 2-days post-exposure because our prior studies indicated the ABR was largely abolished at this time.

Synaptic Plasticity qRT-PCR:

Nine rats (three sham, three at 2 d post-treatment and three at 28 d post-treatment) were used for gene array studies. The qRT-PCR procedures used in the study have been described in our previous publications (Hu et al., 2009, Manohar et al., 2014, Manohar et al., 2016). Rats were euthanized with CO₂, decapitated and the brain quickly extracted from the cranial cavity. The cerebellum was removed to expose the cochlear nuclei. The cochlear nuclei, which protrude from the dorsal-lateral surface of the brainstem were carefully dissected out in an RNase free environment. The protruding edges of the cochlear nuclei along anterior, posterior and dorsal surface of the lateral brainstem along with the entry of the auditory nerve root were used as landmarks for extracting the DCN and VCN. The protruding surface in the dorsolateral region above the entry point of the auditory nerve was used for the DCN analysis whereas the protruding region roughly medial to the auditory nerve was used for the VCN. Total RNA was isolated using an RNeasy lipid tissue extraction kit (Qiagen) as described previously (Manohar et al., 2016).

The noise-induced gene expression changes in the DCN and VCN were evaluated with a synaptic plasticity gene array (RT² Profiler PCR Array Rat Synaptic Plasticity, Cat. no:

PARN-126ZA array, SA Biosciences/Qiagen) according to the manufacturer's instructions. The array contained 84 genes related to synaptic plasticity (Appendix Table A.1). A list of gene abbreviations with outlinks to definitions and annotations is available here: <https://tinyurl.com/y9py9843>. The analysis was performed with a MyiQ™ single color Real-Time PCR Detection System; gene expression was evaluated by monitoring SYBR Green fluorescence during the PCR reaction using our previously described protocol (Manohar et al., 2016). Three samples obtained from three different animals were evaluated for the three experimental conditions (sham control, 2 d post-exposure, and 28 d post-exposure). Ct values were measured and the 2^{-Ct} measures for each gene calculated after normalizing with Ct values with housekeeping genes. The *B2m* housekeeping gene was used to normalize genes in the ipsilateral DCN in the sham control group and the 2 d and 28 d noise-exposed groups. For the VCN, Ct values were normalized using the average of two housekeeping genes *B2m* and *Rplp1*. The statistical analysis of noise-induced changes in mRNA expression levels in the DCN and VCN was performed using the SA Biosciences online data analysis resource (<http://pcrdataanalysis.sabiosciences.com/pcr/arrayanalysis.php>). The software automatically performs all 2^{-Ct} -based fold change (FC) calculations from uploaded raw Ct data and the statistical analysis returns the p value for each gene expression comparison. Noise-induced changes in gene expression were considered significant if: (1) the FC in gene expression increased by more than 50% or decreased by more than 50% and (2) $p < 0.05$ for the statistical comparison. The overall differential expression of mRNAs associated with synaptic plasticity are shown in a volcano plot. Fold changes and p values were converted into $\log_2$ and $-\log_{10}$ values in volcano plot, respectively. In addition, a gene profiling analysis was performed on the sham controls to characterize the relative abundance of the synaptic plasticity genes relative to the housekeeping genes in the DCN and VCN.

RNA *In Situ* Hybridization:

Six rats (two sham rats, two rats at 2 d post-exposure, and two rats at 28 d post-exposure) were used for the RNA *in situ* hybridization studies. The rats were euthanized (86 mg/kg, i.p., Fatal Plus, Vortech Pharmaceutical Ltd.) and perfused with 0.1 M phosphate buffered saline (PBS) followed by 10% formalin in PBS. The brains were removed from the skull, post-fixed in 10% formalin for 24 h, and then cryoprotected in 15% sucrose in PBS for 6 h followed by 30% sucrose in PBS for 12 h. Cryostat sections were cut in the coronal plane at a thickness of 15 μ m, mounted on Superfrost Plus slides (Thermo Fischer Scientific) and then stored at -80°C . Prior to starting the *in situ* procedure, the temperature of the sections was increased to -20°C for 1 h to remove moisture from the sections. Afterward, the slides were processed according to the manufacturer's instructions (Advanced Cell Diagnostics, Newark, CA, RNAscope). Probes designed to detect *Bdnf*, *Homer1*, or *Grin1* mRNA were evaluated using 15 μ m thick cryostat sections from the DCN and VCN. Positive and negative assay control probes included in the kit were also evaluated. The gene-specific *in situ* mRNA labeled sections were counterstained with Gills' hematoxylin to label nuclei (RICC Chemical Co., Arlington, TX). The sections were then visualized with a light microscope (Zeiss Axioskop, 400X), photographed with a digital camera (SPOT Insight, Diagnostic Instruments Inc.) and processed with software (PowerPoint 2010).

Statistics:

For the gene array data, p-values were calculated using a Student's t-test (two-tail distribution and equal variances between the two samples) on the replicate 2⁻ CT values for each gene in each noise exposure group compared to the sham group. The p-values less than 0.05 were considered statistically significant. Each group (including the sham) contained 3 independent samples to calculate p-values. For ABR, a two-way ANOVA, with frequency as the repeated measure was used to identify significant main effects with $p < 0.05$, followed by Bonferroni post-hoc tests to identify frequency specific differences (data normally distributed, equal variance).

Results

To determine if noise-induced gene changes in the DCN and VCN might be affected by initial differences in baseline gene expression levels in these two regions, we measured the relative abundance of the 84 synaptic plasticity genes in the DCN and VCN of the three sham control rats by normalizing the value of each synaptic plasticity gene to the value of the highly abundant actin housekeeping gene in the same region. These results are shown in Appendix Table A.1, with the ten most abundant genes highlighted in bold text. Most of the highly abundant genes expressed in the DCN and VCN fell into the long term depression (LTD) category; these included *Gnai1*, *Gria1*, *PPP1r14a*, *PPP2ca*, *PPP3ca*, and *Mapk1*. *Mapk1*, also considered a long term potentiation (LTP)-associated gene, along with two other LTP-related genes, *Rab3a* and *Ywhaq*, were highly expressed in both the DCN and VCN. In contrast, some of the least abundant genes in the DCN and VCN were in the growth factor category (i.e., *Ngf*, *Tnf*, *Ntf3*, and *Ntf4*).

Figure 1 plots the FC relative abundance of each gene in the VCN against the FC relative abundance of that gene in the DCN. The FC gene expression values in the VCN and DCN were highly ($r^2 = 0.91$) correlated, indicating similar levels of gene expression in these two regions under baseline control conditions. A few genes fell outside the 95% confidence interval. Genes somewhat more highly expressed in the VCN than DCN included *Gabra5* (a GABA receptor subunit), *Ngfr* (nerve growth factor receptor), *Arc* (a cytoskeletal protein), *Inhba* (inhibin beta considered a late response gene), and *Grip1* (a glutamate receptor interacting protein). In contrast, genes expressed slightly higher in the DCN than VCN included *Prkcg* (protein kinase C gamma), *Gria1* (glutamate ionotropic receptor AMPA type subunit 1), *Camk2a* (CaM Kinase II Alpha Subunit), *Igf1* (insulin-like growth factor 1), and *Prkg1* (CGMP-Dependent Protein Kinase). All other genes fell within the 95% confidence interval, indicative of similar expression of these synaptic plasticity genes in the DCN and VCN. The similarity in gene expression levels in these two regions is important information for interpreting the effects of noise-induced gene expression changes in the DCN and VCN.

Noise-Induced Unilateral Hearing Loss:

Our 126 dB SPL (2 h, 12 kHz NBN) unilateral noise exposure caused severe unilateral hearing loss, consistent with the massive hair cell loss and extensive auditory nerve fiber degeneration reported in earlier studies (Kraus et al., 2011, Baizer et al., 2015). Figure 2 compares the mean ($\pm$ SEM, $n=6$) ABR thresholds measured in the sham control group

and the noise-exposed group. ABR thresholds in the noise-exposed group ranged from approximately 85 dB SPL at low frequencies to roughly 100 dB at high frequencies while thresholds in the control group ranged from 25-40 dB SPL. Thresholds in the noise-exposed group were significantly higher than those in the control group at all frequencies ($F_{(1, 30)} = 1211.6$ $p < 0.0001$; Bonferroni post-tests, $p < 0.001$).

Gene Expression Changes 2 d Post-Exposure:

PCR array results for 2 d post-exposure for the DCN and VCN are shown in the volcano plots of Figure 3A-B and in Appendix Table A.2 and Table A.3. At 2 d post-exposure, none of the synaptic plasticity genes were significantly altered in the DCN (Figure 3A, Appendix Table A.2). However, in the VCN, six genes had decreased significantly 2 d post-exposure as seen in Figure 3B and Appendix Table A.3. *Bdnf*, which promotes the survival, growth and differentiation of neurons, decreased 1.528 fold ($p = 0.003$). Early growth response protein 1 (*Egr1*), a transcription factor that regulates differentiation and mitogenesis, decreased 1.165 fold; $p = 0.033$). Protocadherin 8 (*Pcdh8*), which encodes integral membrane proteins involved in cell adhesion in the central nervous system, decreased 1.708 fold ($p = 0.038$). The cAMP response element modulator gene, *Crem*, which regulates transcription in response to stress and development, decreased 0.551 fold ($p = 0.005$). The *Homer1* gene, which encodes proteins widely expressed in postsynaptic structures in the central nervous system, decreased 0.581 fold ($p = 0.020$). The *Cebpb* gene, an interleukin 6-dependent binding protein, decreased by 0.575 ($p = 0.040$).

Gene Expression Changes 28 d Post-Exposure:

At 28 d post-exposure, only two genes in the DCN were significantly upregulated, but none were significantly down regulated (Appendix Table A.4, Figure 4A). The expression of the neurotrophic factor 3 (*Ntf3*) gene, that codes for NT-3, a protein that promotes the differentiation and survival of neurons, increased 1.047 fold ($p = 0.038$). The neurotrophic factor 4 (*Ntf4*) gene, which codes for the NT-4 protein, a growth factor that promotes the survival and differentiation of neurons, was upregulated 1.282 fold ($p = 0.043$). A plethora of 66 genes out of a total of 84 synaptic plasticity genes were significantly downregulated in the VCN at 28 d post-exposure; none were significantly upregulated. The 66 genes that were significantly downregulated are highlighted in blue in Appendix Table A.5 and are shown in the volcano plot of Figure 4B.

For ease of interpretation, these 66 genes were organized into nine categories (Appendix Table A.6): (1) long term depression (LTD), (2) long term potentiation (LTP), (3) immediate early gene (IEG), (4) late response gene (LRG), (5) cell adhesion, (6) extracellular matrix and proteolytic activity, (7) Creb cofactors, (8) neuronal receptors, (9) postsynaptic density and other. Some of the downregulated genes were included in more than one functional category such as all of the Creb cofactors, as well as *Grin2a* and *Grin2b*, which appeared in four categories (LTP, cell adhesion, Creb cofactor, and neuronal receptors). Twenty-three genes in the LTD category were significantly downregulated at 28 d post-exposure, a time at which there is significant loss of afferent neural input caused by auditory nerve fiber degeneration in the VCN (Baizer et al., 2015). LTD, which contributes to the selective weakening and elimination of synapses (Sheng and Erturk, 2014) can be triggered by

sensory deprivation due to reduced neural input from the periphery (Bender et al., 2006). In some cases, LTD results from a decrease in post-synaptic density proteins (Wilkerson et al., 2018). In the VCN, 13 genes that code for postsynaptic density proteins were significantly down regulated. In other cases, LTD can be altered by decreased presynaptic transmitter release (Castillo, 2012, Padamsey et al., 2017); such a decrease would be expected from the noise-induced degeneration of excitatory auditory nerve fibers (Baizer et al., 2015). Another major change was the downregulation of 22 genes involved in LTP, a form of synaptic plasticity regulated by the recent pattern of neural activity (Papatheodoropoulos and Kouvaros, 2016). Five of the LTP genes, *Grin1*, *2a*, *2b*, *2c* and *2d*, are members of the glutamate ionotropic receptor NMDA subtype, that plays a major role in synaptic plasticity. Eighteen neural receptors genes were also downregulated. Three were members of the *Gria* gene family (*Gria2*, *3*, and *4*), whose gene products form ionotropic glutamate receptors (Hoppmann et al., 2008) and which are involved in LTP. Seven neural receptor genes were members of the *Grm* gene family (*Grm1*, *2*, *3*, *4*, *5*, *7*, and *8*), which code for metabotropic glutamate receptors I-III and which play a role in LTP and LTD (Bashir et al., 1993, Tang et al., 2013, Dietz and Manahan-Vaughan, 2017). Nineteen IEG, one LRG, and eight cell adhesion genes were also downregulated significantly.

mRNA in situ Hybridization:

Our gene expression studies provide a global perspective on the noise-induced changes in synaptic gene expression in the entire VCN and DCN. In order to visualize mRNA expression on a cell-by-cell basis, we used a commercial mRNA *in situ* hybridization kit with positive and negative assay controls. Since it was not technically feasible to examine all 66 genes that exhibited a response to noise exposure, we chose particular genes based on the magnitude of change in response to noise exposure, their potential roles in auditory synaptic plasticity, and availability of rat-specific probes. Figure 5A shows a representative photomicrograph from a control section from the VCN labeled with the positive assay control probe included in the kit; the 15 μ m thick sections from the VCN were counterstained with Gills hematoxylin to label the nuclei. In positive assays from controls, numerous reddish/brown puncta were present on and near the nuclei (Figure 5A), but largely absent from the surrounding neuropil. In negative assay controls, the reddish/brown puncta were completely absent (Figure 5B). Together, these validate the *in situ* mRNA methods.

As shown in Appendix Table A.1, *Bdnf* was moderately expressed in controls. Consistent with our gene expression findings, mRNA *in situ* hybridization revealed moderate expression of *Bdnf* reddish/brown puncta around the nuclei of neurons in the VCN (Figure 6A). At 28 d post-exposure, *Bdnf* gene expression had significantly decreased in the VCN (Appendix Table A.3). Consistent with the gene expression data, very few puncta of *Bdnf* mRNA were present in the VCN 28 d post-exposure (Figure 6B).

Grin1 gene expression was relatively abundant (−6.14 FC) in the DCN of sham controls (Appendix Table A.1). Consistent with the gene expression data, many reddish/brown puncta of *Grin1* mRNA were present around the nuclei of most neurons in the DCN (Figure 7A). At 28 d post-exposure, *Grin1* gene expression had not decreased significantly in the DCN (Appendix Table A.4) and many *Grin1* mRNA puncta were still present on DCN neurons at

this time (Figure 7B). At 28 d post-exposure, *Homer1* gene expression had decreased significantly in the VCN (Appendix Table A.5). Very heavy *Homer1* mRNA labeling was evident in most neurons in the VCN of sham controls (Figure 8A) whereas *Homer1* labeling in the VCN was less intense at 28 d post-exposure (Figure 8B). In contrast, there was little change in *Homer1* gene expression in the DCN 28 d post-exposure (Appendix Table A.4) and little difference in *Homer1* mRNA labeling on the VCN of sham controls and those from the 28 d noise-exposed group (Figure 9).

Discussion

There is growing recognition that cochlear hearing loss leads to numerous structural and functional changes in the central auditory pathway. These neuroplastic changes are triggered by the loss of neural activity flowing from the auditory nerve into the DCN and VCN, the first relay stations in the ascending pathway. In cases of severe hearing loss exemplified by our 126 dB SPL exposure, extensive degeneration of auditory nerve fibers occurs in the cochlear nucleus (Morest et al., 1998, Hildebrandt et al., 2011, Kraus et al., 2013, Baizer et al., 2015). Auditory nerve fiber degeneration can continue for many months and is accompanied by reactive axonal outgrowth, synaptogenesis and microglia activation, structural substrates for auditory neuroplasticity (Benson et al., 1997, Hildebrandt et al., 2011, Kraus et al., 2011, Baizer et al., 2015). Acoustic trauma and the ensuing cochlear damage reduces the neural input to the cochlear nucleus and alters spontaneous activity in the DCN and VCN (Kaltenbach and Afman, 2000, Kaltenbach et al., 2000)(Vogler, et al., 2013). Spontaneous activity in the DCN decreased significantly 2 d following a traumatic noise-exposure similar to the one used in this study. However, spontaneous activity became significantly higher than normal 5 d post-exposure and spontaneous activity continued to increase to somewhat higher levels out to 180 d post-exposure (Kaltenbach et al., 2000). These structural and functional changes provide a framework for interpreting the noise-induced gene expression changes in the DCN and VCN.

DCN gene expression changes:

Surprisingly, we failed to see significant increases or decreases in synaptic plasticity gene expression in the DCN at 2 d post-exposure (Figure 3A, Appendix Table A.2) despite the fact that spontaneous activity in the DCN decreases 2 d post-exposure (Kaltenbach et al., 2000). One potential explanation for the lack of change in the DCN is that few of the 84 genes on our array play a major role in regulating spontaneous activity in the DCN. Other alternative explanations include: i) the decrease in spontaneous activity may not be significant enough to change gene expression or ii) changes at the post-translational level may be sufficient to alter spontaneous activity in the DCN. The scope of our study involved changes at the mRNA level of expression, which may not always coincide with changes at the protein level of expression. The decrease in spontaneous activity in the DCN 2d post-exposure could also be due to functional changes in the VCN, but this seems unlikely because cochlear destruction, which largely eliminates spontaneous activity in the VCN, fails to significantly alter spontaneous activity in the DCN (Koerber et al., 1966). Another possibility is that spontaneous activity in the DCN is largely determined by the intrinsic

properties of these neurons and/or inputs from other regions in the central nervous system (Shore, 2005, Zhan and Ryugo, 2007).

At 28 d post-exposure, spontaneous activity in the DCN significantly increased in the DCN in a variety of animal species (Kaltenbach and Afman, 2000, Kaltenbach et al., 2000, Brozoski et al., 2002, Zacharek et al., 2002). Despite this dramatic increase in spontaneous activity, only two genes out of 84 exhibited a significant change in expression (Figure 4A, Appendix Table A.4). *Ntf3*, which codes for neurotrophic factor 3 (NT-3), increased significantly. NT-3, which binds to the TrkC receptor, promotes neural differentiation, survival and synaptogenesis (Ip et al., 1993, McAllister et al., 1995, Staecker et al., 1996, Wang and Green, 2011). NT-3 also increases the frequency of spontaneous activity, enhances neural synchrony, and suppresses GABA-mediated synaptic transmission (Kim et al., 1994), changes consistent with the increased spontaneous activity seen in the DCN a week or more post-exposure. The expression of *Ntf4*, which codes for neurotrophic factor 4 (NT-4) also increased significantly 28 d post-exposure. NT4 binds to TrkB receptors and promotes neuron survival and differentiation (Ip et al., 1993, Zheng et al., 1995, Becker et al., 1998).

VCN gene expression changes:

Glutamate is thought to be the dominant neurotransmitter released by auditory nerve fibers that make synaptic contact with neurons in the cochlear nucleus (Wenthold and Gulley, 1977, Hackney et al., 1996). Therefore, it is not surprising that most of the genes linked to glutamate receptors, post-synaptic densities and glutamate synaptic function (LTP and LTD) were down regulated in the VCN 2 d and 28 d post-exposure (Appendix Table A.3 and Table A.5) when hearing was severely impaired and many auditory nerve fibers were in the process of degenerating; however, the paucity of changes in the DCN was unexpected.

In contrast to lack of change in the DCN, six genes, *Bdnf*, *Egr1*, *Pcdh8*, *Crem*, *Homer1*, and *Cebpb*, were significantly downregulated in the VCN 2 d post-exposure (Figure 2B, Appendix Table A.3). *Bdnf* codes for the expression of brain derived neurotrophic factor (BDNF), which binds to the TrkB receptor, promotes neuron survival, growth, and differentiation. Neurons often become intrinsically more excitable and responsive to synaptic inputs when their activity is suppressed; this increase in excitability can be prevented by treatment with BDNF or enhanced by blocking TrkB (Desai et al., 1999). Thus, the decrease in *Bdnf* gene expression could facilitate the noise-induced increases in spontaneous activity seen in the VCN (Vogler et al., 2011).

The immediate early gene, *Egr1* which codes for the EGR-1 protein, functions as a transcriptional regulator. EGR-1 has been implicated in neural plasticity, exocytosis and modulating the expression of the GABA receptor (Petersohn and Thiel, 1996, Pinaud et al., 2003, Knapska and Kaczmarek, 2004, Mo et al., 2015). *Egr1* gene expression was significantly reduced in the VCN at 2 and 28 d post-exposure; this was associated with decreased expression of *Gabra5* in the VCN 28 d post-exposure (Appendix Table A.5). Our results are consistent with those seen after bilateral cochlear ablation where *Egr1* and *Gabra5* were reduced in the auditory cortex two weeks and 12-weeks post-ablation (Oh et al., 2007). Because GABA receptors containing the Gabra5 subunit play an important role in experience-dependent auditory learning (Jeong et al., 2011), the noise-induced reduction in

Gabra5 expression, putatively mediated by *Egr1*, likely suppresses synaptic plasticity in the VCN.

Pcdh8 gene expression was significantly reduced at 2 and 28 d post-exposure in the VCN (Appendix Table A.3, Table A.5). *Pcdh8* codes for protocadherin 8 (PCDH8), an integral membrane protein highly expressed in the developing central nervous system. PCDH8 has been implicated in synaptic remodeling, neuroplasticity and long term potentiation (Yamagata et al., 1999, Yasuda et al., 2007). The noise-induced reductions in *Pcdh8* transcripts as well as other extracellular matrix genes may be linked to extensive neural degeneration that occurs in the VCN after this intense noise exposure (Baizer et al., 2015).

The *Crem* gene, which encodes the cAMP response element modulator protein (CREM-1), was significantly downregulated in the VCN 2 and 28 d post-exposure. CREM-1 is a member of the CREB protein family and responds to stress and metabolic changes (Sassone-Corsi, 1995). Interestingly, siRNA knock down of CREM-1 suppresses apoptosis (Wu et al., 2012, Xu et al., 2014). This suggests that downregulation of *Crem* may suppress apoptosis and promote the survival of VCN neurons when they are deprived of their auditory nerve afferent inputs (Trune, 1982, Lesperance et al., 1995).

The multifunctional *Homer1* gene was listed in three functional categories, LTD, IEG and PSD. *Homer1*, which codes the Homer1 protein, was significantly downregulated 2 d and 28 d post-exposure consistent with previous results with the same noise exposure (Manohar et al., 2016). The Homer1 protein is highly expressed in post-synaptic structures in the auditory brainstem (Soria Van Hove and Borst, 2010) where it helps anchor mGluR to the post-synaptic membrane. The Homer1a splice variant is induced by neural activity and contributes to synaptic plasticity by modulating mGluR signaling (Tu et al., 1998, Sala et al., 2003). In our study, reduced expression of *Homer1* was associated with significant reductions in six metabotropic glutamate receptor genes (*Grin1*, 2, 3, 4, 5, 7 and 8). The reductions in *Homer1* and *Grin* genes likely occur because noise-induced hearing loss and cochlear degeneration greatly reduce glutamate-mediated synaptic activity in VCN, thereby reducing the need for metabotropic receptors.

The *Cebpb* gene, which codes the CCAAT/enhancer-binding protein beta (CEBPB), regulates genes involved in inflammation, synaptic plasticity, proliferation, differentiation and survival (Taubenfeld et al., 2001, Taubenfeld et al., 2002, Nikitin et al., 2005, Kovacs et al., 2006, Calella et al., 2007, Straccia et al., 2011). Our noise exposure causes significant nerve fiber degeneration and activates microglia in the cochlear nucleus (Baizer et al., 2015). Because the neurotoxic effects of activated microglia are attenuated by loss of CEBPB (Straccia et al., 2011), the noise-induced reduction of *Cebpb* expression could protect the VCN from the neurotoxic effects of activated microglia.

Comparison of DCN and VCN gene expression before and after noise exposure:

Under control conditions, without noise exposure, there was a high degree of similarity in synaptic gene expression between the DCN and VCN (Table A.1 and Figure 1). The gene expression levels of only 10 of 84 genes fell outside the 95% confidence level in our VCN/DCN comparison (Figure 1). Similar gene expression profiles in the DCN and VCN

prior to noise exposure support our conclusions that the sound-mediated changes we observed in the DCN and VCN were largely a consequence of the noise exposure, not due to inherent differences in gene expression between these two adjacent tissues.

After the noise exposure, we observed major differences in gene expression between the DCN and VCN. At 2 d post exposure, no significant changes in gene expression were observed in the DCN, while six of the 88 genes in the VCN were downregulated (*Bdnf*, *Egr1*, *Pcdh8*, *Crem*, *Homer1*, and *Cebpb*). The differences between DCN and VCN were even more striking at 28 d post-exposure. Only two genes in the DCN were upregulated (*Ntf3* and *Ntf4*) whereas 66 genes were downregulated in the VCN. Some of the contrasting responses between DCN and VCN might be due to differing activities and functions of the cell types that comprise these tissues. The DCN contains Golgi, cartwheel, tuberculo-ventral, brush, giant and fusiform cells (Wouterlood et al., 1984, Hackney et al., 1990). In contrast, the VCN has many octopus, bushy, T- and D-stellate cells, each with distinct characteristics. Previous comparisons of the VCN and DCN reveal other important differences in terms of expression of cytoskeletal genes (Friedland et al., 2006) and GAP-43 response to carboplatin (Kraus et al., 2009).

The overall down regulation of synaptic plasticity genes likely reflects a loss of synaptic activity due to the severe hearing loss and significant loss of auditory nerve fibers entering the cochlear nucleus (Baizer et al., 2015). Downregulation of *Bdnf* gene expression in the VCN, but not in the DCN, 28 d post-exposure was confirmed by *in situ* hybridization. Our finding of unchanged *Bdnf* in the DCN differs from other reports, in which increased BDNF protein expression was observed in fusiform cells in the DCN 80 d post-exposure (Wang et al., 2011); however, the noise exposure employed in this earlier study was much less severe and did not cause a permanent hearing loss. Thus, the most likely explanation for this difference is that our noise exposure caused more severe hearing loss and neural degeneration, although other possible explanations could be differences in protein versus gene expression and the specific cells being studied.

Comparative similarities with other regions of the brain:

Some of gene expression changes in the VCN and DCN were similar to those observed in other brain regions in response to stress and trauma. The decreased expression of *Grin1* (also known as *GluN1*) we observed in the VCN at 28 d post-exposure suggests a decline in excitatory neurotransmission. Consistent with these results, decreased expression of *Grin1* was observed in the IC with loss of afferent input from the cochlea in aging mice (Osumi et al., 2012). *Egr1*, an immediate early gene and synaptic plasticity marker, was downregulated in the VCN at both 2 d and 28 d post-exposure. These findings parallel *Egr1* downregulation in the IC and AC after salicylate-induced tinnitus (Hu et al., 2014), suggesting common response pathways. *Homer1* has been linked to downregulation of mGluR1 expression in the rat hippocampus in response to radiation damage (Moore et al., 2014); the decrease is similar to that seen for *Homer1* in the VCN following intense noise exposure.

In summary, our study identified specific synaptic plasticity genes in the DCN and VCN that are highly responsive to noise-induced cochlear damage at both early (2 d) and later (28 d) post-exposure times. These gene expression changes provide mechanistic insights into the

dynamic structural and functional changes that can occur in the VCN and DCN following severe noise-induced cochlear damage; information that could increase our understanding of the plasticity and repair mechanisms in the first stage of the auditory brainstem. These noise-responsive genes represent potential drug targets that may lead us to novel treatments relevant to tinnitus and other auditory disorders.

Acknowledgements:

Supported in part by NIH grant R01-DC011808.

Appendix

Table A.1:

Fold change in relative abundance of synaptic plasticity genes relative to actin (Top 10 most abundant genes in bold)

Gene	DCN	VCN	Gene	DCN	VCN	Gene	DCN	VCN
<i>Adam10</i>	-4.87	-4.71	<i>Grin2a</i>	-7.03	-7.07	<i>Ntf3</i>	-12.28	-13.1
<i>Adcy1</i>	-7.42	-7.13	<i>Grin2b</i>	-6.69	-7.84	<i>Ntf4</i>	-12.89	-13.76
<i>Adcy8</i>	-6.49	-5.53	<i>Grin2c</i>	-11.35	-11.09	<i>Ntrk2</i>	-3.53	-3.55
<i>Akt1</i>	-5.82	-4.92	<i>Grin2d</i>	-9.28	-9.08	<i>Pcdh8</i>	-10.75	-10.73
<i>Arc</i>	-8.8	-7.36	<i>Grip1</i>	-10.31	-8.79	<i>Pick1</i>	-7.61	-7.36
<i>Bdnf</i>	-11.69	-10.6	<i>Grm1</i>	-5.86	-6.17	<i>Pim1</i>	-8.94	-8.76
<i>Camk2a</i>	-5.44	-7.47	<i>Grm2</i>	-8.92	-9.66	<i>Plat</i>	-4.62	-5.13
<i>Camk2g</i>	-3.98	-3.72	<i>Grm3</i>	-5.05	-5.52	<i>Plcg1</i>	-5.78	-5.68
<i>Cdh2</i>	-7.65	-7.29	<i>Grm4</i>	-7.44	-7.81	<i>Ppp1ca</i>	-4.17	-4.19
<i>Cebpb</i>	-11.82	-11.94	<i>Grm5</i>	-10.84	-10.48	<i>Ppp1cc</i>	-6.6	-6.19
<i>Cebpd</i>	-10.42	-10.76	<i>Grm7</i>	-8.26	-8.5	<i>Ppp1r14a</i>	-3.44	-3.88
<i>Cnr1</i>	-6.54	-6.02	<i>Grm8</i>	-6.85	-7.32	<i>Ppp2ca</i>	-2.77	-2.84
<i>Creb1</i>	-6.46	-6.48	<i>Homer1</i>	-5.94	-4.85	<i>Ppp3ca</i>	-3.72	-4.44
<i>Crem</i>	-6.2	-6.56	<i>Igf1</i>	-7.31	-9.34	<i>Prkca</i>	-6.11	-5.76
<i>Dlg4</i>	-5.85	-5.87	<i>Inhba</i>	-9.73	-7.81	<i>Prkcg</i>	-4.71	-7.29
<i>Egr1</i>	-9.81	-10.22	<i>Jun</i>	-5.85	-6.21	<i>Prkg1</i>	-9.15	-11.3
<i>Egr2</i>	-13.28	-13.84	<i>Junb</i>	-10.48	-9.68	<i>Rab3a</i>	-1.94	-1.78
<i>Egr3</i>	-11.53	-13.11	<i>Klf10</i>	-9.64	-10.08	<i>Rela</i>	-7.45	-7.5
<i>Egr4</i>	-9.04	-10.36	<i>Mapk1</i>	-2.96	-3.48	<i>Reln</i>	-3.77	-4.82
<i>Ephb2</i>	-10.19	-9.98	<i>Mmp9</i>	-12.73	-13.74	<i>Kif17</i>	-10.12	-10.52
<i>Fos</i>	-5.9	-6.74	<i>Ncam1</i>	-5.18	-5.28	<i>Rgs2</i>	-5.35	-5.56
<i>Gabra5</i>	-7.44	-5.69	<i>Nfkb1</i>	-7.09	-6.53	<i>Rheb</i>	-3.01	-3.64
<i>Gnai1</i>	-3.59	-3.51	<i>Nfkbib</i>	-11.5	-11.7	<i>Sirt1</i>	-6.42	-6.7
<i>Gria1</i>	-5.21	-7.67	<i>Ngf</i>	-12.48	-12.34	<i>Srf</i>	-7.72	-7.54
<i>Gria2</i>	-3.28	-4.34	<i>Ngfr</i>	-8.5	-7.15	<i>Synpo</i>	-7.82	-8.14
<i>Gria3</i>	-4.81	-4.49	<i>Nos1</i>	-9.96	-8.81	<i>Timp1</i>	-6.19	-7.46
<i>Gria4</i>	-3.8	-3.86	<i>Nptx2</i>	-8.32	-9.63	<i>Tnf</i>	-12.21	-13.22
<i>Grin1</i>	-5.69	-6.14	<i>Nr4a1</i>	-7.17	-7.9	<i>Ywhaq</i>	-2.5	-2.83

Table A.2:

DCN 2 d Post-Exposure Fold Change and p Value re Sham Control

Gene	FC	p value	Gene	FC	p value	Gene	FC	p value
<i>Adam10</i>	0.745	0.728	<i>Grin2a</i>	0.460	0.703	<i>Nr4a1</i>	0.475	0.828
<i>Adcy1</i>	0.895	0.766	<i>Grin2b</i>	0.822	0.733	<i>Ntf3</i>	0.027	0.713
<i>Adcy8</i>	0.688	0.548	<i>Grin2c</i>	1.576	0.295	<i>Ntf4</i>	-0.591	0.068
<i>Akt1</i>	0.743	0.776	<i>Grin2d</i>	0.390	0.908	<i>Ntrk2</i>	0.676	0.557
<i>Arc</i>	0.166	0.590	<i>Grip1</i>	1.716	0.238	<i>Pcdh8</i>	-0.308	0.977
<i>Bdnf</i>	0.081	0.925	<i>Grm1</i>	0.929	0.611	<i>Pick1</i>	0.507	0.706
<i>Camk2a</i>	0.518	0.818	<i>Grm2</i>	0.285	0.836	<i>Pim1</i>	0.770	0.594
<i>Camk2g</i>	0.741	0.560	<i>Grm3</i>	0.533	0.537	<i>Plat</i>	0.627	0.578
<i>Cdh2</i>	0.741	0.431	<i>Grm4</i>	0.234	0.995	<i>Plcg1</i>	0.531	0.721
<i>Cebpb</i>	0.719	0.415	<i>Grm5</i>	0.652	0.630	<i>Ppp1ca</i>	0.574	0.594
<i>Cebpd</i>	0.109	0.873	<i>Grm7</i>	0.951	0.916	<i>Ppp1cc</i>	0.802	0.335
<i>Cnr1</i>	0.907	0.492	<i>Grm8</i>	0.238	0.766	<i>Ppp1r14a</i>	0.023	0.855
<i>Creb1</i>	0.506	0.635	<i>Homer1</i>	0.597	0.653	<i>Ppp2ca</i>	0.446	0.693
<i>Crem</i>	-0.038	0.834	<i>Igf1</i>	0.138	0.929	<i>Ppp3ca</i>	0.415	0.521
<i>Dlg4</i>	1.049	0.493	<i>Inhba</i>	1.510	0.293	<i>Prkca</i>	0.921	0.515
<i>Egr1</i>	-0.067	0.912	<i>Jun</i>	0.600	0.480	<i>Prkcg</i>	0.577	0.748
<i>Egr2</i>	0.059	0.692	<i>Junb</i>	0.596	0.865	<i>Prkg1</i>	0.485	0.716
<i>Egr3</i>	-0.751	0.573	<i>Kif17</i>	1.128	0.370	<i>Rab3a</i>	0.870	0.352
<i>Egr4</i>	-0.638	0.627	<i>Klf10</i>	0.292	0.982	<i>Rela</i>	1.270	0.305
<i>Ephb2</i>	0.926	0.676	<i>Mapk1</i>	0.396	0.484	<i>Reln</i>	0.356	0.667
<i>Fos</i>	-0.700	0.514	<i>Mmp9</i>	-0.499	0.080	<i>Rgs2</i>	0.013	0.942
<i>Gabra5</i>	0.809	0.474	<i>Ncam1</i>	0.945	0.396	<i>Rheb</i>	0.111	0.762
<i>Gnai1</i>	0.388	0.656	<i>Nfkb1</i>	0.622	0.798	<i>Sirt1</i>	0.579	0.589
<i>Gria1</i>	0.645	0.695	<i>Nfkbib</i>	0.499	0.463	<i>Srf</i>	0.760	0.622
<i>Gria2</i>	0.301	0.711	<i>Ngf</i>	1.859	0.139	<i>Synpo</i>	0.894	0.378
<i>Gria3</i>	0.400	0.724	<i>Ngfr</i>	1.313	0.367	<i>Timp1</i>	0.120	0.726
<i>Gria4</i>	0.461	0.631	<i>Nos1</i>	1.303	0.436	<i>Tnf</i>	0.192	0.895
<i>Grin1</i>	0.467	0.495	<i>Nptx2</i>	0.201	0.782	<i>Ywhaq</i>	0.470	0.474

Table A.3:

VCN 2 d Post-Exposure Fold Change and p Value re Sham Control

Gene	FC	p value	Gene	FC	p value	Gene	FC	p value
<i>Adam10</i>	-0.477	0.087	<i>Grin2a</i>	-0.960	0.149	<i>Nr4a1</i>	-0.622	0.351
<i>Adcy1</i>	-0.777	0.147	<i>Grin2b</i>	-0.841	0.196	<i>Ntf3</i>	-0.574	0.340
<i>Adcy8</i>	-0.303	0.113	<i>Grin2c</i>	-0.280	0.508	<i>Ntf4</i>	-0.204	0.793
<i>Akt1</i>	-0.497	0.138	<i>Grin2d</i>	-0.841	0.116	<i>Ntrk2</i>	-0.555	0.107

Gene	FC	p value	Gene	FC	p value	Gene	FC	p value
<i>Arc</i>	-0.912	0.120	<i>Grip1</i>	-0.614	0.186	<i>Pcdh8</i>	-1.708	0.038
<i>Bdnf</i>	-1.528	0.003	<i>Grm1</i>	-0.362	0.391	<i>Pick1</i>	-0.512	0.145
<i>Camk2a</i>	0.060	0.893	<i>Grm2</i>	-0.708	0.161	<i>Pim1</i>	-0.192	0.929
<i>Camk2g</i>	-0.298	0.218	<i>Grm3</i>	-0.325	0.095	<i>Plat</i>	-0.024	0.883
<i>Cdh2</i>	-0.281	0.226	<i>Grm4</i>	-0.728	0.079	<i>Plcg1</i>	-0.469	0.126
<i>Cebpb</i>	-0.575	0.040	<i>Grm5</i>	-1.002	0.073	<i>Ppp1ca</i>	-0.314	0.182
<i>Cebpd</i>	-0.200	0.543	<i>Grm7</i>	-0.567	0.375	<i>Ppp1cc</i>	-0.205	0.404
<i>Cnr1</i>	-0.444	0.160	<i>Grm8</i>	-0.354	0.425	<i>Ppp1r14a</i>	-0.273	0.120
<i>Creb1</i>	-0.370	0.166	<i>Homer1</i>	-0.581	0.020	<i>Ppp2ca</i>	-0.540	0.080
<i>Crem</i>	-0.551	0.005	<i>Igf1</i>	-0.187	0.434	<i>Ppp3ca</i>	-0.225	0.474
<i>Dlg4</i>	-0.347	0.275	<i>Inhba</i>	-0.809	0.056	<i>Prkca</i>	-0.510	0.091
<i>Egr1</i>	-1.165	0.033	<i>Jun</i>	0.183	0.479	<i>Prkcg</i>	0.031	0.908
<i>Egr2</i>	-0.433	0.337	<i>Junb</i>	-0.863	0.119	<i>Prkg1</i>	0.032	0.959
<i>Egr3</i>	-1.442	0.070	<i>Kif17</i>	-0.101	0.839	<i>Rab3a</i>	-0.008	0.877
<i>Egr4</i>	-1.464	0.135	<i>Klf10</i>	-0.675	0.076	<i>Rela</i>	-0.162	0.495
<i>Ephb2</i>	-0.233	0.526	<i>Mapk1</i>	-0.158	0.396	<i>Reln</i>	-0.279	0.446
<i>Fos</i>	-1.444	0.062	<i>Mmp9</i>	-0.210	0.736	<i>Rgs2</i>	-0.647	0.055
<i>Gabra5</i>	-0.561	0.072	<i>Ncam1</i>	-0.223	0.400	<i>Rheb</i>	-0.284	0.222
<i>Gnai1</i>	-0.402	0.025	<i>Nfkb1</i>	-0.743	0.103	<i>Sirt1</i>	-0.414	0.184
<i>Gria1</i>	-0.136	0.634	<i>Nfkbib</i>	-0.386	0.646	<i>Srf</i>	-0.526	0.173
<i>Gria2</i>	-0.444	0.176	<i>Ngf</i>	-0.884	0.271	<i>Synpo</i>	-0.062	0.755
<i>Gria3</i>	-0.390	0.140	<i>Ngfr</i>	0.737	0.058	<i>Timp1</i>	0.350	0.406
<i>Gria4</i>	-0.414	0.157	<i>Nos1</i>	-0.610	0.399	<i>Tnf</i>	-0.594	0.339
<i>Grin1</i>	-0.215	0.426	<i>Nptx2</i>	-0.745	0.279	<i>Ywhaq</i>	-0.189	0.334

Table A.4:

DCN 28 d Post-Exposure Fold Change and p Value re Sham Control

Gene	FC	p value	Gene	FC	p value	Gene	FC	p value
<i>Adam10</i>	0.222	0.813	<i>Grin2a</i>	-1.410	0.309	<i>Nr4a1</i>	-0.185	0.675
<i>Adcy1</i>	-1.725	0.372	<i>Grin2b</i>	-0.164	0.627	<i>Ntf3</i>	1.047	0.038
<i>Adcy8</i>	0.131	0.824	<i>Grin2c</i>	-0.021	0.785	<i>Ntf4</i>	1.282	0.043
<i>Akt1</i>	-0.277	0.889	<i>Grin2d</i>	-0.547	0.509	<i>Ntrk2</i>	-0.394	0.601
<i>Arc</i>	-0.391	0.665	<i>Grip1</i>	0.946	0.357	<i>Pcdh8</i>	0.885	0.406
<i>Bdnf</i>	0.674	0.542	<i>Grm1</i>	-1.205	0.380	<i>Pick1</i>	-1.016	0.490
<i>Camk2a</i>	0.078	0.885	<i>Grm2</i>	-1.205	0.375	<i>Pim1</i>	-0.043	0.929
<i>Camk2g</i>	0.031	0.822	<i>Grm3</i>	-0.247	0.769	<i>Plat</i>	0.197	0.916
<i>Cdh2</i>	-0.323	0.574	<i>Grm4</i>	-0.946	0.432	<i>Plcg1</i>	-0.406	0.807
<i>Cebpb</i>	0.506	0.562	<i>Grm5</i>	0.685	0.550	<i>Ppp1ca</i>	-0.762	0.500

Gene	FC	p value	Gene	FC	p value	Gene	FC	p value
<i>Cebpd</i>	0.226	0.991	<i>Grm7</i>	-0.229	0.692	<i>Ppp1cc</i>	-1.795	0.126
<i>Cnr1</i>	-0.444	0.505	<i>Grm8</i>	-0.832	0.311	<i>Ppp1r14a</i>	-0.440	0.946
<i>Creb1</i>	0.129	0.935	<i>Homer1</i>	-0.053	0.794	<i>Ppp2ca</i>	-0.294	0.678
<i>Crem</i>	-0.334	0.479	<i>Igf1</i>	-0.099	0.817	<i>Ppp3ca</i>	-0.175	0.698
<i>Dlg4</i>	-1.405	0.390	<i>Inhba</i>	0.487	0.895	<i>Prkca</i>	-0.269	0.836
<i>Egr1</i>	-0.597	0.436	<i>Jun</i>	-0.620	0.710	<i>Prkcg</i>	-0.933	0.451
<i>Egr2</i>	1.045	0.150	<i>Junb</i>	0.216	0.605	<i>Prkg1</i>	0.869	0.331
<i>Egr3</i>	0.916	0.719	<i>Kif17</i>	0.738	0.530	<i>Rab3a</i>	-0.564	0.515
<i>Egr4</i>	-1.705	0.162	<i>Klf10</i>	-0.231	0.656	<i>Rela</i>	0.213	0.830
<i>Ephb2</i>	0.406	0.982	<i>Mapk1</i>	-0.194	0.611	<i>Reln</i>	-0.728	0.461
<i>Fos</i>	-1.040	0.428	<i>Mmp9</i>	1.671	0.132	<i>Rgs2</i>	-0.560	0.365
<i>Gabra5</i>	0.493	0.748	<i>Ncam1</i>	0.115	0.923	<i>Rheb</i>	-0.362	0.337
<i>Gnai1</i>	-0.066	0.793	<i>Nfkb1</i>	0.096	0.951	<i>Sirt1</i>	-0.254	0.555
<i>Gria1</i>	-0.535	0.564	<i>Nfkbib</i>	-0.124	0.914	<i>Srf</i>	0.323	0.955
<i>Gria2</i>	-0.159	0.733	<i>Ngf</i>	1.116	0.196	<i>Synpo</i>	0.097	0.984
<i>Gria3</i>	-0.497	0.470	<i>Ngfr</i>	0.297	0.771	<i>Timp1</i>	0.387	0.531
<i>Gria4</i>	-0.369	0.511	<i>Nos1</i>	-0.041	0.671	<i>Tnf</i>	0.572	0.605
<i>Grin1</i>	-1.593	0.228	<i>Nptx2</i>	-1.199	0.258	<i>Ywhaq</i>	-0.386	0.561

Table A.5

VCN 28 d Post-Exposure Fold Change and p Value re Sham Control

Gene	FC	p value	Gene	FC	p value	Gene	FC	p value
<i>Adam10</i>	-2.358	0.007	<i>Grin2a</i>	-2.744	0.037	<i>Nr4a1</i>	-2.616	0.074
<i>Adcy1</i>	-4.011	0.010	<i>Grin2b</i>	-3.097	0.048	<i>Ntf3</i>	0.233	0.780
<i>Adcy8</i>	-2.463	0.003	<i>Grin2c</i>	-2.364	0.009	<i>Ntf4</i>	0.149	0.789
<i>Akt1</i>	-2.897	0.010	<i>Grin2d</i>	-3.105	0.019	<i>Ntrk2</i>	-2.682	0.008
<i>Arc</i>	-3.636	0.017	<i>Grip1</i>	-2.841	0.008	<i>Pcdh8</i>	-2.512	0.019
<i>Bdnf</i>	-2.318	0.001	<i>Grm1</i>	-3.829	0.026	<i>Pick1</i>	-3.232	0.004
<i>Camk2a</i>	-1.457	0.100	<i>Grm2</i>	-3.078	0.025	<i>Pim1</i>	-1.559	0.044
<i>Camk2g</i>	-2.265	0.004	<i>Grm3</i>	-1.846	0.004	<i>Plat</i>	-1.755	0.010
<i>Cdh2</i>	-1.724	0.006	<i>Grm4</i>	-2.871	0.010	<i>Plcg1</i>	-2.582	0.011
<i>Cebpb</i>	-1.519	0.028	<i>Grm5</i>	-1.846	0.024	<i>Ppp1ca</i>	-3.111	0.006
<i>Cebpd</i>	-0.927	0.630	<i>Grm7</i>	-2.614	0.032	<i>Ppp1cc</i>	-3.375	0.003
<i>Cnr1</i>	-2.441	0.006	<i>Grm8</i>	-1.311	0.031	<i>Ppp1r14a</i>	-1.493	0.026
<i>Creb1</i>	-1.937	0.006	<i>Homer1</i>	-2.238	0.001	<i>Ppp2ca</i>	-2.643	0.007
<i>Crem</i>	-1.162	0.003	<i>Igf1</i>	0.289	0.404	<i>Ppp3ca</i>	-1.695	0.069
<i>Dlg4</i>	-3.944	0.005	<i>Inhba</i>	-2.623	0.031	<i>Prkca</i>	-2.640	0.008
<i>Egr1</i>	-2.392	0.008	<i>Jun</i>	-2.141	0.015	<i>Prkcg</i>	-2.426	0.126

<i>Egr2</i>	1.636	0.163	<i>Junb</i>	-2.686	0.011	<i>Prkg1</i>	-0.055	0.959
<i>Egr3</i>	-0.786	0.590	<i>Kif17</i>	-1.424	0.050	<i>Rab3a</i>	-3.022	0.013
<i>Egr4</i>	-1.394	0.096	<i>Klf10</i>	-2.129	0.003	<i>Rela</i>	-2.522	0.007
<i>Ephb2</i>	-1.993	0.011	<i>Mapk1</i>	-0.785	0.041	<i>Reln</i>	-2.179	0.041
<i>Fos</i>	-2.755	0.031	<i>Mmp9</i>	0.417	0.957	<i>Rgs2</i>	-1.954	0.012
<i>Gabra5</i>	-3.231	0.004	<i>Ncam1</i>	-2.590	0.009	<i>Rheb</i>	-1.225	0.027
<i>Gnai1</i>	-1.709	0.002	<i>Nfkb1</i>	-2.800	0.008	<i>Sirt1</i>	-2.538	0.010
<i>Gria1</i>	-1.713	0.125	<i>Nfkbib</i>	-0.537	0.458	<i>Srf</i>	-2.940	0.013
<i>Gria2</i>	-2.195	0.015	<i>Ngf</i>	-0.991	0.205	<i>Synpo</i>	-1.915	0.004
<i>Gria3</i>	-2.291	0.009	<i>Ngfr</i>	-1.800	0.303	<i>Timp1</i>	1.610	0.052
<i>Gria4</i>	-2.405	0.008	<i>Nos1</i>	-2.230	0.032	<i>Tnf</i>	-0.554	0.527
<i>Grin1</i>	-2.942	0.006	<i>Nptx2</i>	-2.029	0.079	<i>Ywhaq</i>	-2.299	0.006

Table A.6.

Significant changes in gene expression in VCN 28 d post-exposure. Genes organized by gene category; some genes in more than one category. Bottom row lists number of genes in each category

LTD (long-term depression)	LTP (long-term potentiation)	IEG (immediate early gene)	LRG (late response gene)	Cell Adhesion	Extracellular matrix & proteolytic	Creb Cofactors	Neuronal receptors	Postsynaptic density	Other
<i>Camk2g</i>	<i>Adcy1</i>	<i>Arc</i>	<i>Inhba</i>	<i>Adam10</i>	<i>Adam10</i>	<i>Akt1</i>	<i>Ephb2</i>	<i>Adam10</i>	<i>Sirt1</i>
<i>Gnai1</i>	<i>Adcy8</i>	<i>Bdnf</i>		<i>Cdh2</i>	<i>Plat</i>	<i>Camk2g</i>	<i>Gabra5</i>	<i>Arc</i>	
<i>Gria1</i>	<i>Bdnf</i>	<i>Cebpb</i>		<i>Grin2a</i>	<i>Reln</i>	<i>Grin1</i>	<i>Gria2</i>	<i>Dlg4</i>	
<i>Gria2</i>	<i>Camk2g</i>	<i>Creb1</i>		<i>Grin2b</i>		<i>Grin2a</i>	<i>Gria3</i>	<i>Gria3</i>	
<i>Gria3</i>	<i>Cdh2</i>	<i>Creb1</i>		<i>Ncam1</i>		<i>Grin2b</i>	<i>Gria4</i>	<i>Gria4</i>	
<i>Gria4</i>	<i>Cnr1</i>	<i>Egr1</i>		<i>Pcdh8</i>		<i>Grin2c</i>	<i>Grin1</i>	<i>Grin1</i>	
<i>Gria4</i>	<i>Gabra5</i>	<i>Fos</i>		<i>Ppp2ca</i>		<i>Grin2d</i>	<i>Grin2a</i>	<i>Grin2</i>	
<i>Grip1</i>	<i>Gnai1</i>	<i>Homer1</i>		<i>Reln</i>		<i>Mapk1</i>	<i>Grin2b</i>	<i>Grin2a</i>	
<i>Grm1</i>	<i>Gria2</i>	<i>Jun</i>				<i>Ppp1ca</i>	<i>Grin2c</i>	<i>Grin2b</i>	
<i>Grm2</i>	<i>Grin1</i>	<i>Junb</i>				<i>Ppp1cc</i>	<i>Grin2d</i>	<i>Grm1</i>	
<i>Homer1</i>	<i>Grin2a</i>	<i>Klf10</i>					<i>Grm1</i>	<i>Grm3</i>	
<i>Klf10</i>	<i>Grin2b</i>	<i>Nfkb1</i>					<i>Grm2</i>	<i>Homer1</i>	
<i>Mapk1</i>	<i>Grin2c</i>	<i>Pcdh8</i>					<i>Grm3</i>	<i>Pick1</i>	
<i>Ncam1</i>	<i>Grin2d</i>	<i>Pim1</i>					<i>Grm4</i>		
<i>Nos1</i>	<i>Mapk1</i>	<i>Plat</i>					<i>Grm5</i>		
<i>Ntrk2</i>	<i>Ntrk2</i>	<i>Rela</i>					<i>Grm7</i>		
<i>Pick1</i>	<i>Plcg1</i>	<i>Rgs2</i>					<i>Grm8</i>		
<i>Plat</i>	<i>Ppp1ca</i>	<i>Rheb</i>					<i>Ntrk2</i>		
<i>Ppp1ca</i>	<i>Ppp1cc</i>	<i>Srf</i>							
<i>Ppp1cc</i>	<i>Prkca</i>								
<i>Ppp1r14a</i>	<i>Rab3a</i>								

LTD (long-term depression)	LTP (long-term potentiation)	IEG (immediate early gene)	LRG (late response gene)	Cell Adhesion	Extracellular matrix & proteolytic	Creb Cofactors	Neuronal receptors	Postsynaptic density	Other
<i>Ppp2ca</i>	<i>Ywhaq</i>								
<i>Prkca</i>									
23	22	19	1	8	3	10	18	13	1

List of uncommon abbreviations

ABR	Auditory brain stem response
ANOVA	Analysis of variance
Ct values	Cycle threshold values (PCR)
dB	Decibels
DCN	Dorsal cochlear nucleus
FC	Fold change
GABA	Gamma-Aminobutyric Acid
Hz	Hertz
IEG	Immediate early gene
LRG	Late response gene
LTD	Long term depression
LTP	Long term potentiation
SEM	Standard error of the mean
SPL	Sound pressure level
VCN	Ventral cochlear nucleus

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Highlights NSC-18-1229

- 1.** Noise damage causes changes in synaptic plasticity genes in the cochlear nucleus
- 2.** More noise-induced changes occurred in the ventral vs. the dorsal cochlear nucleus
- 3.** Most changes in synaptic plasticity genes were seen 28 days after noise damage

Figure 1:

Fold change in expression of synaptic plasticity genes relative to actin housekeeping gene in DCN compared to VCN. Relative abundance of synaptic plasticity genes in the DCN was similar to the VCN as indicated by blue open circles within the 95% confidence interval for VCN. The thick dashed line shows the linear regression fit to the data and the shaded area represents the 95% confidence interval. Red open circles along with gene symbol identify genes that were more abundant in VCN than DCN (above 95% confidence interval) or less abundant in the VCN than DCN (below the 95% confidence interval).

Figure 2:

Mean ($\pm$ SEM, $n=6$) auditory brainstem response thresholds measured in the right ear of the sham control group and the right ear of the noise exposed group 28 d post-exposure. Results were analyzed by two-way ANOVA, frequency repeated measure, $F(1,30)=1211$, $p<0.0001$, Bonferroni post-hoc, $p<0.001$). Asterisks ($p<0.001$) indicate thresholds that were significantly different between the control group and noise-exposed group.

Figure 3:

Volcano plots showing the negative logarithm base 10 of the p-value (left ordinate) or p value (right ordinate) versus log 2 fold change in synaptic plasticity gene expression in the 2 d post-exposure group relative to the sham control group. Data shown for the DCN (A) and VCN (B). To be considered significant, the fold change had to be greater than 0.5 fold (i.e., 50% increase, red circles) or less than 0.5 (i.e., 50% decrease, blue circles) as indicated by the red or blue dashed vertical lines respectively. In the VCN, six genes, identified with their gene symbols, met the criteria for a significant increase whereas none of the genes in the DCN met the criteria for significant increase or decrease at 2 d post-exposure. See Appendix Table A.2 and Table A.3 for details.

Figure 4:

Volcano plots showing the negative logarithm base 10 of the p-value (left ordinate) or p value (right ordinate) versus log 2 fold change in synaptic plasticity gene expression in the 28 d post-exposure group relative to the sham control group. Data shown for the DCN (A) and VCN (B). To be considered significant, the fold change had to be greater than 0.5 fold (i.e., 50% increase, red circles) or less than 0.5 (i.e., 50% decrease, blue circles) as indicated by the red or blue dashed vertical lines respectively. In the DCN, two genes, identified with their gene symbols met the criteria for a significant increase while in the VCN, 66 genes met the criteria for a significant decrease at 28 d post-exposure. See Appendix Table A.4 and Table A.5 for details.

Figure 5.

Representative photomicrographs of sections from the posterior ventral cochlear nucleus (PVCN) showing (A) positive and (B) negative assay controls for the *in situ* hybridization kit. Note reddish/brown spots (arrows) of *in situ* mRNA probe in panel A and complete absence of reddish/brown dots in panel B. Sections counterstained with Gills hematoxylin. Scale bar shown in each panel.

Figure 6:

Representative photomicrographs of sections from the ventral portion of cochlear nucleus close to auditory nerve root entry showing reddish/brown puncta of *in situ* *Bdnf* mRNA probe (arrows). Sections counterstained with Gills hematoxylin. (A) Many reddish/brown puncta present around nuclei of sham control whereas few puncta were present on section from 28d post-exposure group (B) consistent with the noise-induced decrease in *Bdnf* gene expression (see Appendix Table A.4). Scale bar shown in each panel.

Figure 7:

Representative photomicrographs of sections from the middle layer of dorsal cochlear nucleus (DCN) showing reddish/brown puncta (arrows) of *in situ* *Grin1* mRNA probe. Sections counterstained with Gills hematoxylin. (A) Many reddish/brown puncta present around the nuclei of sham control and (B) 28d post-exposure group consistent with an absence of significant change in *Grin1* gene expression (see Appendix Table A.4). Scale bar shown in each panel.

Figure 8:

Representative photomicrographs of sections from the posterior ventral cochlear nucleus (PVCN) close to auditory nerve root entry showing reddish/brown puncta (arrows) of *in situ* *Homer1* mRNA probe. Sections counterstained with Gills hematoxylin. (A) Many reddish/brown puncta of *Homer1A* mRNA present around nuclei of sham controls whereas at 28 d post-exposure, fewer *Homer1A*-labeled puncta were present in the VCN, consistent with *Homer1* gene expression (see Appendix Table A.5). Scale bar shown in each panel.

Figure 9:

Representative photomicrographs of sections from the middle layer of the dorsal cochlear nucleus (DCN) showing reddish/brown puncta of in situ *Homer1* mRNA probe. Sections counterstained with Gills hematoxylin. (A) Many reddish/brown *Homer1* puncta present around nuclei of neurons in the DCN of sham controls and (B) nuclei of DCN neurons in the 28 d post-exposure group consistent with an absence of significant change in *Homer 1* gene expression. Scale bar shown in each panel.