Published in final edited form as:

Hear Res. 2016 April ; 334: 20–29. doi:10.1016/j.heares.2015.06.005.

Tinnitus: Maladaptive Auditory-Somatosensory Plasticity

Calvin Wu, Roxana A. Stefanescu, David T. Martel, and Susan E. Shore*

Department of Otolaryngology, Kresge Hearing Research Institute, University of Michigan, Ann Arbor, MI, USA

Abstract

Tinnitus, the phantom perception of sound, is physiologically characterized by an increase in spontaneous neural activity in the central auditory system. However, as tinnitus is often associated with hearing impairment, it is unclear how a decrease of afferent drive can result in central hyperactivity. In this review, we first assess methods for tinnitus induction and objective measures of the tinnitus percept in animal models. From animal studies, we discuss evidence that tinnitus originates in the cochlear nucleus (CN), and hypothesize mechanisms whereby hyperactivity may develop in the CN after peripheral auditory nerve damage. We elaborate how this process is likely mediated by plasticity of auditory-somatosensory integration in the CN: the circuitry in normal circumstances maintains a balance of auditory and somatosensory activities, and loss of auditory inputs alters the balance of auditory somatosensory integration in a stimulus timing dependent manner, which propels the circuit towards hyperactivity. Understanding the mechanisms underlying tinnitus generation is essential for its prevention and treatment.

1. Introduction

Tinnitus, the phantom perception of sound, affects 50 million adults in the U.S, with 12 million experiencing disruption of daily lives and suffering severe psychological stress (Rizzardo et al., 1998). Associative risks for tinnitus include intense noise exposure (Nicolas-Puel et al., 2006), ototoxic insults (Seligmann et al., 1996), head and neck injuries (Folmer and Griest, 2003), and age-related hearing impairment (Sataloff et al., 1987). Peripheral trauma causes partial deafferentation of the auditory nerve fibers (ANF), which reduces afferent drive to its central target, the cochlear nucleus (CN). However, in experimental animal models (*Section 2*), noise-trauma paradoxically induces elevated spontaneous activity in ventral (VCN) and dorsal cochlear nuclei (DCN) (Bledsoe et al., 2009; Brozoski et al., 2002; Dehmel et al., 2012; Koehler and Shore, 2013a; Vogler et al., 2011; Zhang and Kaltenbach, 1998).

A large body of evidence supports DCN as the site of tinnitus induction, where diminished auditory nerve input initiates hyperactivity, which then spreads to higher areas (Brozoski and

*Corresponding Author: Tel. 734 647-2116, Fax. 734 764-0014, Sushore@umich.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Bauer, 2005; Brozoski et al., 2012; Dehmel et al., 2012; Kaltenbach et al., 2005; Schaette and Kempster, 2006; Zacharek et al., 2002). How does a “loss-of-input” from the cochlea cause tinnitus? In this review, we show that the DCN is primed for such an induction process. The principle output neurons, fusiform cells, express a high degree of synaptic plasticity (*Section 4.4*), which largely involves integration of multisensory information (*Section 4.5–4.6*). Somatosensory inputs provide a reservoir of excitatory drive that can be tapped during homeostatic changes (*Section 4.3*). It is therefore not surprising that more than two thirds of tinnitus patients are able to modulate tinnitus perception by somatic maneuvers (Levine et al., 2003; Shore et al., 2007): an epitome of maladaptive auditory-somatosensory plasticity.

2. Animal model

Tinnitus can be induced through several methods. Noise overexposure is the major cause of tinnitus in humans and serves as a plausible method for generating an animal model (Eggermont and Roberts, 2004). Early studies of noise-induced tinnitus in humans revealed several consistent relationships between the characteristics of the noise exposure spectrum and the resulting tinnitus pitch and quality (Heffner and Heffner, 2012; Loeb and Smith, 1967). Tinnitus tends to present at frequencies up to 1.5 octaves higher than the exposure frequency (Konig et al., 2006; Roberts et al., 2008; Schaette and Kempster, 2009). Subjects in these studies tended to report tinnitus having a tone-like quality, similar to the exposure sound. Extended frequency audiograms on patients with tinnitus and hearing loss show a correlation between the frequency of the falling edge or a maximum threshold shift and the tinnitus frequency (Moore et al., 2010; Schecklmann et al., 2012). These results suggest that noise exposure that damages hearing, even temporarily (Kujawa and Liberman, 2009; Schaette and McAlpine, 2011), can induce tinnitus, and that the noise damaging spectra correlate with the tinnitus spectra. However, noise damage does not always result in tinnitus (Lockwood et al., 2002; Roberts et al., 2010). Similarly, manipulation of the facial or neck muscles can induce or alter tinnitus in individuals without a clinically measurable threshold shift (Levine et al., 2007).

2.1. Gap Detection Paradigm and Procedure for Assessing Tinnitus

To make claims about the neural mechanisms of tinnitus, animals must be determined to have tinnitus, preferably with an estimate of their tinnitus spectra. The first behavioral model of tinnitus relied on operant conditioning techniques that trained animals to discriminate between sound and no-sound (Jastreboff et al., 1988). Following salicylate administration, which is known to induce tinnitus in humans, animals with a decreased ability to detect a no-sound condition were classified with tinnitus. This technique, along with salicylate administration, have served as a “gold standard” for tinnitus assessment. As pioneering as this test was, it has several limitations. Training animals is a time-intensive process, and the behavioral outcomes of animals are dependent on additional factors such as learning and memory, which may contaminate the results. To overcome these limitations, the gap detection paradigm was developed (Turner et al., 2006). This method does not require training, and can be applied without timing constraints. The paradigm was derived from

procedures used in schizophrenia, and has been cross validated against several operant conditioning techniques (Turner et al., 2006; Yang et al., 2007).

Gap detection is based upon two behavioral responses (Fig. 1). First, animals startle in response to an intense, rapid onset sound. Second, when a gap in the narrow-band background noise is inserted shortly prior to the startle pulse, the amplitude of the startle response is reduced, as the salience of the gap “warns” of the impending startle pulse. To quantify this behavior for comparisons within and across animals over time, the gap startle amplitude is normalized by the no gap startle amplitude, to produce a normalized startle response. Similarly, a noise pre-pulse can be used as a replacement for the gap to produce similar results. The pre-pulse is presented at a lower sound level than the startle pulse, warning the animal of the impending startle pulse. Pre-pulse inhibition has been used to ascertain whether the animal has hearing or temporal processing impairments. These stimuli are presented across the animals hearing range to establish a baseline profile of the animal’s ability to process sounds. However, when a gap is introduced to animals with tinnitus that presents in the same frequency and intensity as the narrow-band background noise, they will startle as if there was no gap present. This frequency-specificity allows for a particular animal to be classified with tinnitus as well as estimates the perceived pitch of the tinnitus, as long as the PPI has been shown to be normal. Deficits in PPI could indicate a hearing loss at that frequency and thus, those animals would be excluded from the study (see below).

Limitations—The gap detection paradigm is an increasingly popular technique within the tinnitus research community. However, there are several perceived limitations to the technique (Campolo et al., 2013; Hickox and Liberman, 2014; Lobarinas et al., 2013). Noise exposure carries the potential confounding factor of permanent damage to the auditory pathway (Liberman and Dodds, 1984). This can occur even with a temporary threshold shift (Kujawa and Liberman, 2009). This suggests that gap detection and other pitch estimating forms of tinnitus assessment might be measuring hearing loss instead.

However, the gap detection procedure corrects for hearing loss through two experimental controls (Turner et al., 2006). One method is to include a frequency matched prepulse train in addition to the gap train. A prepulse contains the same temporal structure as a gap in noise, serving as an assessment of animal’s temporal hearing. Animals that fail to detect a noise prepulse are correspondingly removed from the study. Another is to unilaterally expose animals: this ensures that at least one ear is functional to process the stimulus.

The correlation between hearing loss edge and tinnitus spectrum suggests that exposed animals, following a transiently induced hearing loss edge, could present with tinnitus that has a spectrum similar to the noise exposure (Schaeffer and McAlpine, 2011). The gap detection paradigm requires the subject to process information within a frequency band. Frequency-specific gap impairments indicate the presence of tinnitus under the assumption that the tinnitus “fills the gap”. However, recent studies in humans question the mechanism of reduced gap detection in tinnitus patients (Hebert et al., 2013). Therefore, to validate impaired gap detection as an indication of tinnitus, we also measure elevation in spontaneous firing rate which is a physiological correlate of tinnitus, in the same animals, that receive gap detection testing (Section 3).

3. Hyperactivity and Tinnitus

3.1. Relationship to behavioral results

Frequency-specific hyperactivity as a result of noise trauma is a principal neural correlate of tinnitus (Brozoski et al., 2002; Kaltenbach and Afman, 2000; Kaltenbach and Godfrey, 2008; Kaltenbach et al., 2004; Middleton et al., 2011). Thus, behavioral tinnitus assessment techniques can be validated by examining the correlation between a noise exposure spectrum, tinnitus pitch estimation and hyperactivity at a corresponding best frequency of the neuron (Kaltenbach et al., 2004). A demonstration of this relationship utilizing the gap detection technique is demonstrated in Dehmel et al, 2012 (Fig. 2). A bandwidth limited, unilateral 97 dB SPL sound was applied to guinea pigs to induce a temporary threshold shift in the animal's hearing. This method of induction ensured that the resulting tinnitus was unilateral localized to a specific region within the tonotopic map of the DCN. The temporary nature of the threshold shift also ensured that the animals maintained otherwise normal hearing thresholds. This is demonstrated in Figure 2 by the overlapping frequency distributions of the temporary threshold shifts in auditory brainstem response (ABR) thresholds for the exposed ear, which closely match the spectrum of the tinnitus-inducing sound. Animals were tested for tinnitus using the gap detection paradigm, and a majority of animals presented with tinnitus at 8 kHz or higher. This is coincident with the peak ABR threshold shift, implying that noise damage responsible for tinnitus was located at that region. Finally, the spontaneous rates of fusiform cells had a distribution overlaying the exposure spectrum. This result ties together a behavioral prediction with electrophysiological data, validating the gap detection method against a physiological correlate of tinnitus (Dehmel et al., 2012; Koehler and Shore, 2013a).

3.2. Hyperactivity in IC and its dependence on DCN

Inferior colliculus (IC) hyperactivity is also an observed physiological correlate of tinnitus (Ma et al., 2006; Manzoor et al., 2013b; Manzoor et al., 2012; Mulders et al., 2010; Robertson et al., 2013). However, several lines of evidence show that IC cannot independently generate hyperactivity. First, DCN ablation before noise damage prevented hyperactivity and tinnitus (Brozoski et al., 2012), precluding the involvement of IC in tinnitus development. Second, DCN ablation after noise damage immediately and completely abolishes IC hyperactivity (Manzoor et al., 2012). However, it must be noted that this finding contradicts those of Brozoski and Bauer (2005), wherein tinnitus persists after DCN ablation, suggesting the possibility that higher structures are recruited in tinnitus maintenance. Nevertheless, it is unlikely that IC independently maintains hyperactivity, as reduction of peripheral inputs can abolish tinnitus-related hyperactivity in IC for up to 6 weeks post exposure (Mulders and Robertson, 2009), contrasting with DCN wherein established hyperactivity is not affected by subsequent elimination of efferent or afferent inputs 4–6 weeks post exposure (Zacharek et al., 2002; Zhang et al., 2006). Finally, the developmental time course and tonotopicity of hyperactivity in IC mimics that of DCN (Manzoor et al., 2013b). Together, these findings suggest that elevated spontaneous activity in DCN is transmitted to IC, and perhaps independently to the medial geniculate via direct projections (Anderson et al., 2006; Malmierca et al., 2002; Schofield et al., 2014), where tinnitus-related hyperactivity is also observed (Kalappa et al., 2014).

4. Multisensory mechanisms in CN and their contribution to tinnitus-related hyperactivity

4.1. Convergence of auditory and somatosensory inputs in CN and IC

The CN is the first site of sensory convergence in the auditory system. Tract tracing studies have demonstrated extensive projections originating from two separate somatosensory afferent pathways (see Table 1). The trigeminal pathway innervates the face, including oral structures, while the dorsal column pathway innervates the back of the head and pinnae as well as the neck and upper limbs. Axonal terminals in the CN are derived from both first-order (dorsal root ganglion) and trigeminal ganglion (TG)), and second-order projections (spinal trigeminal nucleus (Sp5)) and dorsal column nucleus (DCoN)). Input patterns from these somatosensory neurons are similar: the majority of anterogradely labeled axons are located in the granule cell domain (GCD; Table 1). The GCD is typically referred to as the area comprising the marginal area of VCN, the small cell cap, and interspersing with the fusiform cell layer (DCN2) of DCN (Fig. 3A: grey region) (Mugnaini et al., 1980; Weedman et al., 1996; Zhou and Shore, 2004). Somatosensory projections to the granule cells form mossy fibers (Weedman et al., 1996), whose synapses contain small, spherical vesicles, indicate excitatory, glutamatergic transmission (Shore et al., 2000; Wright and Ryugo, 1996).

Some mossy fiber inputs are found outside of the GCD. In rats and guinea pigs, projections from Sp5 and TG are also found in the magnocellular area of VCN (VCNm) (Haenggeli et al., 2005; Shore et al., 2000; Zhou and Shore, 2004), and likely innervate the dendrites of bushy cells (Gomez-Nieto and Rubio, 2011). Furthermore, somatosensory inputs to the molecular (DCN1) and deep layers (DCN3) of DCN are mostly en passant boutons different from granule cell synapses (Zhou and Shore, 2004), though the target cell types of these inputs are yet to be determined.

Convergence of auditory and somatosensory inputs also occurs in the midbrain. The external cortex of the IC receives contralateral Sp5 and DCoN inputs via axonal collaterals from their CN projections (Li and Mizuno, 1997). In anterograde tracing studies, Sp5 projections are found primarily in the ventral region IC, bordering central and external IC, where there is a significant overlap with auditory inputs from DCN (Zhou and Shore, 2006).

4.2. Differential synaptic markers for auditory and somatosensory terminals in CN

ANF inputs from the cochlea and somatosensory inputs from Sp5 and the cuneate nucleus express different isoforms of vesicular glutamate transporters (VGLUTs; Fig. 3B–C) (Zeng et al., 2011; Zhou et al., 2007). VGLUTs package glutamate for vesicular release, and are traditionally used to identify excitatory synapses. VGLUT1 is expressed heavily in the cochlear root, VCNm, and DCN1 (Fig. 3B), while VGLUT2 is expressed in the GCD, including DCN2 (Fig. 3C). The expression pattern correlates with the expected axonal terminal locations of ANF and Sp5 inputs. Double immuno-labeling of VGLUTs and anterograde tracers reveals significant co-localization: VGLUT1 co-labels with ANF (Fig. 3D) and not Sp5 terminals (Fig. 3E), while VGLUT2 co-labels with Sp5 (Fig. 3F) and not ANF terminals (Fig. 3G). Most Sp5–VGLUT2 co-labeling occurs on mossy fibers in GCD,

but on boutons in VCNm (Fig. 3H; also see Gomez-Nieto and Rubio (2011)). Almost no Sp5 terminals are co-labeled with VGLUT1.

4.3. Redistribution of VGLUT expression after hearing loss: evidence for cross-modal compensation

Synaptic transmission of auditory and somatosensory inputs in CN can be estimated by measuring their corresponding VGLUT1 and 2 expression levels. The balance of auditory and somatosensory inputs is disrupted by cochlear damage and ANF degeneration (Zeng et al., 2009; Zeng et al., 2012). As expected, VGLUT1 expression decreases dramatically in magnocellular AVCN and PVCN one and two weeks following an ipsilateral cochlear injection of neomycin (Fig. 3I). Some decreases are observed in DCN1, shell (GCD), and DCN3 after two weeks. With a slight time lag, VGLUT2 expression increases in all CN subdivisions, but primarily in the GCD and DCN2, two weeks after deafness. The complementary changes in expression of VGLUT1 and VGLUT2 correspond to the down- and up-regulation of co-labeled ANF and somatosensory terminals respectively, and this pattern of redistribution persists for at least 6 weeks after cochlear ablation (Zeng et al., 2012). These results suggest that somatosensory inputs compensate for ANF deafferentation (Fig. 3A, 3I), and the upregulation of excitatory neurotransmission from somatosensory projections likely contributes to a mechanism for resulting hyperactivity that is evident in tinnitus.

4.4. Synaptic plasticity of the DCN circuitry

In the DCN, the major output neurons, fusiform cells, integrate auditory information relayed by ANFs with somatosensory input relayed by the parallel fiber axons of the granule cells (Fig. 4). Early *in vitro* studies (Fujino and Oertel, 2003) describe robust plasticity only at the parallel fiber synapses on fusiform and cartwheel cells, which is mediated primarily by NMDA and metabotropic glutamate (groups 1, 2 and 3) receptors, and suggest that other factors may contribute as well.

In general, the presence of NMDA receptors at a synaptic site allows modifications of synaptic strength by means of spike-timing dependent plasticity (STDP). In fusiform and cartwheel cells, changes in synaptic strength depend on the order of the pre- and post-synaptic spiking within a critical window of tens of milliseconds. The systematic evaluation of these changes for different pre-post spiking time intervals leads to a “learning rule” which is classified as “Hebbian” or “anti-Hebbian”, when the changes in synaptic strength are consistent with the Hebbian theory of learning (Cooper, 2005; Hebb, 1949). Thus, in a Hebbian learning rule the synapse would be strengthened if the pre-synaptic spike is followed by a post-synaptic potential and weakened when the opposite order occurs. The anti-Hebbian learning rule would be characterized by opposite synaptic changes from the Hebbian learning rule.

In vitro studies investigating STDP in DCN (Tzounopoulos et al., 2004) demonstrated Hebbian learning rules in fusiform cells and anti-Hebbian learning rules cartwheel cells. While these changes are mediated mostly by the NMDA receptor, the endocannabinoid receptor CB1 was shown to control the LTD component in cartwheel cells (Tzounopoulos et

al., 2007). Additional evidence suggests that the differential expression of this receptor at the parallel fiber synapses on fusiform and cartwheel cells, and thus the selective engagement of the endocannabinoid system, may partially explain the difference in the types of learning rules observed in these cells (Tzounopoulos et al., 2007). Furthermore, M1/M3 muscarinic acetylcholine receptors (mAChR) were shown to interact with the endocannabinoid system to control the size and sign of the associative long-term synaptic plasticity in fusiform cells by converting the postsynaptic Hebbian long-term potentiation (LTP) to presynaptic anti-Hebbian long-term depression (LTD) (Zhao and Tzounopoulos, 2011). This conversion is also dependent on NMDA receptors and a rise in the postsynaptic Ca^{2+} concentration. Synaptic plasticity can also depend on the cell's degree of intrinsic excitability. This quality is usually controlled by potassium channels and is linked to the typical response the unit displays in response to standard tone and noise presentations (Kanold and Manis, 2001).

4.5. Somatosensory inputs induce persistent changes in CN activity

Plasticity of fusiform cell activity can be evoked *in vivo* by means of well-timed bimodal stimulation in which auditory stimuli are presented in temporal proximity to electrical stimulation of the trigeminal ganglion or spinal trigeminal nucleus (Sp5). For instance, bipolar electrical pulses delivered to the trigeminal ganglion preceding broadband noise bursts by as much as 95 ms were found to suppress or facilitate the sound-driven firing responses of 78% recorded fusiform cells in DCN (Shore, 2005). Similarly, responses to tones (Koehler et al., 2011) or rate-level functions (RLFs) (Dehmel et al., 2012) paired with electrical stimulation of the Sp5 nucleus lead to long-term changes in the tone-evoked firing activity of the fusiform cells. Interestingly, similar to the long-term changes in firing activity of fusiform cells, auditory-Sp5 bimodal stimulation induced persistent changes in the tone and RLF responses of primary-like cells in VCN (Fig. 5A and B) but not in the IC (Fig. 5C and D). The long-term effects correspond to the upregulation of somatosensory innervation in CN (Section 4.3), and likely underlie induction of hyperactivity and tinnitus (Dehmel et al., 2012). Indeed, as IC does not exhibit long-term bimodal plasticity, a probable induction mechanism (Fig. 5D), IC hyperactivity probably cannot occur independently of DCN hyperactivity (Section 3.2).

4.6. Auditory-somatosensory stimulus timing-dependent plasticity in fusiform cells

Stimulation of the ANFs and the somatosensory system at various bimodal intervals, i.e. the onset difference between the auditory and somatosensory stimulation, produces different effects on the tone-evoked firing activity of fusiform cells. Systematic evaluation of this change for positive and negative bimodal intervals (when the auditory stimulus precedes and follows the Sp5 stimulation, respectively) leads to stimulus-timing dependent learning rules in fusiform cells, the macromolecular correlate of STDP (Koehler and Shore, 2013a; Koehler and Shore, 2013b). While the predominant pattern remains Hebbian in healthy (Koehler and Shore, 2013a; Koehler and Shore, 2013b) or sham-exposed animals (Fig. 6A, gray curve; Fig. 6C, left distribution), other types of rules including enhancing, suppressive and anti-Hebbian timing rules can be found in the fusiform cell population *in vivo*. Interestingly, the stimulus-timing rules *in vivo* correlate with the amount of inhibitory inputs, as reflected in the fusiform cell frequency response maps. Thus, Hebbian-like learning rules are more likely to be found in units with Type I (no inhibition) than units with

Type III and IV response areas (significant inhibition off- best frequency or at higher intensities) (Koehler and Shore, 2013b). Additionally, it is likely that variation in the distribution of receptors (e.g. mAChR, CB1, NMDA) influences the type of timing rules observed.

The same bimodal stimulation can alter the spontaneous rates of the fusiform cells as well. Across the fusiform cell population in healthy or sham exposed animals, the learning rule for the spontaneous activity has a Hebbian profile, consistent with the tone-evoked learning rule (Fig. 6B, gray curve). While the mechanisms leading to these changes are not entirely understood, it is likely that STDP inducing stimulation mirrors changes in the degree of neural excitability of the pre- and post-synaptic neurons similar to those documented in hippocampus (Daoudal and Debanne, 2003; Debanne and Poo, 2010; Xu et al., 2005). One possible factor mediating simultaneous plasticity of the tone evoked responses and spontaneous activity of the fusiform cells could be the mAChRs. As discussed in Section 4.4 cholinergic activation changes the Hebbian LTP in anti-Hebbian LTD in fusiform cells. In addition, activating the mAChR by applying carbachol *in vivo* on the DCN surface leads to suppression or suppression accompanied by transient activation of the fusiform cell's spontaneous activity (Zhang and Kaltenbach, 2000).

4.7. Alteration of stimulus-timing dependent plasticity underlies fusiform cell hyperactivity

In sham-exposed animals, bimodal stimulation not only alters the spontaneous activity (Fig. 6B – gray curve; (Koehler and Shore, 2013a)) but also the gain and maximum amplitude of the fusiform cell's RLFs (Stefanescu et al., 2014), with learning rules that are very similar to the ones determined by the tone-evoked firing activity. How do these learning rules change in noise exposed and tinnitus animals? In contrast to sham-exposed animals, noise-exposed animals with and without tinnitus display predominantly anti-Hebbian and suppressive tone-evoked timing rules, respectively (Fig. 6C middle and right distributions; (Koehler and Shore, 2013a)). Anti-Hebbian bimodal enhancement is broader in animals with behaviorally-confirmed tinnitus (Fig. 6A, red curve), while noise-exposed animals without tinnitus show broader suppressive bimodal plasticity (Fig. 6A, pink curve). Similar changes occur for RLF maximum amplitude and gain in sham and noise- exposed animals with and without tinnitus (Stefanescu et al., 2014) strengthening the argument that tinnitus pathology is associated with changes in DCN plasticity mechanisms.

Of particular importance is the remarkable similarity between the timing rules of the spontaneous rates and the ones established by tone-evoked responses in sham and tinnitus animals (Fig. 6A and B). The consistency in the transition towards an anti-Hebbian profile in tinnitus animals strongly suggests that the changes in auditory-somatosensory mechanisms implicated in tinnitus are linked with changes in the spontaneous rates of the fusiform cells. Together with the spontaneous baseline hyperactivity reported in the fusiform cells for noise-exposed and tinnitus animals these findings suggests that cartwheel cells could be at least one potential contributors mediating these changes. Decreased activity of these inhibitory interneurons following anti-Hebbian LTD of their parallel fiber synapses (Tzounopoulos et al., 2004) could increase the spontaneous firing rates of the fusiform cells and assist in altering the learning rules from a Hebbian to an anti-Hebbian profile.

4.8. Molecular mechanisms of tinnitus-related changes in DCN plasticity

A number of factors could mediate the functional changes induced by noise exposure and tinnitus. Redistribution of the NMDA receptor subtype 2B (Marianowski et al., 2000; Sato et al., 2000) together with an increased number of glutamatergic somatosensory projections (Zeng et al., 2012) may change DCN stimulus-timing rules in noise exposed animals with and without tinnitus. A recent study in cerebellar slices, known for functional and structural similarities to DCN (Oertel and Young, 2004), suggests that under normal physiological conditions, NMDA receptors could inhibit the spontaneous activity of fusiform cells by enhancing the spontaneous activity of their inhibitory partners (Roberts et al., 2008). Thus, the redistribution of NMDA receptors following noise exposure and tinnitus (Dong et al., 2010), towards a predominantly dendritic localization (Forster and Illing, 1998), could alter both learning rules and spontaneous activities of fusiform cells. Furthermore, reduction in glycinergic (Suneja et al., 1998; Wang et al., 2009) and GABAergic (Middleton et al., 2011) inhibition following noise exposure and tinnitus could also alter the excitatory-inhibitory intrinsic balance of the DCN circuitry controlling sound evoked responses. Additional mechanisms mediating reduced inhibition of the fusiform cells activity including plasticity-induced suppression of cartwheel cell activity (Tzounopoulos et al., 2004) could be contributing factors.

As discussed in Section 4.4, cholinergic input can mediate conversion of the learning rules of fusiform cells from Hebbian to anti-Hebbian (Zhao and Tzounopoulos, 2011). Up-regulation of cholinergic input following noise exposure has been demonstrated in various regions of the cochlear nucleus including the fusiform layer of DCN (Jin et al., 2006). Under normal physiological conditions, pharmacological application of the cholinergic agonist, carbachol showed diverse effects on fusiform cell spontaneous activity, including suppression, activation or a combination of both (Zhang and Kaltenbach, 2000) suggesting a complex interaction between various cholinergic modulatory factors influencing fusiform cell excitability. Interestingly, carbachol could also suppress DCN noise-induced hyperactivity (Manzoor et al., 2013a; Zhang and Kaltenbach, 2000), suggesting a role of the inhibitory circuitry in mediating the effects of acetylcholine on fusiform cell spontaneous activity.

Factors that control primarily the intrinsic excitability of fusiform cells may also be altered by noise exposure and tinnitus and could contribute to changes in the STDP induced learning rules. For instance, tinnitus-specific hyperactivity of fusiform cells has been linked with a reduction in Kv7.2/3 activity (Li et al., 2013). Moreover, following acoustic overexposure, fusiform cells that showed hyperactivity also exhibited a change in the firing pattern from regular spiking to bursting activity, a change that was mediated by a down regulation of high voltage activated potassium channels (Pilati et al., 2012). Because induction of LTP/LTD may depend on the cell's intrinsic excitability (Felix-Oliveira et al., 2014), it is possible that such changes could contribute to the observed alterations in STDP plasticity following noise exposure and tinnitus.

Finally, it is worth noting that the CN receives serotonergic inputs from the dorsal raphe nucleus (Thompson and Thompson, 2001), which are activated during salicylate-induced tinnitus (Caperton and Thompson, 2011). Thus, serotonergic neuromodulation could also be

a potential factor influencing the changes in stimulus-timing rules of the fusiform cells of tinnitus animals.

5. Concluding Remarks

Currently, tinnitus remains a debilitating public health problem without an effective treatment. Understanding the pathophysiology of tinnitus is essential but challenging, as animal studies are limited by the disease's subjective nature. To overcome this, the gap detection paradigm was established as an objective measure of animal's tinnitus percept. Together with a consistent induction method – namely, frequency-specific noise exposure that produces a temporary threshold shift, animal models can be physiologically tested for neural signatures of hyperactivity. In this review, we discussed mechanisms of auditory-somatosensory plasticity, that is, persistent, cross-modal changes, and alterations in bimodal plasticity, which likely underlie induction of hyperactivity in the CN. However, other mechanisms, such as changes in intrinsic excitability (Li et al., 2013), inhibition (Potashner et al., 2000), or contributions from other neuromodulators (Kaltenbach, 2006), may also influence hyperactivity. The interplay of these factors with auditory-somatosensory plasticity raises interesting questions for future research.

Acknowledgments

This work was supported by National Institutes of Health Grants R01 DC004825 and P30 DC05188, and the Tinnitus Research Consortium. We thank Amarins Heeringa for her helpful comments on the manuscript.

Abbreviations

ABR	Auditory brainstem response
CN	Cochlear nucleus
DCN	Dorsal cochlear nucleus
VCN	Ventral cochlear nucleus
DCoN	Dorsal column nuclei
GCD	Granule cell domain
IC	Inferior colliculus
LTP/LTD	Long-term potentiation/depression
RLF	Rate-level functions
Sp5	Spinal trigeminal nucleus
TG	Trigeminal ganglion
VGLUT	Vesicular glutamate transporters

References

- Anderson LA, Malmierca MS, Wallace MN, Palmer AR. Evidence for a direct, short latency projection from the dorsal cochlear nucleus to the auditory thalamus in the guinea pig. *Eur J Neurosci.* 2006; 24:491–8. [PubMed: 16836634]
- Bledsoe SC Jr, Koehler S, Tucci DL, Zhou J, Le Prell C, Shore SE. Ventral cochlear nucleus responses to contralateral sound are mediated by commissural and olivocochlear pathways. *Journal of neurophysiology.* 2009; 102:886–900. [PubMed: 19458143]
- Brozoski TJ, Bauer CA. The effect of dorsal cochlear nucleus ablation on tinnitus in rats. *Hear Res.* 2005; 206:227–36. [PubMed: 16081010]
- Brozoski TJ, Bauer CA, Caspary DM. Elevated fusiform cell activity in the dorsal cochlear nucleus of chinchillas with psychophysical evidence of tinnitus. *J Neurosci.* 2002; 22:2383–90. [PubMed: 11896177]
- Brozoski TJ, Wisner KW, Sybert LT, Bauer CA. Bilateral dorsal cochlear nucleus lesions prevent acoustic-trauma induced tinnitus in an animal model. *J Assoc Res Otolaryngol.* 2012; 13:55–66. [PubMed: 21969021]
- Campolo J, Lobarinas E, Salvi R. Does tinnitus “fill in” the silent gaps? *Noise & health.* 2013; 15:398–405. [PubMed: 24231418]
- Caperton KK, Thompson AM. Activation of serotonergic neurons during salicylate-induced tinnitus. *Otology & neurotology: official publication of the American Otological Society, American Neurotology Society [and] European Academy of Otology and Neurotology.* 2011; 32:301–7.
- Cooper SJ, Donald O. Hebb’s synapse and learning rule: a history and commentary. *Neuroscience and biobehavioral reviews.* 2005; 28:851–74. [PubMed: 15642626]
- Daoudal G, Debanne D. Long-term plasticity of intrinsic excitability: learning rules and mechanisms. *Learning & memory (Cold Spring Harbor, NY).* 2003; 10:456–65.
- Debanne D, Poo MM. Spike-timing dependent plasticity beyond synapse – pre- and post-synaptic plasticity of intrinsic neuronal excitability. *Frontiers in synaptic neuroscience.* 2010; 2:21. [PubMed: 21423507]
- Dehmel S, Pradhan S, Koehler S, Bledsoe S, Shore S. Noise overexposure alters long-term somatosensory-auditory processing in the dorsal cochlear nucleus—possible basis for tinnitus-related hyperactivity? *J Neurosci.* 2012; 32:1660–71. [PubMed: 22302808]
- Dong S, Mulders WH, Rodger J, Woo S, Robertson D. Acoustic trauma evokes hyperactivity and changes in gene expression in guinea-pig auditory brainstem. *The European journal of neuroscience.* 2010; 31:1616–28. [PubMed: 20525074]
- Eggermont JJ, Roberts LE. The neuroscience of tinnitus. *Trends in neurosciences.* 2004; 27:676–82. [PubMed: 15474168]
- Felix-Oliveira A, Dias RB, Colino-Oliveira M, Rombo DM, Sebastiao AM. Homeostatic plasticity induced by brief activity deprivation enhances long-term potentiation in the mature rat hippocampus. *Journal of neurophysiology.* 2014; 112:3012–22. [PubMed: 25210161]
- Folmer RL, Griest SE. Chronic tinnitus resulting from head or neck injuries. *The Laryngoscope.* 2003; 113:821–7. [PubMed: 12792317]
- Forster CR, Illing RB. Redistribution of NMDA receptors in the cochlear nucleus following cochleotomy. *Neuroreport.* 1998; 9:3531–5. [PubMed: 9855312]
- Fujino K, Oertel D. Bidirectional synaptic plasticity in the cerebellum-like mammalian dorsal cochlear nucleus. *Proc Natl Acad Sci U S A.* 2003; 100:265–70. [PubMed: 12486245]
- Gomez-Nieto R, Rubio ME. Ultrastructure, synaptic organization, and molecular components of bushy cell networks in the anteroventral cochlear nucleus of the rhesus monkey. *Neuroscience.* 2011; 179:188–207. [PubMed: 21284951]
- Haenggeli CA, Pongstaporn T, Doucet JR, Ryugo DK. Projections from the spinal trigeminal nucleus to the cochlear nucleus in the rat. *J Comp Neurol.* 2005; 484:191–205. [PubMed: 15736230]
- Hebb, DO. *The Organization of Behavior.* New York: Wiley&Sons; 1949.
- Hebert S, Fournier P, Norena A. The auditory sensitivity is increased in tinnitus ears. *J Neurosci.* 2013; 33:2356–64. [PubMed: 23392665]

- Heffner, H.; Heffner, R. Behavioral Tests for Tinnitus in Animals. In: Eggermont, JJ.; Zeng, F-G.; Popper, AN.; Fay, RR., editors. Tinnitus. Vol. 44. Springer; New York: 2012. p. 21-58.
- Hickox AE, Liberman MC. Is noise-induced cochlear neuropathy key to the generation of hyperacusis or tinnitus? *Journal of neurophysiology*. 2014; 111:552–64. [PubMed: 24198321]
- Itoh K, Kamiya H, Mitani A, Yasui Y, Takada M, Mizuno N. Direct projections from the dorsal column nuclei and the spinal trigeminal nuclei to the cochlear nuclei in the cat. *Brain Res*. 1987; 400:145–50. [PubMed: 2434184]
- Jastreboff PJ, Brennan JF, Coleman JK, Sasaki CT. Phantom auditory sensation in rats: an animal model for tinnitus. *Behavioral neuroscience*. 1988; 102:811–22. [PubMed: 3214530]
- Jin YM, Godfrey DA, Wang J, Kaltenbach JA. Effects of intense tone exposure on choline acetyltransferase activity in the hamster cochlear nucleus. *Hearing research*. 2006:216–217. [PubMed: 16597491]
- Kalappa BI, Brozoski TJ, Turner JG, Caspary DM. Single unit hyperactivity and bursting in the auditory thalamus of awake rats directly correlates with behavioural evidence of tinnitus. *J Physiol*. 2014; 592:5065–78. [PubMed: 25217380]
- Kaltenbach JA. The dorsal cochlear nucleus as a participant in the auditory, attentional and emotional components of tinnitus. *Hearing research*. 2006:216–217. [PubMed: 16597491]
- Kaltenbach JA, Afman CE. Hyperactivity in the dorsal cochlear nucleus after intense sound exposure and its resemblance to tone-evoked activity: a physiological model for tinnitus. *Hearing research*. 2000; 140:165–72. [PubMed: 10675644]
- Kaltenbach JA, Godfrey DA. Dorsal cochlear nucleus hyperactivity and tinnitus: are they related? *American journal of audiology*. 2008; 17:S148–61. [PubMed: 18978198]
- Kaltenbach JA, Zhang J, Finlayson P. Tinnitus as a plastic phenomenon and its possible neural underpinnings in the dorsal cochlear nucleus. *Hear Res*. 2005; 206:200–26. [PubMed: 16081009]
- Kaltenbach JA, Zacharek MA, Zhang J, Frederick S. Activity in the dorsal cochlear nucleus of hamsters previously tested for tinnitus following intense tone exposure. *Neurosci Lett*. 2004; 355:121–5. [PubMed: 14729250]
- Kanold PO, Manis PB. A physiologically based model of discharge pattern regulation by transient K⁺ currents in cochlear nucleus pyramidal cells. *Journal of neurophysiology*. 2001; 85:523–38. [PubMed: 11160490]
- Koehler SD, Shore SE. Stimulus timing-dependent plasticity in dorsal cochlear nucleus is altered in tinnitus. *J Neurosci*. 2013a; 33:19647–56. [PubMed: 24336728]
- Koehler SD, Shore SE. Stimulus-timing dependent multisensory plasticity in the guinea pig dorsal cochlear nucleus. *PLoS One*. 2013b; 8:e59828. [PubMed: 23527274]
- Koehler SD, Pradhan S, Manis PB, Shore SE. Somatosensory inputs modify auditory spike timing in dorsal cochlear nucleus principal cells. *Eur J Neurosci*. 2011; 33:409–20. [PubMed: 21198989]
- Konig O, Schaette R, Kempter R, Gross M. Course of hearing loss and occurrence of tinnitus. *Hearing research*. 2006; 221:59–64. [PubMed: 16962270]
- Kujawa SG, Liberman MC. Adding insult to injury: cochlear nerve degeneration after “temporary” noise-induced hearing loss. *J Neurosci*. 2009; 29:14077–85. [PubMed: 19906956]
- Levine RA, Abel M, Cheng H. CNS somatosensory-auditory interactions elicit or modulate tinnitus. *Experimental brain research. Experimentelle Hirnforschung. Experimentation cerebrale*. 2003; 153:643–8. [PubMed: 14600798]
- Levine RA, Nam EC, Oron Y, Melcher JR. Evidence for a tinnitus subgroup responsive to somatosensory based treatment modalities. *Progress in brain research*. 2007; 166:195–207. [PubMed: 17956783]
- Li H, Mizuno N. Single neurons in the spinal trigeminal and dorsal column nuclei project to both the cochlear nucleus and the inferior colliculus by way of axon collaterals: a fluorescent retrograde double-labeling study in the rat. *Neuroscience research*. 1997; 29:135–42. [PubMed: 9359462]
- Li S, Choi V, Tzounopoulos T. Pathogenic plasticity of Kv7.2/3 channel activity is essential for the induction of tinnitus. *Proc Natl Acad Sci U S A*. 2013; 110:9980–5. [PubMed: 23716673]
- Liberman MC, Dodds LW. Single-neuron labeling and chronic cochlear pathology. II. Stereocilia damage and alterations of spontaneous discharge rates. *Hearing research*. 1984; 16:43–53. [PubMed: 6511672]

- Lobarinas E, Hayes SH, Allman BL. The gap-startle paradigm for tinnitus screening in animal models: limitations and optimization. *Hearing research*. 2013; 295:150–60. [PubMed: 22728305]
- Lockwood AH, Salvi RJ, Burkard RF. Tinnitus. *The New England journal of medicine*. 2002; 347:904–10. [PubMed: 12239260]
- Loeb M, Smith RP. Relation of induced tinnitus to physical characteristics of the inducing stimuli. *The Journal of the Acoustical Society of America*. 1967; 42:453–5. [PubMed: 6075938]
- Ma WL, Hidaka H, May BJ. Spontaneous activity in the inferior colliculus of CBA/J mice after manipulations that induce tinnitus. *Hear Res*. 2006; 212:9–21. [PubMed: 16307852]
- Malmierca MS, Merchan MA, Henkel CK, Oliver DL. Direct projections from cochlear nuclear complex to auditory thalamus in the rat. *The Journal of neuroscience: the official journal of the Society for Neuroscience*. 2002; 22:10891–7. [PubMed: 12486183]
- Manzoor NF, Chen G, Kaltenbach JA. Suppression of noise-induced hyperactivity in the dorsal cochlear nucleus following application of the cholinergic agonist, carbachol. *Brain Res*. 2013a; 1523:28–36. [PubMed: 23721928]
- Manzoor NF, Gao Y, Licari F, Kaltenbach JA. Comparison and contrast of noise-induced hyperactivity in the dorsal cochlear nucleus and inferior colliculus. *Hear Res*. 2013b; 295:114–23. [PubMed: 22521905]
- Manzoor NF, Licari FG, Klapchar M, Elkin RL, Gao Y, Chen G, Kaltenbach JA. Noise-induced hyperactivity in the inferior colliculus: its relationship with hyperactivity in the dorsal cochlear nucleus. *J Neurophysiol*. 2012; 108:976–88. [PubMed: 22552192]
- Marianowski R, Liao WH, Van Den Abbeele T, Fillit P, Herman P, Frachet B, Huy PT. Expression of NMDA, AMPA and GABA(A) receptor subunit mRNAs in the rat auditory brainstem. I. Influence of early auditory deprivation. *Hearing research*. 2000; 150:1–11. [PubMed: 11077189]
- Middleton JW, Kiritani T, Pedersen C, Turner JG, Shepherd GM, Tzounopoulos T. Mice with behavioral evidence of tinnitus exhibit dorsal cochlear nucleus hyperactivity because of decreased GABAergic inhibition. *Proc Natl Acad Sci U S A*. 2011; 108:7601–6. [PubMed: 21502491]
- Moore BC, Vinay Sandhya. The relationship between tinnitus pitch and the edge frequency of the audiogram in individuals with hearing impairment and tonal tinnitus. *Hearing research*. 2010; 261:51–6. [PubMed: 20103482]
- Mugnaini E, Osen KK, Dahl AL, Friedrich VL Jr, Korte G. Fine structure of granule cells and related interneurons (termed Golgi cells) in the cochlear nuclear complex of cat, rat and mouse. *J Neurocytol*. 1980; 9:537–70. [PubMed: 7441303]
- Mulders WH, Robertson D. Hyperactivity in the auditory midbrain after acoustic trauma: dependence on cochlear activity. *Neuroscience*. 2009; 164:733–46. [PubMed: 19699277]
- Mulders WH, Seluakumaran K, Robertson D. Efferent pathways modulate hyperactivity in inferior colliculus. *J Neurosci*. 2010; 30:9578–87. [PubMed: 20631186]
- Nicolas-Puel C, Akbaraly T, Lloyd R, Berr C, Uziel A, Rebillard G, Puel JL. Characteristics of tinnitus in a population of 555 patients: specificities of tinnitus induced by noise trauma. *The international tinnitus journal*. 2006; 12:64–70. [PubMed: 17147043]
- Oertel D, Young ED. What's a cerebellar circuit doing in the auditory system? *Trends Neurosci*. 2004; 27:104–10. [PubMed: 15102490]
- Pilati N, Large C, Forsythe ID, Hamann M. Acoustic over-exposure triggers burst firing in dorsal cochlear nucleus fusiform cells. *Hear Res*. 2012; 283:98–106. [PubMed: 22085487]
- Potashner SJ, Suneja SK, Benson CG. Altered glycinergic synaptic activities in guinea pig brain stem auditory nuclei after unilateral cochlear ablation. *Hear Res*. 2000; 147:125–36. [PubMed: 10962179]
- Rizzardo R, Savastano M, Maron MB, Mangialaio M, Salvadori L. Psychological distress in patients with tinnitus. *The Journal of otolaryngology*. 1998; 27:21–5. [PubMed: 9511115]
- Roberts LE, Moffat G, Baumann M, Ward LM, Bosnyak DJ. Residual inhibition functions overlap tinnitus spectra and the region of auditory threshold shift. *J Assoc Res Otolaryngol*. 2008; 9:417–35. [PubMed: 18712566]
- Roberts LE, Eggermont JJ, Caspary DM, Shore SE, Melcher JR, Kaltenbach JA. Ringing ears: the neuroscience of tinnitus. *J Neurosci*. 2010; 30:14972–9. [PubMed: 21068300]

- Robertson D, Bester C, Vogler D, Mulders WH. Spontaneous hyperactivity in the auditory midbrain: relationship to afferent input. *Hear Res.* 2013; 295:124–9. [PubMed: 22349094]
- Sataloff J, Sataloff RT, Lueneburg W. Tinnitus and vertigo in healthy senior citizens without a history of noise exposure. *The American journal of otology.* 1987; 8:87–9. [PubMed: 3591925]
- Sato K, Shiraishi S, Nakagawa H, Kuriyama H, Altschuler RA. Diversity and plasticity in amino acid receptor subunits in the rat auditory brain stem. *Hearing research.* 2000; 147:137–44. [PubMed: 10962180]
- Schaette R, Kempster R. Development of tinnitus-related neuronal hyperactivity through homeostatic plasticity after hearing loss: a computational model. *Eur J Neurosci.* 2006; 23:3124–38. [PubMed: 16820003]
- Schaette R, Kempster R. Predicting tinnitus pitch from patients' audiograms with a computational model for the development of neuronal hyperactivity. *Journal of neurophysiology.* 2009; 101:3042–52. [PubMed: 19357344]
- Schaette R, McAlpine D. Tinnitus with a normal audiogram: physiological evidence for hidden hearing loss and computational model. *J Neurosci.* 2011; 31:13452–7. [PubMed: 21940438]
- Schecklmann M, Vielsmeier V, Steffens T, Landgrebe M, Langguth B, Kleinjung T. Relationship between Audiometric slope and tinnitus pitch in tinnitus patients: insights into the mechanisms of tinnitus generation. *PloS one.* 2012; 7:e34878. [PubMed: 22529949]
- Schofield BR, Motts SD, Mellott JG, Foster NL. Projections from the dorsal and ventral cochlear nuclei to the medial geniculate body. *Front Neuroanat.* 2014; 8:10. [PubMed: 24634646]
- Seligmann H, Podoshin L, Ben-David J, Fradis M, Goldsher M. Drug-induced tinnitus and other hearing disorders. *Drug safety: an international journal of medical toxicology and drug experience.* 1996; 14:198–212.
- Shore S, Zhou J, Koehler S. Neural mechanisms underlying somatic tinnitus. *Progress in brain research.* 2007; 166:107–23. [PubMed: 17956776]
- Shore SE. Multisensory integration in the dorsal cochlear nucleus: unit responses to acoustic and trigeminal ganglion stimulation. *Eur J Neurosci.* 2005; 21:3334–48. [PubMed: 16026471]
- Shore SE, Vass Z, Wys NL, Altschuler RA. Trigeminal ganglion innervates the auditory brainstem. *J Comp Neurol.* 2000; 419:271–85. [PubMed: 10723004]
- Stefanescu RA, Koehler SD, Shore SE. Stimulus-timing dependent modifications of rate-level functions in animals with and without tinnitus. *J Neurophysiol.* 2015 Feb 1; 113(3):956–70. [PubMed: 25392166]
- Suneja SK, Benson CG, Potashner SJ. Glycine receptors in adult guinea pig brain stem auditory nuclei: regulation after unilateral cochlear ablation. *Experimental neurology.* 1998; 154:473–88. [PubMed: 9878183]
- Thompson AM, Thompson GC. Serotonin projection patterns to the cochlear nucleus. *Brain research.* 2001; 907:195–207. [PubMed: 11430903]
- Turner JG, Brozoski TJ, Bauer CA, Parrish JL, Myers K, Hughes LF, Caspary DM. Gap detection deficits in rats with tinnitus: a potential novel screening tool. *Behav Neurosci.* 2006; 120:188–95. [PubMed: 16492129]
- Tzounopoulos T, Kim Y, Oertel D, Trussell LO. Cell-specific, spike timing-dependent plasticities in the dorsal cochlear nucleus. *Nat Neurosci.* 2004; 7:719–25. [PubMed: 15208632]
- Tzounopoulos T, Rubio ME, Keen JE, Trussell LO. Coactivation of pre- and postsynaptic signaling mechanisms determines cell-specific spike-timing-dependent plasticity. *Neuron.* 2007; 54:291–301. [PubMed: 17442249]
- Vogler DP, Robertson D, Mulders WH. Hyperactivity in the ventral cochlear nucleus after cochlear trauma. *J Neurosci.* 2011; 31:6639–45. [PubMed: 21543592]
- Wang H, Brozoski TJ, Turner JG, Ling L, Parrish JL, Hughes LF, Caspary DM. Plasticity at glycinergic synapses in dorsal cochlear nucleus of rats with behavioral evidence of tinnitus. *Neuroscience.* 2009; 164:747–59. [PubMed: 19699270]
- Weedman DL, Pongstaporn T, Ryugo DK. Ultrastructural study of the granule cell domain of the cochlear nucleus in rats: mossy fiber endings and their targets. *The Journal of comparative neurology.* 1996; 369:345–60. [PubMed: 8743417]

- Wright DD, Ryugo DK. Mossy fiber projections from the cuneate nucleus to the cochlear nucleus in the rat. *J Comp Neurol*. 1996; 365:159–72. [PubMed: 8821448]
- Xu J, Kang N, Jiang L, Nedergaard M, Kang J. Activity-dependent long-term potentiation of intrinsic excitability in hippocampal CA1 pyramidal neurons. *The Journal of neuroscience: the official journal of the Society for Neuroscience*. 2005; 25:1750–60. [PubMed: 15716411]
- Yang G, Lobarinas E, Zhang L, Turner J, Stolzberg D, Salvi R, Sun W. Salicylate induced tinnitus: behavioral measures and neural activity in auditory cortex of awake rats. *Hearing research*. 2007; 226:244–53. [PubMed: 16904853]
- Zacharek MA, Kaltenbach JA, Mathog TA, Zhang J. Effects of cochlear ablation on noise induced hyperactivity in the hamster dorsal cochlear nucleus: implications for the origin of noise induced tinnitus. *Hear Res*. 2002; 172:137–43. [PubMed: 12361876]
- Zeng C, Shroff H, Shore SE. Cuneate and spinal trigeminal nucleus projections to the cochlear nucleus are differentially associated with vesicular glutamate transporter-2. *Neuroscience*. 2011; 176:142–51. [PubMed: 21167260]
- Zeng C, Nannapaneni N, Zhou J, Hughes LF, Shore S. Cochlear damage changes the distribution of vesicular glutamate transporters associated with auditory and nonauditory inputs to the cochlear nucleus. *J Neurosci*. 2009; 29:4210–7. [PubMed: 19339615]
- Zeng C, Yang Z, Shreve L, Bledsoe S, Shore S. Somatosensory projections to cochlear nucleus are upregulated after unilateral deafness. *J Neurosci*. 2012; 32:15791–801. [PubMed: 23136418]
- Zhan X, Pongstaporn T, Ryugo DK. Projections of the second cervical dorsal root ganglion to the cochlear nucleus in rats. *J Comp Neurol*. 2006; 496:335–48. [PubMed: 16566003]
- Zhang JS, Kaltenbach JA. Increases in spontaneous activity in the dorsal cochlear nucleus of the rat following exposure to high-intensity sound. *Neurosci Lett*. 1998; 250:197–200. [PubMed: 9708866]
- Zhang JS, Kaltenbach JA. Modulation of spontaneous activity by acetylcholine receptors in the rat dorsal cochlear nucleus in vivo. *Hearing research*. 2000; 140:7–17. [PubMed: 10675631]
- Zhang JS, Kaltenbach JA, Godfrey DA, Wang J. Origin of hyperactivity in the hamster dorsal cochlear nucleus following intense sound exposure. *J Neurosci Res*. 2006; 84:819–31. [PubMed: 16862546]
- Zhao Y, Tzounopoulos T. Physiological activation of cholinergic inputs controls associative synaptic plasticity via modulation of endocannabinoid signaling. *J Neurosci*. 2011; 31:3158–68. [PubMed: 21368027]
- Zhou J, Shore S. Projections from the trigeminal nuclear complex to the cochlear nuclei: a retrograde and anterograde tracing study in the guinea pig. *J Neurosci Res*. 2004; 78:901–7. [PubMed: 15495211]
- Zhou J, Shore S. Convergence of spinal trigeminal and cochlear nucleus projections in the inferior colliculus of the guinea pig. *J Comp Neurol*. 2006; 495:100–12. [PubMed: 16432905]
- Zhou J, Nannapaneni N, Shore S. Vesicular glutamate transporters 1 and 2 are differentially associated with auditory nerve and spinal trigeminal inputs to the cochlear nucleus. *J Comp Neurol*. 2007; 500:777–87. [PubMed: 17154258]

Highlights

Gap detection assessments of tinnitus are supported by spontaneous rates

Tinnitus originates in the cochlear nucleus

Up-regulation of somatosensory inputs contributes to tinnitus-related hyperactivity

Auditory-somatosensory plasticity contributes to tinnitus generation.

Fig. 1. Gap pre-pulse inhibition of acoustic startle measures tinnitus

Animals respond with a large startle in response to an unexpected noise pulse, the magnitude of which diminishes when a gap (or prepulse; not shown) precedes the startle. Frequency-matched tinnitus will inhibit the salience of the gap (but not the prepulse), as indicated by increases in normalized startle amplitudes in exposed animals with tinnitus (Exp-Tinn, tinnitus shown by star) compared to exposed, no tinnitus animals (Exp-No Tinn) or sham exposed animals (Sham). Figure adapted from Koehler and Shore (2013a).

Fig. 2. Gap detection predicts tinnitus-related hyperactivity

The noise-exposure spectrum (grey) and spontaneous rate distribution across DCN unit best frequencies (red trace) overlap. Maximum temporary threshold shift for left ear (black filled circles) is slightly higher than the peak intensity frequency and recovers after one week (white filled circles). Tinnitus assessed by gap deficits, occurs at the spectrum center frequency and up to 2 octaves higher (black box). Right (non-exposed) ear ABRs were normal (not shown). From Dehmel et al. (2012).

Fig. 3. Vesicular glutamate transporter (VGLUT) isoforms 1 and 2 are differentially expressed in auditory and somatosensory terminals in CN, and undergo redistribution following auditory deafferentation

(A–C) VGLUT1 and 2 immuno-reactivity is shown by white dots on CN images (image width: 2 mm). The schematic diagram in (A) shows the CN regions. Grey: GCD. (D) Co-localization (yellow) of VGLUT1 (green) and anterogradely labeled auditory nerve fibers (ANF; red) in VCNm. Image width: 50 μ m. (E) Sp5 terminals and VGLUT1 are not co-localized in VCNm. (F) Co-localization of VGLUT2 and anterogradely labeled Sp5 terminals in GCD. (G) ANF terminals and VGLUT2 are not co-localized. (H) Proportions of Sp5 terminal endings labeled with VGLUT1 or VGLUT2 in GCD or VCNm. M: mossy fiber endings; B: bouton endings; T: total co-labeled endings. (I) Changes (normalized ratios) of VGLUT1 and 2 puncta density in each CN subdivision 1 and 2 weeks following cochlear ablation. DCN1, SCC (small cell cap: adjacent to GCD), DCN2 are regions receiving non-auditory projections and where VGLUT2 predominates, while DCN3, AVCN, and PVCN are regions receiving ANF inputs with predominantly VGLUT1 expression. Redistribution

of VGLUTs shows similar patterns across all subdivision. As a result of cochlear ablation, ANF terminal endings (VGLUT1) in CN are decreased, reflecting deafferentation, while during the same time course, Sp5 terminals (VGLUT2) increase to compensate for ANF loss. Figures **B–H** are adopted from Zhou et al. (2007) and **I** from Zeng et al. (2009).

Fig. 4. Multisensory circuitry of DCN

Fusiform cells, the principle neurons that output to IC via the dorsal acoustic atria (DAS), receive auditory nerve fiber (ANF) and inhibitory interneuron inputs (vertical and D-stellate cells) at their basal dendrites and cell bodies. These synapses exhibit lower degrees of plasticity than the parallel fiber (axon of granule cell) inputs at the fusiform and inhibitory interneurons, the cartwheel cells' apical dendrites. The fusiform cell bodies integrate somatosensory information from trigeminal ganglion (TG), spinal trigeminal nucleus (Sp5), cervical dorsal root ganglion (C2), and dorsal column nucleus (DCoN).

Fig. 5. Bimodal stimulation induces persistent effects in DCN, VCN, but not IC

(A) An example of enhanced tone-evoked activity in a primary-like cell in VCN quantified by changes in its peri-stimulus time histogram (PSTH) evaluated 15 minutes after bimodal stimulation (right panel) relative to control (left panel). (B) Example of changes in the RLFs of VCN units 15 minutes after bimodal stimulation. (C) Bimodal stimulation produces no persistent effects in a representative IC unit as measured by its PSTH evaluated 15 minutes after stimulation (right panel) in comparison with control PSTH (left panel). (D) While both DCN and IC both show immediate changes (BI), plastic changes in tone evoked firing rate evaluated 15 minutes after bimodal stimulation are significant in DCN units (right panel) but not significant in IC units (left panel). Significance was evaluated by paired t-test at $p < 0.05$.

Fig. 6. Stimulus-timing dependent plasticity is altered in animals with tinnitus

(A) Mean tone-evoked timing rules showing stimulus-timing induced plasticity for fusiform cell single units in Sham (gray) ENT (expose/no-tinnitus; pink) and ET (exposed/tinnitus; red) guinea pigs. Diagram at top left and right corner illustrates the relative order of the tone (sinusoid curve) and Sp5 stimulation (vertical bar). (B) Mean timing rules of spontaneous firing rates color represented as in A. Error bars indicate SEM. (C) Percentage of units showing Hebbian-like (H), anti-Hebbian (aH), enhancing (E) and suppressing (S) timing rules from Sham (left), ENT (middle) and ET (right) animals. Significant differences

between Sham, ENT and ET groups are indicated by $^{*}(p<0.05; \text{Tukey-Kramer's post-hoc test})$. From Koehler and Shore (2013a).

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Distribution of somatosensory inputs in CN. GCD: granule cell domain (shell and small cell cap); DCN1: molecular layer; DCN2: fusiform cell layer; VCNm: magnocellular area of VCN.

Table 1

	GCD	DCN1	DCN 2	VCNm	Species	Reference
Cervical DRG (C2)	+				Rat	Zhan et al. (2006)
Cuneate, gracile (DCoN)	+		+		Rat	Wright and Ryugo (1996)
	+		+		Guinea pig	Zeng et al. (2011)
Trigeminal ganglion	+			+	Guinea pig	Shore et al. (2000)
Sp5	+		+	+	Rat	Haenggeli et al. (2005)
	+	+	+	+	Guinea pig	Zhou and Shore (2004); Zhou et al. (2007)
Sp5 + DCoN*	+		+		Cat	Itoh et al. (1987)

* Anterograde tracer injection overlaps both Sp5 and DCoN regions