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Auditory Nerve Damage and Adaption in the Central Auditory System – A Potential Cause for Tinnitus

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Contents

Abbreviations

ABR	Auditory brainstem response
AN	Auditory nerve
AVCN	Anterior ventral cochlear nucleus
BF	Best frequency
BM	Basliar membrane
BW	Bandwidth
CF	Characteristic frequency
CN	Cochlear nucleus
DCN	Dorsal cochlear nucleus
EFR	Evoked firing rate
FRA	Frequency response area
HL	Hearing loss
HHL	Hidden hearing loss
IC	Inferior colliculus
ICc	Central inferior colliculus
IHC	Inner hair cell
i.p.	Intraperitoneal
LSO	Lateral superior olivary complex
MGB	Medial geniculate body
MNTB	Medial nucleus of the trapezoid body
MSO	Medial superior olivary complex
NE	Noise exposure
OHC	Outer hair cell
PVCN	Posterior ventral cochlear nucleus
PSTH	Post stimulus time histogram
PTS	Permanent threshold shift
RLF	Rate level function
s.c.	Subcutaneous
SFR	Spontaneous firing rate
SOC	Superior olivary complex
SPL	Sound pressure level
TDT	Tucker-Davis Technologies
TTS	Temporary threshold shift

1. Introduction and Research Questions

1.1 In Search of the Neurophysiological Correlate of Tinnitus

Tinnitus is defined as the perception of a sound or noise in absence of an external or internal acoustic or electric stimulus (Møller, 2007b). It is a common and often agonising symptom (Axelsson and Ringdahl, 1989; Pilgramm et al., 1999; Nondahl et al., 2011; McCormack et al., 2014), frequently associated with exposure to loud noise, the use of ototoxic drugs, hearing loss (HL) and hyperacusis (Pilgramm et al., 1999; Nicolas-Puel et al., 2002). These findings indicate a positive (if not a causal) relation between cochlear damage and the appearance of tinnitus.
However, the exact relation between cochlear damage and tinnitus remains unknown. Not all tinnitus patients show signs of HL and not all subjects with HL simultaneously have tinnitus (Lockwood et al., 2002; Schaette and McAlpine, 2011; Vielsmeier et al., 2015). The same applies to hyperacusis (Sheldrake et al., 2015).

The question remains whether a particular kind of cochlear damage is necessary for the development of tinnitus or if all kinds of reduced cochlear output can potentially cause tinnitus. Furthermore, does the condition of the central neuronal system — concerning alertness, expectation, learning and memory — determine whether tinnitus is perceived?

In cases where hearing thresholds appear unaffected, it has been hypothesised that tinnitus might be caused by “hidden” cochlear damage that does not necessarily lead to a measurable increase of the hearing thresholds (Schaette and McAlpine, 2011). Recent studies showed that mild noise exposure (NE), which does not cause a permanent HL and no inner hair cell (IHC) loss, can nevertheless cause presynaptic and postsynaptic damage of synapses between IHCs and auditory nerve (AN) fibres and can lead to a loss of AN fibres (Kujawa and Liberman, 2009; Furman et al., 2013). Might this “hidden” cochlear damage, also called “hidden hearing loss” (HHL) be sufficient to cause tinnitus?

In fact, auditory brainstem response (ABR) measurements in tinnitus patients with normal hearing thresholds showed reduced amplitudes of wave I in the ABR (Barnea et al., 1990; Schaette and McAlpine, 2011), indicating a pronounced loss or damage of synapses between IHCs and AN fibres and/or a loss of AN fibres (Buchwald and Huang, 1975; Kujawa and Liberman, 2009; Schaette and McAlpine, 2011; Bourien et al., 2014). Simultaneously, wave V amplitudes in the ABR were not significantly different in the tinnitus group in comparison to the control group (Schaette and McAlpine, 2011), indicating similar synchronous activity of afferent fibres to the inferior colliculus (IC) in both groups (Møller, 2007a). The ABR results suggest increased neuronal gain in the auditory brainstem in the tinnitus group, which could compensate for the reduced input from AN fibers, but also produce neuronal hyperactivity as a side-effect (Schaette and McAlpine, 2011). This increased spontaneous activity could then lead to the perception of a sound or noise in absence of an external or internal acoustic or electric stimulus: tinnitus.

Indeed, hyperactivity in the central auditory system — in particular increased spontaneous firing rates (SFRs) — has frequently been discussed as the neurophysiological correlate of tinnitus (Jastreboff, 1990; Eggermont and Roberts, 2004; Rauschecker et al., 2010). De facto, various animal studies positively correlated increased SFRs in the central auditory system and behavioural signs for tinnitus (Brozoski et al., 2002; Kaltenbach et al., 2004; Bauer et al., 2008; Koehler et al., 2013; Mulders et al., 2014). However, in these studies neuronal hyperactivity and tinnitus were usually induced by strong cochlear damage, causing hair cell loss and permanent hearing threshold shifts. When mild noise exposure was applied, only causing temporary hearing threshold shifts, a more detailed analysis of cochlear damage was not performed (Dehmel et al., 2012). Accordingly, the exact relationship between different degrees of cochlear damage — including “hidden” cochlear damage — and the development of central neuronal hyperactivity and tinnitus remains unclear.

Is mild cochlear damage which causes only temporary hearing threshold shifts sufficient to cause hyperactivity in the central auditory system, potentially causing tinnitus? Does it lead to a decrease of wave I but a normal wave V in the ABR, as seen in tinnitus patients? On the level of the cochlea, does it lead to a damage of synapses between IHCs and AN fibres and to a loss of AN fibres, as observed after mild NE before? How does its effect differ from more pronounced cochlear damage, leading to a permanent hearing threshold shift? I will further investigate these questions in this thesis.

To further introduce the subject, I will subsequently describe the auditory pathway, tinnitus characteristics and epidemiology and current tinnitus research in more detail. At the end of this section, I will list the research questions.

1.2 The Auditory Pathway

In this chapter, I give an overview on the healthy auditory system in mammals. I put an emphasis on the cochlea and its innervation and on the IC, as these structures are mainly investigated in this work.

1.2.1 An Overview

The auditory system enables hearing by translating sound waves into neuronal activity. Generally, a sound wave enters the outer ear and is transmitted to the cochlea via the middle ear (Figure 1). The essential function of the middle ear is the effective transduction of sound waves from air to the fluids in the cochlea. Within the cochlea, the sound wave is transduced to an electric signal by the IHCs. These subsequently pass on the signal to the AN, the auditory part of the vestibulocochlear nerve. Further processing stages include the cochlear nucleus (CN), the trapezoid body, the superior olivary complex (SOC), the lateral lemniscus including its nuclei, the IC, the brachium of the IC, the auditory nuclei of the thalamus, thalamocortical auditory radiation and the auditory portion of the cerebral cortex and connected areas (Figure 2; reviewed by Moore, 2008).

1.2.2 The Cochlea

The spiral cochlea is located in the temporal bone (Figure 1). From its base to its apex, the Reissner's membrane and the basilar membrane (BM) divide the cochlea into two outer chambers, the scala vestibuli and the scala tympani, and one inner chamber, the scala media (Figure 4). All chambers are filled with nearly incompressible fluid. The scala vestibuli and the scala tympani are connected at the apex of the cochlea. The Organ of Corti, the auditory organ, is situated on the BM (Figure 4). In mammals it usually contains one row of IHCs and three rows of outer hair cells (OHCs) along the length of the cochlea. The name "hair cell" is derived from hair-like structures — more precisely called stereocilia — which are located at the apical site of IHCs and OHCs and directly below the tectorial membrane — a gelatinuous membrane — located directly above the Organ of Corti (Figure 4). IHCs are the auditory sensory cells; OHC activity influences the mechanical properties of the BM and the tectorial membrane (reviewed by Moore,

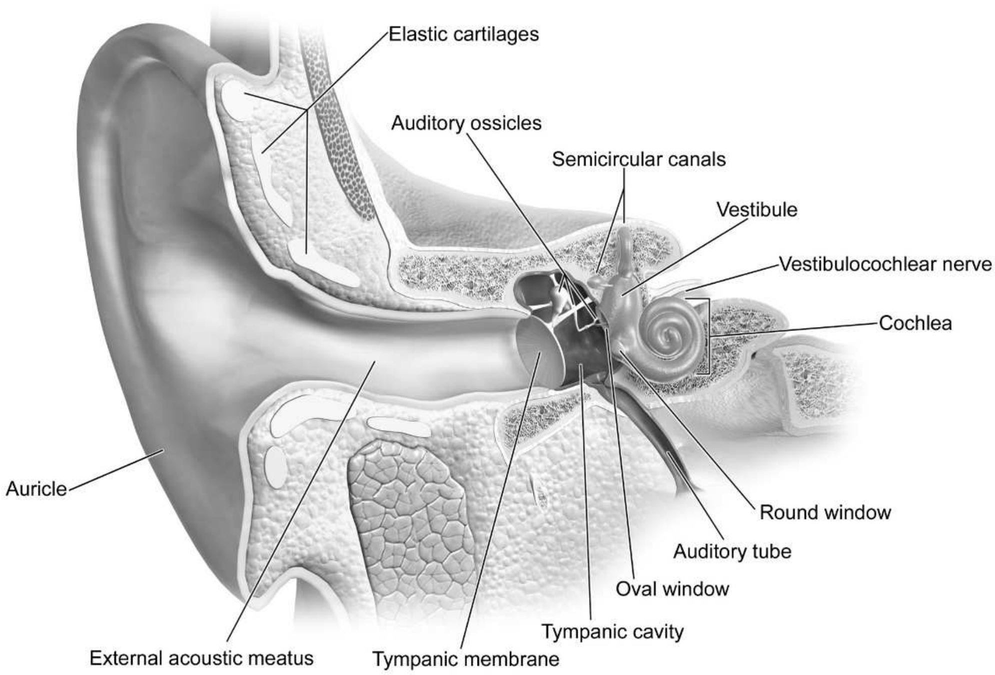


Figure 1: The outer, middle and inner ear; coronal illustration. The meatus routes the incoming sound waves to the middle ear and there they cause a vibration of the tympanic membrane. Its vibration is transduced via the three ossicles of the middle ear, the malleus, incus and stapes, to the oval window, a membrane-covered opening, to the cochlea. Figure from https://en.wikipedia.org/wiki/Ear (2015).

2008). In humans, each cochlea contains approximately 3.500 IHCs and 12.000 OHCs (reviewed by Ryugo, 1992). A mouse cochlea comprises approximately 750 IHCs and 2.500 OHCs (reviewed by Ryugo, 1992). From the oval window, sound waves are conducted to the fluid of the scala vestibuli. The resulting pressure difference between the scala vestibuli and the scala tympani causes a movement of the BM, e.g. a travelling wave for a sinusoidal acoustical signal. Because the BM is rather stiff and narrow at the base of the cochlea and more flexible and wider at the apex, high frequencies lead to a maximum deflection near the base and low frequencies to a maximum deflection near the apex. The vibration pattern of the BM is also actively influenced by the action of the OHCs for amplification of weak signals and sharpening of frequency tuning (reviewed in Moore, 2008).

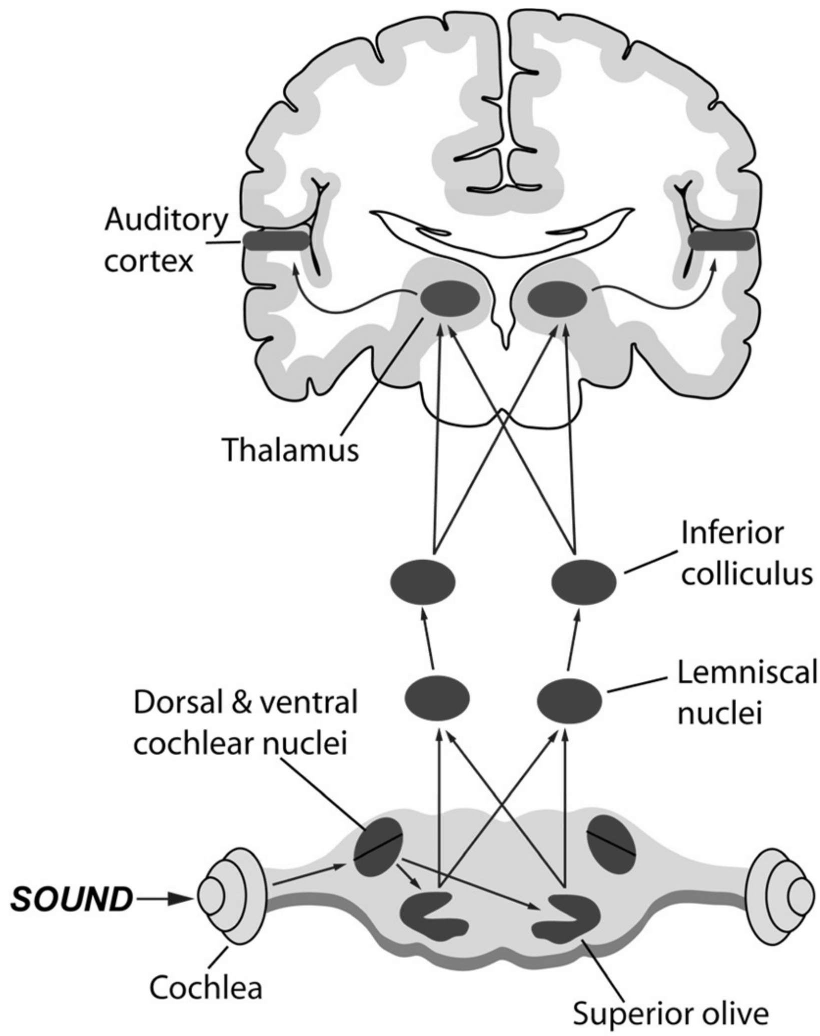


Figure 2: Schematic overview of major brain structures involved in the auditory pathway. Figure from Bender and Trussell (2011) with permission from K.J. Bender, L.O. Trussell and S. Blatrix.

Pronounced deflection of the BM leads to a movement of the tectorial membrane and consecutively to a displacement of stereocilia. This displacement causes a biochemical cascade in the IHCs which finally leads to a rapid glutamate release at the ribbon synapses of IHCs with AN fibres at the basal side of the IHCs. Glutamate consecutively binds to postsynaptic AMPA-receptors on AN fibres, thus triggering action potentials of AN fibres (reviewed by Moore, 2008). The ribbon synapses of IHCs with AN fibres are described in more detail in the following section. The frequency-specific spatial arrangement of AN fibre activation is called tonotopy and is maintained throughout the auditory system.

1.2.3 Cochlear innervation

Hair cells are innervated by three types of fibres: afferent auditory fibres, efferent auditory fibres and autonomic fibres (reviewed by Ryugo, 1992 and Møller, 2006).

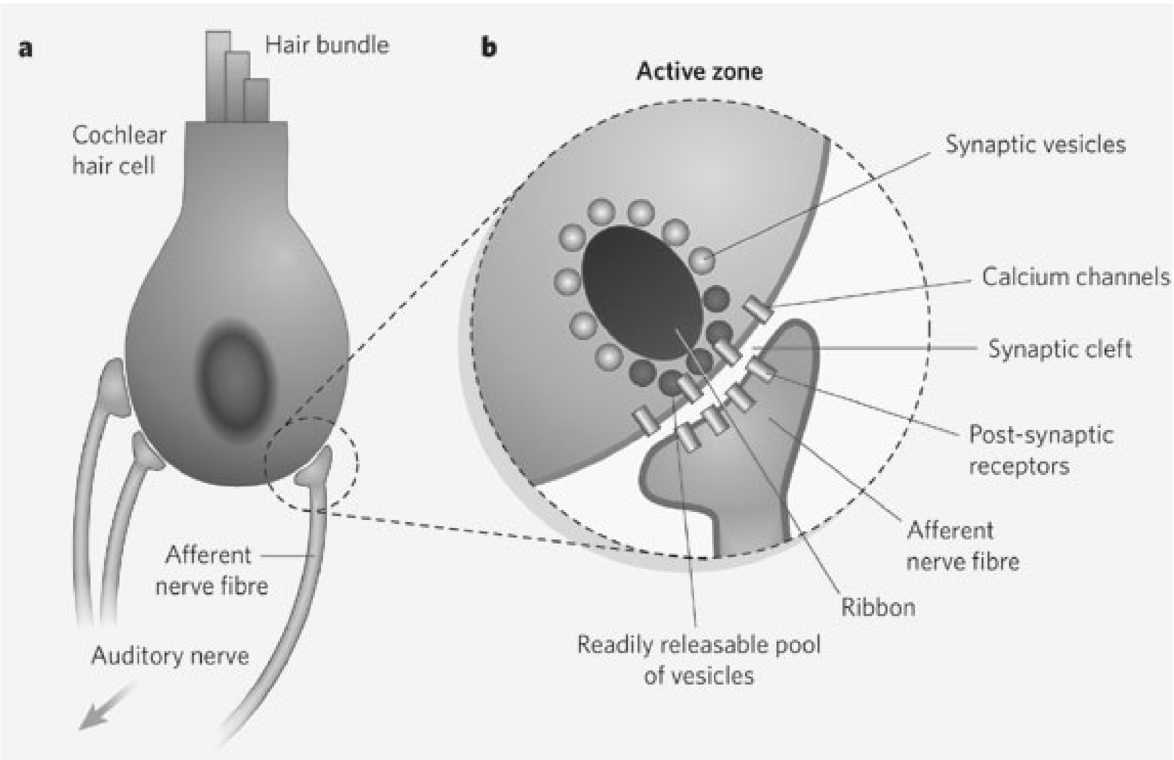


Figure. 3: a: Schematic overview of afferent cochlear hair cell innervation; **b:** a more detailed overview of a ribbon synapse between cochlear hair cells and an afferent auditory nerve fibre. Synapses are located at the basal, subnuclear site of inner hair cells (a). The hair bundle marks the stereocilia. Figure from Parsons (2006), with permission.

1.2.3.1 Cochlear Afferences: The Auditory Nerve

AN fibres are the primary afferent auditory fibres, transmitting auditory-sensory information from the cochlea to the central auditory system. AN fibres are bipolar neurons. Their distant axons synapse with IHCs and OHCs; cell bodies form the spiral ganglion which is located within the modiolus, the central axis of the cochlea; their central axons leave the cochlea at the internal auditory meatus, enter the brain at the cerebellopontine angle as a part of the vestibulocochlear nerve and synapse with neurons in the CN (reviewed by Ryugo, 1992 and Møller, 2006).

In humans, the AN contains approximately 30.000 fibres; in mice, there are around 12.000 AN fibres (reviewed by Ryugo, 1992). The activity of AN fibres is driven by IHCs and OHCs via ribbon synapses (reviewed by Nicolson, 2012). Up to 95% of AN fibres build synapses with IHCs (Spoendlin, 1972). These fibres are myelinated and are called type I fibres. Each type I fibre synapses with only one IHC via a single ribbon synapse (Liberman et al., 1990); however, IHCs have synapses with around twenty AN fibres (Liberman, 1982; reviewed by Møller, 2006). 5-10% of AN fibres synapse with OHCs and are called type II fibres (they branch several times). Type II fibres are not myelinated and they are smaller than type I fibres. One type II fibre innervates several OHCs; also one OHC has synapses with many type II fibres (reviewed by Ryugo, 1992 and Møller, 2006).
Synapses between IHCs and AN fibres are located at the subnuclear, basal side of the hair cells. The region with the most synapses is positively correlated with the region of most sensitive hearing. In mice, this is the region which detects sounds between 10-20 kHz (Meyer et al., 2009; Liberman et al., 2011).

Ribbon synapses between IHCs and AN fibres enable rapid multivesicular transmitter release. Depending on the strength of stereocilia deflection and consequently on sound intensity, L-type calcium channels mediate a gradated, seemingly linear influx of calcium into IHCs, a consecutively gradated fusion of synaptic vesicles and finally a gradated release of glutamate at the synapses with AN fibres (Figure 3; reviewed by Nicolson, 2012). Sufficient amounts of glutamate which bind to AMPA-receptors of AN fibres will consecutively trigger action potentials in AN fibres (reviewed by Nicolson, 2012). Synaptic ribbons are surrounded by a pool of transmitter vehicles and presumably enable fusion of vehicles and rapid multivesicular release of glutamate (reviewed by Nicolson, 2012). Synaptic vehicles are also released spontaneously, possibly to enable good responsiveness to small changes in membrane potential (reviewed by Nicolson, 2012).

Synapses between IHCs and AN fibres are congenerously structured from the base to the apex of the cochlea, concerning the size of synaptic ribbons, the amount of Ca^{2+} Channels and the AMPA-Receptors on the AN-endings (Matsubara et al., 1996; Meyer et al., 2009; Liberman et al., 2011). However, synapses on individual IHCs show heterogeneity, concerning the size of presynaptic ribbons and postsynaptic AMPA-patches (Liberman, 1982; Meyer et al., 2009; Liberman et al., 2011). This could provide the cause for the differing response properties of afferent AN fibres: AN fibres are spontaneously active but SFRs can vary by more than a factor of 1000 between AN fibres (Liberman et al., 2011). According to their SFR, AN fibres in mammals are often divided into three groups: high SFR AN fibres, medium SFR AN fibres and low SFR AN fibres (in the cat: SFR >18 spikes/s, 0.5 < SFR < 18 spikes/s and SFR < 0.5 spikes/s; Liberman, 1978). High SFR AN fibres have low response thresholds and evoked firing rates seem to rapidly saturate with the increase in sound pressure level (Winter et al., 1990; Bourien et al., 2014); medium SFR AN fibres have slightly higher thresholds and sound evoked firing rates do not fully saturate with increasing sound pressure level (SPL), showing pronounced increases in firing rates at low intensities but little increases in response to high intensities (Bourien et al., 2014). Low SFR AN fibres are only recruited at high sound intensities with firing rates continuously increasing with SPL (Winter et al., 1990). Evoked firing of high and medium SFR AN fibres seems to be highly synchronous (Bourien et al., 2014); low SFR AN fibres seem to fire less synchronously and show elongated and more broadly distributed latencies in comparison to high and medium SFR AN fibres (Bourien et al., 2014).

1.2.3.2 Cochlear Efferences: The Olivocochlear Bundle

By modifying the mechanical properties of OHCs, the olivocochlear system plays an important part in noise protection, mediation of selective attention, improving signal to noise ratio, auditory adaption and frequency selectivity (reviewed by Ciuman, 2010).

In humans approximately 500 efferent fibres — originating in the SOC — innervate the cochlea: most fibres innervate the OHCs. Olivocochlear efferent fibres can be divided into two types: the medial and the lateral olivocochlear fibres. The medial olivocochlear fibres are predominantly myelinated; they innervate the OHCs and originate in the medial superior olivary (MSO) complex, mostly in the contralateral MSO. Medial olivocochlear fibres build synapses with several OHCs and each OHC synapses with several medial olivocochlear fibres. Lateral olivocochlear fibres are small and unmyelinated; they synapse with type I AN fibres and originate in the ipsilateral lateral superior olivary (LSO) complex (reviewed by Møller, 2006).

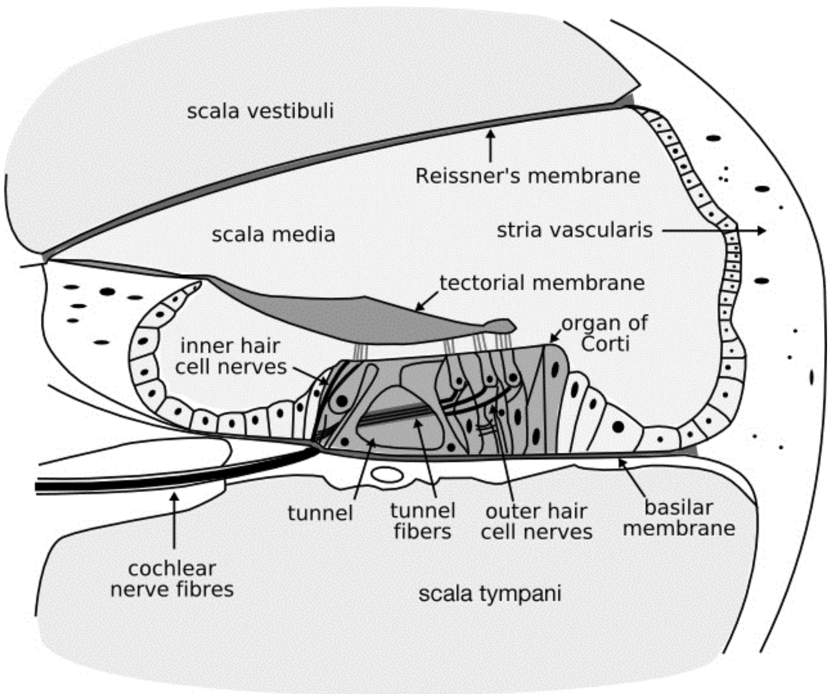


Figure 4: Axial illustration of a cochlea turn. Figure from: https://en.wikipedia.org/wiki/Cochlea (2015)

1.2.3.3 Autonomic innervation of the cochlea

Autonomic, adrenergic nerve fibres mostly innervate cochlear blood vessels. However, some fibres also synapse with hair cells (reviewed by Møller, 2006).

1.2.4 The Cochlear Nucleus Complex

The cochlear nuclei form the first central stage of the ascending auditory pathway. From here cochlear information is distributed to other brainstem nuclei. Different information on sound such as timing and sound localisation is split into multiple separate pathways (reviewed by Young and Oertel, 2003).

Located at the cerebellopontine angle, the CNs are brainstem nuclei and they receive their main input from the ipsilateral AN (Figure 2). However, they also receive various other input from the contralateral CN (Davis, 2005), the SOC (reviewed by Brugge, 1992 and by Young and Oertel, 2003) and the somatosensory system (Shore, 2008). Projections mainly go to the contralateral IC (reviewed by Møller, 2006). Fibres also project to the ipsilateral IC, the SOC and various other cell groups in the pons, the medulla and the midbrain (reviewed by Brugge, 1992 and by Young and Oertel, 2003). Also CN neurons connect with other ipsilateral CN neurons (reviewed by Brugge, 1992).

The CN can be divided into three subdivisions, according to differences in cellular architecture: the dorsal CN (DCN), the posterior ventral CN (PVCN) and the anterior ventral CN (AVCN; reviewed by Brugge, 1992 and by Young and Oertel, 2003). Depending on the species, the CN contains several morphologically different types of neurons, e.g. in the cat, twenty morphologically different cell types exist (reviewed by Brugge, 1992).

Type I AN fibres branch and synapse with neurons in all three subdivisions, regardless of their SFR, evoked response and threshold patterns. However, the branching patterns might vary between subgroups (reviewed by Brugge, 1992). It is assumed that excitatory and inhibitory input to CN neurons is mediated by various different neurotransmitters (reviewed by Brugge, 1992). Few data on type

II fibres suggests that type II fibres also split and potentially codetermine with type I fibres (reviewed by Brugge, 1992). However the role of type II fibres remains unknown (reviewed by Young and Oertel, 2003). Each subdivision receives cochleotopic information (reviewed by Brugge, 1992) which is maintained up to auditory cortex areas (reviewed by Brugge, 1992 and by Young and Oertel, 2003).).

CN neurons may also be divided into subtypes according to their electrical properties. In the CN, differences in electrical properties are associated with morphological differences (reviewed by Brugge, 1992). Electrical differences between CN neurons can be described by characterising temporal and spatial differences in spike patterns, depending on afferent input and the electrical properties of the CN neurons (for further detail see Brugge, 1992 and Young and Oertel, 2003).

1.2.5 The Superior Olivary Complex

SOC neurons are involved in sound localisation and in the efferent cochlear pathway. For sound localisation binaural input converges in the SOC.
The SOC contains a number of closely grouped nuclei which are located in the ventrolateral pons (Figure 2; reviewed by Brugge, 1992). The three major nuclei are: the MSO, the LSO and the medial nucleus of the trapezoid body (MNTB; reviewed by Brugge, 1992).

SOC neurons receive their main input from AVCN and PVCN neurons (reviewed by Brugge, 1992) and projections mainly go to the midbrain. MSO and LSO neurons also project to the cochlea, as mentioned above; however, they mainly project to the central nucleus of the IC (ICc), whereas MNTB neurons mainly project to LSO neurons.

1.2.6 The Inferior Colliculus

The ICs form the main hub of the ascending auditory pathway, routing auditory input and integrating somatosensory information (Shore, 2008; reviewed by Ono and Ito, 2015). Approximately twenty pathways from auditory and non-auditory brainstem nuclei — as well as projections from the auditory cortex, the auditory thalamus and other non-auditory cortical and thalamical projections — converge here, sending excitatory and inhibitory input (Hage and Ehret, 2003; Shore, 2008; reviewed by Ono and Ito, 2015). Excitatory and inhibitory projections mainly go to the ipsilateral medial geniculate body (MGB; reviewed by Ono and Ito, 2015).

The ICs are located in the midbrain, forming the inferior part of the corpora quadrigemina and can be divided into three major subdivisions: a central nucleus (ICc), a dorsal cortex and an external cortex — also called the lateral cortex (Figure 7; Loftus et al., 2008; Shore, 2008). The ICc receives its main input from the contralateral CN, the contralateral and ipsilateral SOC and from the contralateral and ipsilateral nuclei of the lateral lemniscus, forming the main hub of the ascending auditory pathway (reviewed by Ono and Ito, 2015). The cortex of the IC receives its main input from descending cortical neurons and from ICc neurons (reviewed by Ono and Ito, 2015). The dorsal cortex of the IC may be important for detecting novel sounds in the environment (Lumani and Zhang 2010). The external cortex of the IC also receives visual and somatosensory input and is involved in multisensory integration (Loftus et al., 2008; Ono and Ito, 2015).
In the ICc, neurons are organised tonotopically with medio-ventral neurons responding to high frequencies and latero-dorsal neurons responding to low frequencies (Stiebler and Ehret, 1985). In more detail, dendrites of ICc neurons form fibrodendritic laminae, whereas neurons forming a lamina have similar best frequencies (reviewed by Ono and Ito, 2015). Within the laminae, synapses with afferent neurons are organised in zones, e.g. DCN neurons synapse more dorsomedially and LSO neurons synapse more ventrolaterally (reviewed by Ono and Ito, 2015). Nevertheless, each ICc neuron receives various excitatory and inhibitory input from several brainstem nuclei. Besides, intrinsic neuronal properties such as the pattern of potassium and calcium channel expression and input resistance, vary between ICc neurons. In the ICc, differences in intrinsic properties could not be correlated to morphological differences or linked to particular ICc areas; intrinsic properties seem to vary heterogeneously within the ICc (reviewed by Ono and Ito, 2015).

Similar to CN neurons, ICc neurons may also be divided into subtypes, according to their electrical properties. According to their characterising temporal and spatial differences in spike patterns, up to eight types of ICc neurons have been determined (reviewed by Ono and Ito, 2015).

1.2.7 The Auditory Thalamus and the Auditory Cortex

The MGB, the lateral division of the posterior group and the auditory sector of the reticular nucleus form the auditory nuclei of the thalamus (Jones, 1985). The main projections to the auditory thalamus originate in the ipsilateral IC; projections from the auditory thalamic nuclei mainly go to auditory cortex areas (reviewed by Brugge, 1992; and Winer, 1992).

The auditory cortex is located in the temporal lobe around the Brodman areas 41 and 42. It can roughly be divided into a primary auditory cortex and secondary and association areas. The primary auditory cortex mainly receives input from ventral MGB neurons and is clearly organised tonotopically. Neurons in secondary and association areas receive more diffuse input from the MGB and their tonotopy is less clear (reviewed by Winer, 1992).

1.3 Tinnitus Characteristics and Epidemiology

In this work I investigate pathophysiological changes in the cochlea and in the auditory midbrain, potentially causing or contributing to the development of tinnitus. To provide a better understanding of the significance of tinnitus as a symptom, I provide more information about the characteristics of tinnitus, its epidemiology and associated symptoms and diseases in this chapter.

Tinnitus can be defined as the perception of a sound in the absence of an external acoustic or electric stimulus (Møller, 2007b). If, in addition, no internal acoustic or electric stimulus is verifiable, the phantom sound is called subjective tinnitus. In rare cases an internal sound source can be detected (Møller, 2007b); this form of tinnitus is called objective tinnitus. The following text deals with the common subjective tinnitus.

As a percept, tinnitus is difficult to measure. Its name derives from the Latin word *tinnire*, which means to ring. In fact, the perceived tones or noises can be very variable: sound levels range from barely perceivable to drowning out all ambient noise; all audible frequencies can be affected; sound characteristics vary from continuous whistling to intermittent humming in either one or both ears — and furthermore the percept can change in the course of time (Pilgramm et al., 1999). In the majority of cases, the tinnitus percept is matched to pure tones with frequencies above 3 kHz (Baguley, 2006).

In Germany approximately 25% of the population have experienced tinnitus at least once and about 4% have had to endure tinnitus that lasted longer than one month (Pilgramm et al., 1999). Data concerning the prevalence of chronic tinnitus varies between 3-30% in the revised studies (Axelsson and Ringdahl, 1989; Pilgramm et al., 1999; Sanchez, 2004; Nondahl et al., 2011), depending on the varying definition of acute and chronic tinnitus and the age of studied subjects, as tinnitus incidence increases with age (Baigi et al., 2011). Altogether, the data demonstrates that tinnitus is a very common phenomenon.

Several risk factors for the development of tinnitus have been identified. The most important risk factor identified so far is hearing loss (Nicolas-Puel et al., 2002; Sanchez et al., 2004). Other important risk factors are exposure to loud noise, the use of ototoxic drugs (e.g. cisplatin, salicylates and aminoglycoside antibiotics) and hyperacusis (Kaltenbach et al., 2002; Nicolas-Puel et al., 2002; Sanchez et al., 2004). A further domain frequently associated with having a major impact on tinnitus development is the psyche: stress, depression and anxiety disorders are associated with an increased risk for tinnitus (Mazurek et al., 2010; de Ridder et al., 2011). However, they can also be the consequence of tinnitus (Mazurek et al., 2010). Furthermore, a history of head injury, headache, otitis media and cardiovascular disease can be associated with tinnitus development (Pilgramm et al., 1999; Sanchez, 2004). The influence of genetic factors for the development of tinnitus remains unclear (Sand et al., 2007; Kvestadt et al., 2010).

1.4 Models and Studies on Tinnitus

So far, the exact pathophysiology of tinnitus has remained unclear. However, most tinnitus models and studies in animals and humans suggest that in in the majority of cases the development of tinnitus is triggered by cochlear damage (reviewed by Eggermont and Roberts, 2015 and Noreña, 2015). Though cochlear damage and thereby reduced cochlear output initiate the development of tinnitus, neuronal changes in the central auditory system appear to ultimately generate it (reviewed by Eggermont and Roberts, 2015 and Noreña, 2015). Evidence suggests that central neuroplastic changes causing tinnitus include increased spontaneous firing rates, increased neuronal synchrony and tonotopic map reorganisation (reviewed by Eggermont and Roberts, 2015).

Besides changes in the auditory system, alterations in non-auditory brain structures have also been linked to tinnitus; in particular, structures involved in alertness, emotion and memory appear to be changed in subjects with tinnitus. However, it remains unknown whether these non-auditory changes preexist and reveal a vulnerability to tinnitus, whether they are necessary for the perception of tinnitus or whether they are linked to associated symptoms such as stress and discomfort (reviewed by Eggermont and Roberts, 2015).

1.4.1 Modelling Central Neuronal Changes in Tinnitus Subjects

Several models have been constructed to explain the development of tinnitus through neuronal changes in the central auditory system. A common presumption is that neuronal hyperactivity in the central auditory system may cause the tinnitus percept. Hyperactivity could arise as a consequence of a stabilisation of the mean firing rates of central auditory neurons after reduced input from the periphery through homeostatic plasticity: decreased AN activity after cochlear damage or reduced cochlear output for other reasons is counterbalanced by an increase in neuronal response gain (Schaette and Kempter, 2006; Noreña, 2011); this restores the mean rate but may also lead to increased SFRs, potentially causing an auditory phantom perception (Schaette and Kempter, 2006).

Other commonly debated theories focus on the role of non-auditory structures in the development of tinnitus: De Ridder et al. (2011) suggested that hyperactivity in the central auditory system after reduced cochlear output is only perceived if non-auditory brain networks involved in (self-)awareness are coactivated. They also pointed out that involvement of the nonspecific distress network and memory might be important for the persistence of a tinnitus percept. Similarly, Rauschecker et al. (2010) suggested that tinnitus-related changes in the central auditory system after reduced cochlear output or other auditory lesioning might only be perceived if they are not cancelled out by feedback activity from limbic structures.

1.4.2 Studies on Cochlear Damage, Neuronal Plasticity and Tinnitus

In accordance with the presumption that tinnitus is related to hyperactivity in the central auditory system, various animal studies indicate that indeed increased spontaneous and evoked neuronal activity in the central auditory system can be linked to behavioural signs of tinnitus (Brozoski et al., 2002; Kaltenbach et al., 2004; Bauer et al., 2008; Koehler and Shore, 2013; Mulders et al., 2014).

Increased spike rates were found in the dorsal CN (Kaltenbach et al., 1998; Kaltenbach et al., 2004; Koehler and Shore, 2013), the ventral CN (Vogler et al., 2011), the IC (Ma et al., 2006; Mulders and Robertson, 2009; Mulders et al., 2011; Vogler et al., 2014), the auditory thalamus (Kalappa et al, 2014) and the auditory cortex (Noreña and Eggermont, 2003) after inducing cochlear damage by exposure to loud noise (Brozoski et al., 2002; Kaltenbach et al., 2004; Ma et al., 2006 Bauer et al., 2008; Finlayson and Kaltenbach, 2009) or the use of ototoxic drugs (Ma et al., 2006; Bauer et al., 2008). The degree to which spontaneous activity was increased could be related to the strength of the behavioural evidence of tinnitus (Kaltenbach et al., 2004). Furthermore, hyperactivity appeared to be restricted to neurons that responded to frequencies where hearing was impaired (Mulders and Robertson, 2009).

Auditory nerve recordings have shown no signs of increased spontaneous firing rates of AN fibres after acoustic trauma (Liberman and Kiang, 1978; Liberman and

Dodds, 1984) and the CN appears to be the first stage in the ascending auditory pathway where hyperactivity develops after cochlear trauma. Interestingly, hyperactivity of CN neurons persists even after ablation of the AN, suggesting that the hyperactivity of CN neurons is not dependent on input from the AN (Zacharek et al., 2002).

Inhibition appears to be weakened after cochlear damage in the DCN, the SOC and the IC by a reduction of inhibitory transmitter release and receptor binding (Buras et al. 2006; Dong et al. 2009; Milbrandt et al. 2000; Wang et al. 2009). Also excitation is increased in these nuclei (Potashner et al. 1997; Shore 2011; Zeng et al. 2009*).* Simultaneously, inhibitory and excitatory innervation seem to change after cochlear damage by a modified formation of synaptic connections (Bilak et al, 1997; Kim et al., 1997; Morest et al., 1998).

Also in functional imaging studies in both animals and humans with tinnitus, signs of hyperactivation were found in several structures involved in the auditory pathway up to the auditory cortex and also in non-auditory structures — in particular in limbic structures, which are involved in emotional processing, and in the cerebellum (Lockwood et al., 1998; Brozoski et al., 2007; Eichhammer et al., 2007; Lanting et al., 2008; Melcher et al., 2009; reviewed by Norena, 2015 and by Eggermont and Roberts, 2015). In addition to hyperactivity, hypersynchrony, changes in thalamocortical and corticolimbical oscillatory activity and cortical map reorganisation in the primary auditory cortex were found (Noreña and Eggermont, 2005; Weisz et al., 2007; Adjamian, 2012; reviewed by Noreña, 2015, and by Eggermont and Roberts, 2015).

Whether and exactly how hyperactivity is transmitted along the auditory system remains unclear. However, bottom-up and top-down mechanisms seem to be involved as well as non-auditory inputs (Shore et al., 2007; Caspary et al., 2008; Roberts et al., 2010; Noreña, 2015).

Small amounts of data do not align with the assumption that tinnitus is related to an increase in central gain and spontaneous neuronal activity in the central auditory system (Singer et al., 2013; Coomber et al., 2014); however, it should be considered that the observed changes might be more closely linked to associated symptoms such as hyperacusis or hearing loss.

Furthermore, the meaning of neuronal changes in non-auditory brain structures is still unknown: are these changes specific to tinnitus or are they associated with conditions, positively correlated with tinnitus, such as hyperacusis or distress (reviewed by Eggermont and Roberts, 2015)?

1.4.3 Revisiting the Relationship of Cochlear Damage, Neuronal Hyperactivity and Tinnitus

As mentioned above, a connection between cochlear damage, neuronal changes in the central auditory system and the development of tinnitus has been shown extensively. However, it has also been shown that cochlear damage is not necessary for the development of tinnitus: conductive HL or auditory deprivation, for example, do not affect cochlear structures but are sufficient to cause tinnitus (Ayache et al., 2003; Schaette et al., 2012). Thus, possibly any reduced auditory sensory input, regardless of its cause, might be sufficient to trigger the development of tinnitus and related central neuronal changes.

Nevertheless, in the majority of cases, the development of tinnitus can be linked to cochlear damage: several studies show a positive correlation between tinnitus and cochlear damage through exposure to loud noise or the use of ototoxic drugs (Nicolas-Puel et al., 2002; Sanchez et al., 2004). Alongside this, the majority of patients with tinnitus suffer from HL (Nicolas-Puel et al., 2002); the pitch of tinnitus usually falls into the frequency range where hearing is impaired and the tinnitus percept is usually lateralised to the ear that is affected or that is more affected by HL (Henry et al., 1999; Noreña et al., 2002; Koenig et al., 2006; Martines et al., 2010; ScheckImann et al., 2012; Vielsmeier et al., 2015). On the other hand, HL does not automatically lead to tinnitus: the prevalence of HL is four times higher than the prevalence of tinnitus (Lockwood et al., 2002). Also, some tinnitus patients do not show apparent signs of HL in conventional pure-tone audiometry (Barnea et al., 1990; Schaette and McAlpine, 2011). As conventional pure-tone audiometry only tests hearing from 125 Hz–8 kHz and accepts thresholds up to 20 dB hearing level as normal (reviewed by Eggermont and Roberts, 2015), it has to be considered that small increases in threshold as well as high-frequency hearing loss are not detected. However, even when hearing is tested up to 16 kHz with high frequency audiometry, not all subjects with tinnitus also show signs of HL (Vielsmeier et al., 2015).

Both findings — tinnitus without HL and HL without tinnitus — lead to the question whether a certain type of cochlear damage that cannot be detected by hearing threshold measurements is necessary for the development of central neuronal hyperactivity and tinnitus.

Interestingly, some tinnitus patients with a normal audiogram show impairments indicative of cochlear damage in psychophysical tests that assess hearing function at supra-threshold levels (Weisz et al., 2006). Also, other recent studies showed that substantial cochlear damage might be present despite normal hearing thresholds in the audiogram (Kujawa and Liberman, 2009; Lin et al., 2011; Furman et al., 2013): acoustic trauma, leading to a moderate but completely reversible hearing threshold elevation in the ABR caused an immediate loss of synaptic ribbons in IHCs, an acute loss of afferent nerve terminals and a delayed degeneration of the AN. Excitotoxicity of glutamate from IHCs after acoustic overexposure was discussed as a reason for the acute loss of afferent nerve terminals and the delayed degeneration of AN fibres. This kind of cochlear damage also caused a chronic reduction of the amplitude of wave I in the ABR. ABR wave I amplitudes reflect the number of synchronously activated AN fibres (Buchwald and Huang, 1975; Bourien et al., 2014); a reduction of ABR wave I amplitudes thus indicates a loss of activity in a fraction of AN fibers, and/or a decrease in the degree of synchrony of their responses. Correspondingly, reduced wave I amplitudes in the ABR were found in tinnitus subjects with a normal audiogram (Schaette and McAlpine, 2011). Interestingly, in the tinnitus group wave V amplitudes in the ABR — reflecting input from the SOC to the IC in humans (Møller, 2007b) — did not differ from wave V amplitudes in the control group. Accordingly, the results suggest that tinnitus subjects with a normal audiogram nevertheless might be affected by AN fibre deafferentation; however, central auditory activity appears normal, indicating an increase in neuronal response gain, which could explain the presence of tinnitus (Schaette and McAlpine, 2011).

These findings raise a number of important questions: could AN fibre deafferentation be sufficient to cause hyperactivity in the central auditory system and potentially trigger tinnitus, even without an increase in hearing thresholds? How do central changes differ after "hidden" cochlear damage with no elevated hearing thresholds and "obvious" cochlear damage including elevated hearing thresholds? These questions have not been investigated yet and will be addressed in this study.

1.5 Research Questions

In this work I investigate the effect of acoustic trauma on cochlear and auditory brainstem structures, asking the following questions:

- Is it mild NE that only leads to a temporary but not a permanent shift of ABR thresholds sufficient to cause hyperactivity in the central auditory system?
- If hyperactivity can be found, is it accompanied by a reduction of the amplitude of ABR wave I and histological signs of AN fibre degeneration, as predicted?
- Does mild acoustic trauma have a different effect on central neuronal activity compared to more severe acoustic trauma that leads to a permanent elevation of ABR thresholds?

2. Methods

2.1 Animals and Groups

Twenty-three male CBA/Ca mice were used for this study. CBA/Ca mice were chosen as they show late onset of age-related hearing loss with normal hearing thresholds up to two years of age. Also they are commonly used in auditory research (Hultcrantz, 1993*)*.

Mice were divided into three groups:

- **Group NE100** (**mild binaural NE**, n=6): Mice were exposed to an 8-16 kHz band noise, presented at 100 dB SPL for two hours.
- **Group NE105** (**moderate binaural NE**, n=10): Mice were exposed to an 8-16 kHz band noise, presented at 105 dB SPL for two hours.
- **Group C** (**control**, n=7): Mice in group C served as controls and were not exposed to noise prior to the experiment.

In the NE groups, ABRs were recorded once 1-14 days before, 1 day after and once 28-32 days after NE. Extracellular recordings in the IC were also performed 28-32 days after NE, on the same day as the final ABR recordings (Figure 5). Mice in the NE groups were 7-19 weeks old at the time of NE. Control mice were age-matched. In the control group ABRs were recorded once and followed by extracellular IC recordings. At the end of the IC recordings mice were euthanised.
Experiments were equally performed by me and by Warren Bakay.

All experiments were performed in accordance with the United Kingdom Animal Scientific Procedure Act (ASPA) of 1986.

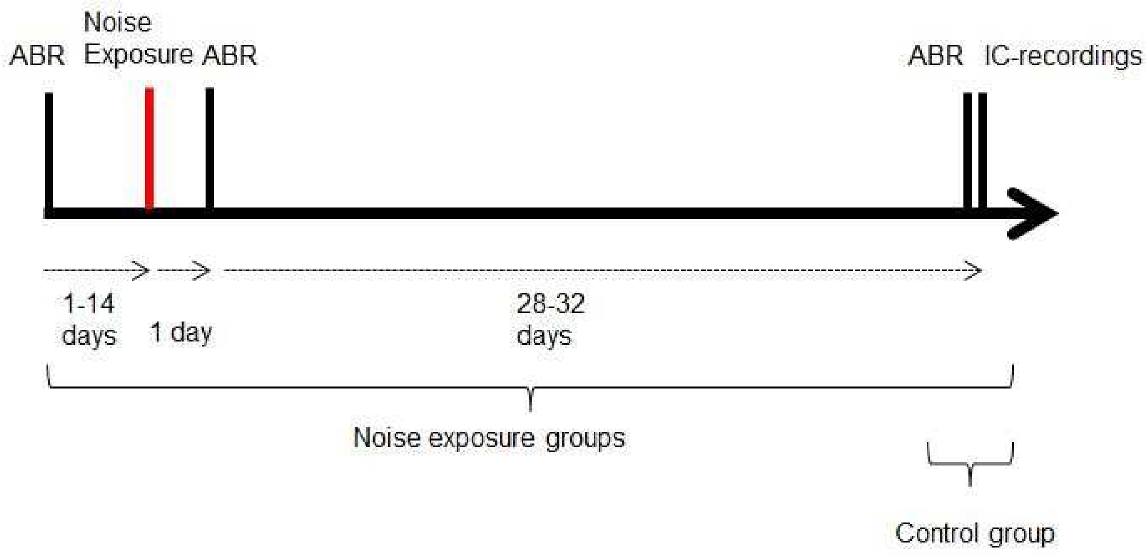


Figure 5: Schematic overview of the time course of experiments. ABR= auditory brainstem response recordings, IC-recordings= multiunit recordings in the central inferior colliculus.

2.2 Noise Exposure

NEs were designed to cause:

- a temporary ABR threshold shift: NE100 group: 100 dB SPL, 8-16 kHz octave band noise, 2 hours; or
- a permanent ABR threshold shift: NE105 group: 105 dB SPL, 8-16 kHz octave band noise, 2 hours.

2.2.1 Anaesthesia and Vital Parameters

Mice received an intraperitoneal (i.p.) injection of Ketamine and Medetomidine (0.01 ml/g body weight; Ketamine: 10 mg/ml; Medetomidine: 0.083 mg/ml). When showing whisker movements, they received a subcutaneous (s.c.) top-up of 0.00375 ml Ketamine/Medetomidine-cocktail. During experiments eyes were kept moist with eye ointment. To stabilise the body temperature, mice were put on a heating pad and were covered with cotton wool and aluminium foil throughout the experiment. Breathing rates were checked every 30-45 minutes. At the end of the NE mice received Atipamezole (0,002 ml/g body weight body weight, 0.5 mg/ml) s.c. for recovery.

2.2.2 Animal and Speaker Position

NE was performed in a sound-attenuating booth. The mice heads were positioned in the centre of the booth, resting on head stands, made of Blu-tack. The noise-delivering speaker was located 45 cm above the animal's heads at the ceiling of the booth.

2.2.3 Noise Generation and Calibration

Noise was generated using a RX6 processor from Tucker-Davis Technologies (TDT). Attenuation was done as required, using a TDT PA5. For amplification, a TDT SA2 was used. Sound was delivered by a Stage Line PA Horn Tweeter MHD-220N/RD. Calibration was performed prior to each use for target SPL and frequency band using a Brüel and Kjær calibration microphone. Sound pressure levels were ensured to vary less than ±1 dB between recordings. Codes for noise generation and calibration were written by Dr. Roland Schaette.

2.3 The Auditory Brainstem Response

The ABR was used to evaluate hearing thresholds and neuronal activity at the level of the peripheral and central auditory system. As described above, wave I of the ABR reflects the synchronous activity of AN fibres. Wave IV, which next to wave I is the most stable wave in the ABR in mice, reflects activity of SOC neurons, projecting to the IC (Henry, 1979; Parham et al., 2001).

2.3.1 Anaesthesia and Vital Parameters

For ABR recordings, anaesthesia with Ketamine/Medetomidine and monitoring of the vital parameters was done in the same way as for the NE. Before inserting ABR electrodes into the skin, mice received an additional local injection of Metacam s.c. as an analgesic.

2.3.2 Experimental Setup

ABR recordings took place in a sound-proof booth. Sound was presented free-field. The speaker was positioned in a 45° angle to the animal's axis in a distance of approximately 15 cm, aiming directly at the tested ear. The contralateral ear was plugged with a sound-attenuating plug.

For recordings, subdermal needle electrodes (Rochester Medical) were inserted at the vertex (electrode 1) and behind both pinnae (electrode 2 ipsilateral to sound presentation and reference electrode contralateral to sound presentation).

2.3.3 Sound Generation and Calibration

For click and tone generation, a TDT RX6 processor was used. Attenuation was performed by a TDT PA5 and amplified by a TDT SA1. Sound was presented by a TDT FF1 speaker. Calibration was done for target SPL and frequency band using a Brüel and Kjær calibration microphone. Sound pressure levels were ensured to vary less than ±1 dB between recordings. Codes for click and tone generation and calibration were written by Dr. Gestur Bjoern Christianson and Dr. Jennifer Linden.

2.3.4 Auditory Brainstem Response Recordings

Click-evoked ABRs were recorded for both ears and tone-evoked ABRs were recorded for the left ear. Clicks had a duration of 50 µs and tone pips a duration of 5 ms, with a rise/fall time of 1.5 ms. Click and tone stimuli were presented from 0-80 dB SPL in 5 dB steps. Both click and tone stimuli were presented with an onset delay of 2 ms and a rate of 20 stimuli/s and were repeated 500 times. Tone-evoked ABRs were recorded separately with stimuli centred at 6, 8, 11, 16, 32 and 48 kHz.

2.3.5 Data Analysis

Electrode signals were low-pass-filtered (7.5 kHz cut-off frequency, 12 dB per octave) and digitised with a sampling rate of 24 kHz. For analysis, a bandpass filter (100-3000 Hertz, 5^{th} order Butterworth filter) was applied.

ABR thresholds were determined visually: the lowest sound level — where ABR waveforms could be identified — was chosen as the threshold. As normal distribution could not be assumed, Mann-Whitney U tests were used for statistical testing. When multiple comparisons were performed, Bonferroni corrections were applied. For ABR wave analysis, ABR time windows were chosen visually and peaks and troughs were determined within the chosen window (see Figure 6). Wave amplitudes were calculated from wave peak to the following through. For statistical analysis of differences in ABR wave amplitudes between groups, multiway analysis of variance (ANOVA) was applied. Wave I and wave IV amplitudes were compared between the control group and both NE groups before NE and four weeks after NE. Because ABR measurements from the control group were included in two analyses, Bonferroni corrections were performed.

The code for ABR threshold analysis was written by me, while the code for ABR wave analysis was written by Dr. Roland Schaette and partially altered by me. I also performed the data analysis. For data analysis, a two hour consultation was done by the Institut für Medizinische Biometrie und Statistik, University of Lübeck.

2.4 Extracellular Recordings in the Inferior Colliculus

To investigate neuronal activity in the central auditory system, multiunit-recordings in the ICc were performed. The ICc was chosen for multi-unit recordings as it is the principal nucleus for ascending auditory information. Moreover, recordings can be performed across the full extent of the central ICc with a linear electrode array, allowing recordings to take place across the whole tonotopical map simultaneously.

2.4.1 Anaesthesia and Vital Parameters

For IC recordings, anaesthesia was maintained by a continuous i.p. infusion of Ketamine/Medetomidine (rate: 0.00375 ml/g body weight/h; 10 mg/ml Ketamine; 0.083 mg/ml Medetomidine). To ensure deep anaesthesia, infusion rates were adjusted until the pedal withdrawal reflex was erased.

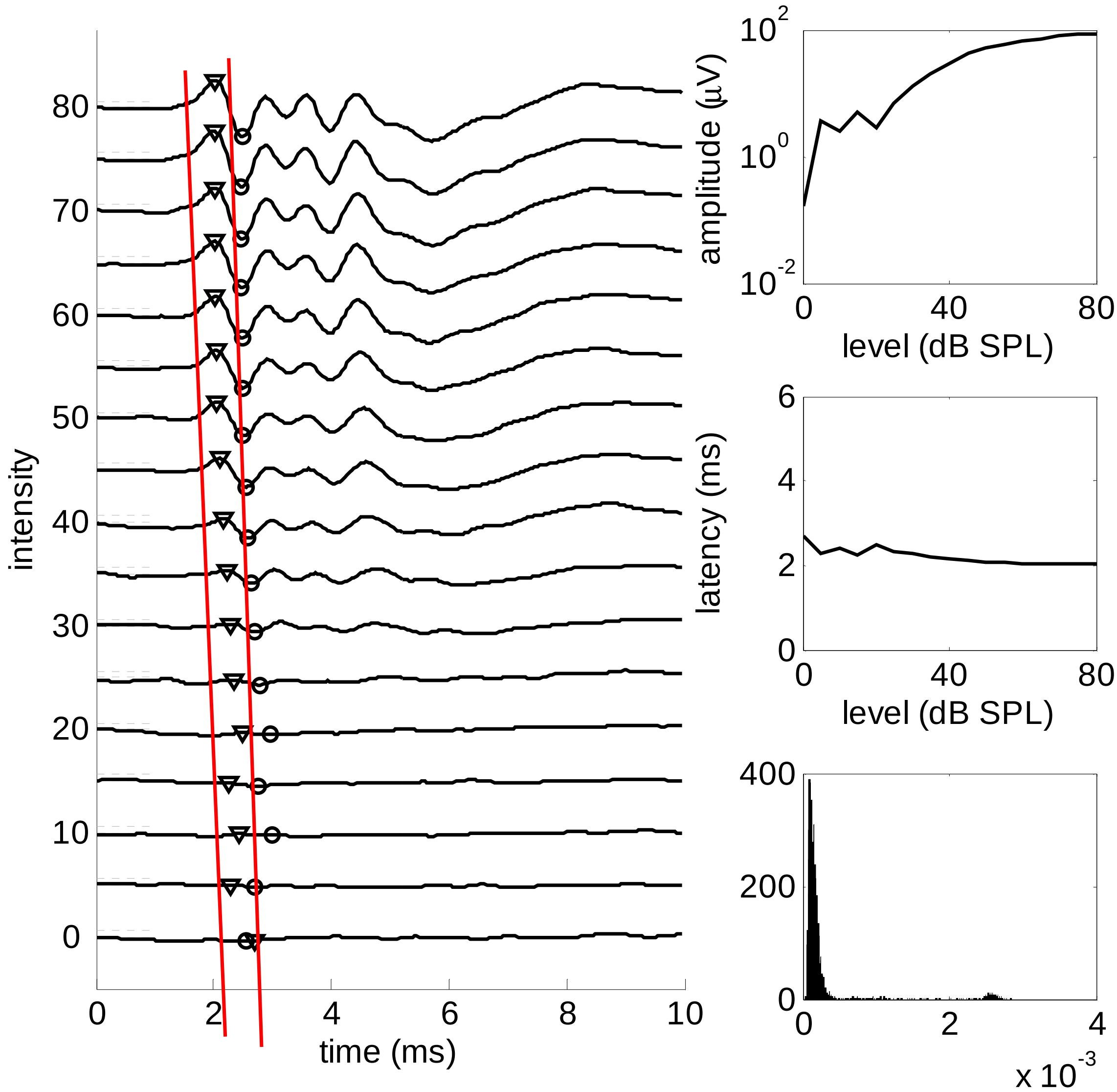


Figure 6: Example of an auditory brainstem response (ABR). **Left figure:** ABR amplitudes in response to clicks presented at different sound levels (y-axis: presented intensity in dB SPL and ABR amplitude), plotted against time (x-axis: time in ms, 0=stimulus onset). Triangles mark wave I peaks, calculated within the visually determined time window (red lines); circles mark wave I troughs, determined in another time window (not shown). **Right, upper figure:** Wave I amplitude plotted against sound level; **Right, middle figure**: Wave I latency plotted against sound level. **Right, lower figure**: electric potential (in µV) measured in individual trials plotted against number of trials.

To minimise respiratory secretions, Atropin (0.00083 ml/g body weight; 0.06 mg/ml Atropin) was given s.c. For hydration, lactated ringer`s solution was injected s.c. (0.005 ml/g body weight) every two hours and to reduce brain oedema, animals received an initial injection of Dexamethason s.c. (0.0033 ml/g body weight; 3 mg/ml). In order to keep eyes moist, eye ointment was applied every 1-2 hours.

Temperature, breathing rate and pedal withdrawal reflex were checked every 30-60 minutes. For temperature control, a heating pad controlled by a rectal thermistor was used — with the temperature held at 37.5° Celsius. The breathing rate was checked and confirmed to be between 100-150 breaths/minute; if it dropped below 80 breaths/minute, animals received an injection of Dopram s.c. (for further detail, see Linden et al.[2003]). At the end of the experiments, mice were euthanised with an overdose of Pentobarbitol i.p.

2.4.2 Experimental Setup and Animal Preparation

IC recordings were performed in the same sound-proof booth as the ABR recordings and sound was presented free-field. The speaker was positioned at a 45° angle to the animal's axis at a distance of app roximately 15 cm, aimed at the left ear. The right ear was plugged with a sound-attenuating plug.

Before surgery, the ear canals were checked to make sure they were clear and they were cleaned, if necessary. When the pedal withdrawal reflex was abolished, the animal's head was fixed to a nose clamp to secure its position and facilitate breathing. The area from behind the ears up to the nose was shaved; a skin incision was made in the midline from the ears up to the area above the nose while skin was moved aside and the skull cleaned. A craniotomy was performed (circa 4.5-6.5 mm posterior to Bregma and 0.5-2.5 mm right to midline), using a drill and scalpel to expose the complete surface of the right IC. For grounding, a screw was inserted into the skull in front of the left coronar suture and connected to the booth with wire and clamps.

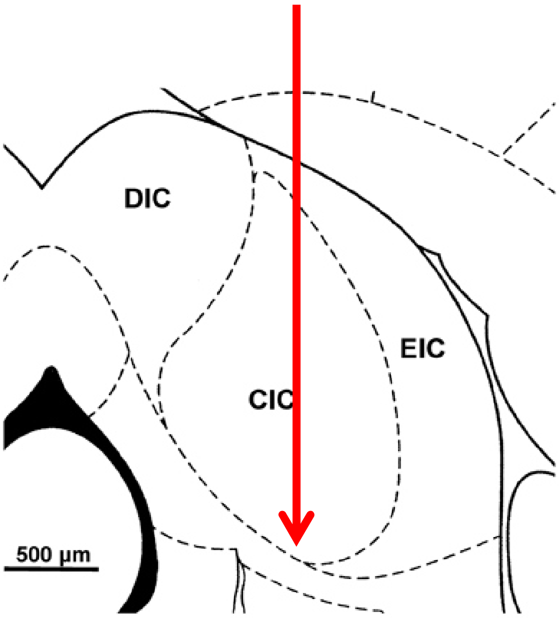
For extracellular multi-unit recordings, single shank silicon multi-electrodes with sixteen recording sites (1x16 linear array with 100 µm spacing from NeuroNexus) were used to ensure that they took place across the full extent of the ICc. Probes were positioned manually until the tip of the probe touched the central surface of the IC (5.2 mm posterior to Bregma and 1.5 mm right to midline). Probes were then advanced rapidly by 2000 µm using a remote hydraulic microdrive (Neurocraft, FHC Inc.) and then retraced rapidly by circa 400 µm to minimise neuronal damage by compression of brain tissue (electrode positioning: see Figure 7).

2.4.3 Sound Generation and Calibration

For click and tone generation, a TDT RX6 processor was used. Attenuation was performed by a TDT PA5 and amplification by a TDT SA1. Sound was presented by a TDT FF1 speaker and calibration was done for target SPL and frequency band. Sound pressure levels were ensured to vary less than ±1 dB between recordings. Codes for click and tone generation and calibration were written by Dr. Gestur Bjoern Christianson and Dr. Jennifer Linden.

2.4.4 Extracellular Recordings in the Inferior Colliculus

To investigate neuronal activity patterns in the ICc, action potentials of multiple neurons (multi-units) were recorded at each electrode recording site. Evoked firing rates were recorded in response to tone pips, presented in random order from 4-70 kHz in $1/8^{th}$ octave steps and from 0-80 dB SPL in 5 dB steps. Tone pips had a duration of 100 ms, a rise/decay time of 5 ms and an inter-stimulus-interval of 400 ms. Sound presentation was repeated 3 times for all frequencies and sound levels. To obtain SFRs of multi-units, hundred 500 ms intervals without sound presentation were recorded. During the recording of spontaneous activity, the speaker was disconnected.



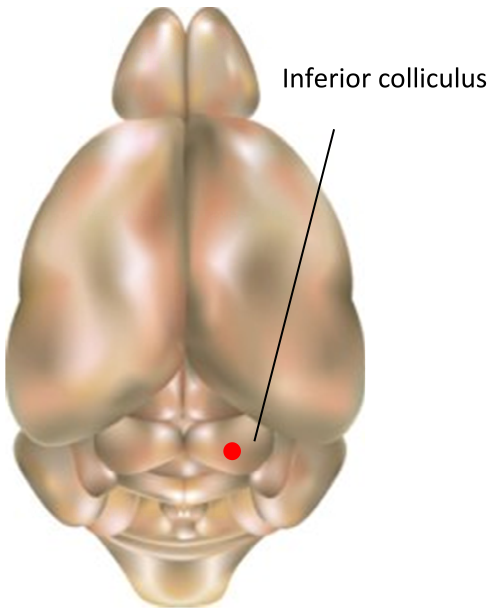


Figure 7: Top left figure: Schematic overview of a mouse inferior colliculus, coronal plane. CIC= central interior colliculus, DIC=dorsal cortex of the inferior colliculus, EIC= external cortex of the inferior colliculus. The red arrow marks the position where multi-electrodes were inserted: Figure taken from Ouda and Syka, 2012 with permission. **Top right figure**: Schematic image of a mouse brain, viewed from above. The red dot marks the position where electrodes were inserted into the inferior colliculus. Figure from http://neurosciencenews.com/neuroscience-brain-size-cognition-143/mouse-brain-size/. **Bottom left figure:** Schematic illustration of the electrode site map. Numbers mark electrode channels. Figure from http://neuronexus.com/images/Electrode%20-Site %20Map/A16_Maps_20150512.pdf, used with permission from Neuronexus. The spacing of 100 µm between recording sites enabled multiunit-recordings across the full span of the central inferior colliculus.

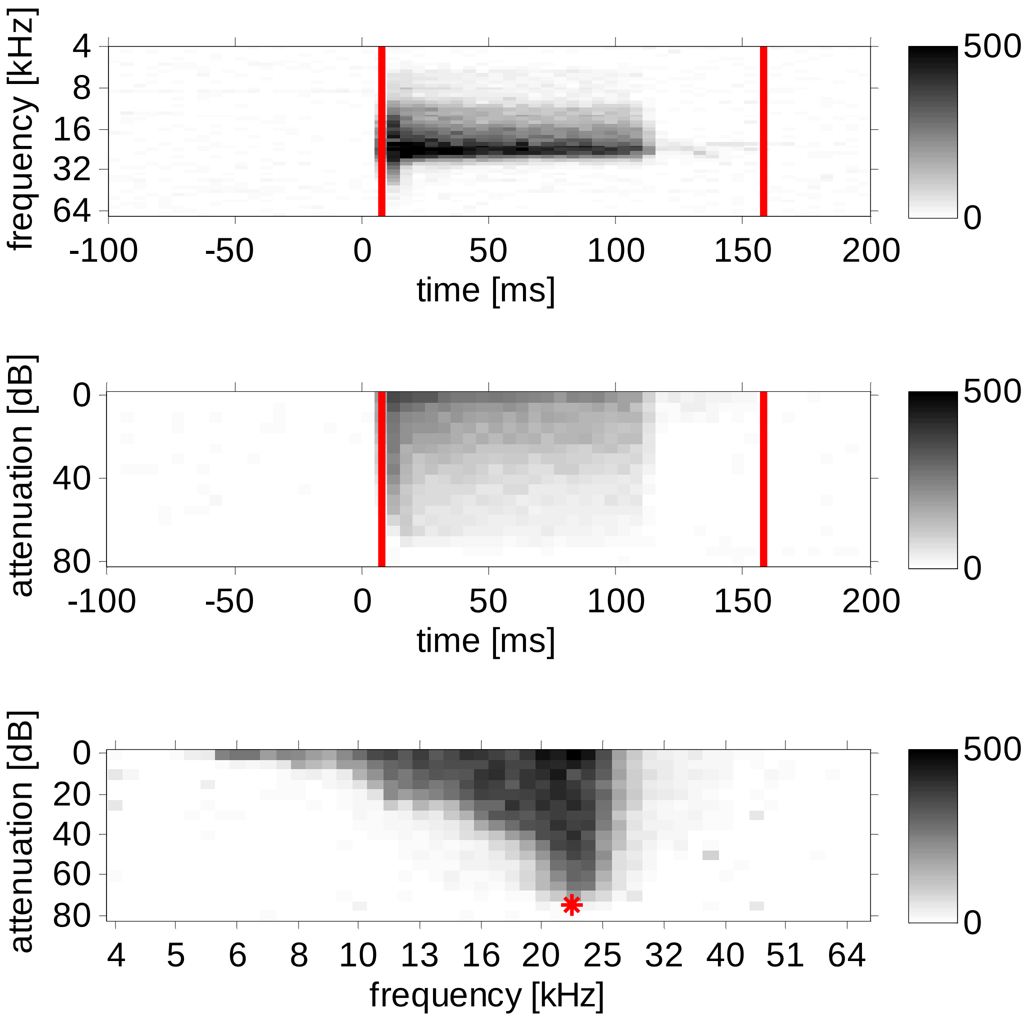


Figure 8: Example of a multi-unit response in the central inferior colliculus of a control mouse**.** The bars on the right side indicate mean spike rate scaling (black: 500 spikes/s, white: 0 spikes/s). Red lines mark the analysed time window (the response onset was defined as mean spike rate before the stimulus onset plus ten times the standard deviation; time window used for analysis: response onset in ms plus 150 ms). **Top figure**: Spike rates across time (y-axis in ms, 0: time of stimulus onset) in response to tone pip presentation from 4-70 kHz, averaged across sound level presentation (0-80 dB SPL in 5 dB steps). The highest and longest lasting responses can be seen in response to tone pip presentations at around 22 kHz. **Middle figure**: Spike rates across time (y-axis in ms, 0: time of stimulus onset) in response to 0-80 dB SPL in 5 dB steps tone pip presentation, averaged across all presented tone frequencies. Spike rates increase with loudness. **Bottom figure**: Frequency response area: Spike rate across frequencies and sound pressure levels, averaged across the analysed time window. The red * marks the frequency with the lowest response threshold, also called characteristic frequency (CF), in this example 22 kHz.

2.4.5 Data Analysis

During recordings, electrode signals (16 electrodes per mouse; Figure 7) were sampled at 24 kHz and bandpass-filtered between 300-9000 Hz. After recordings, the local field potential was removed using a high-pass filter cut-off at 600 Hz. Multi-units were separated from the background noise using a latent-variable spike sort algorithm (Sahani, 1999).

For further analysis, spike rates were averaged across intensities, frequencies or time and plotted across time against frequency, time against attenuation and frequency against attenuation (see Figure 8 for an example). Electrode signals that did not show stimulus dependent spiking activity were excluded from further analysis.

Frequency response areas (FRAs, spike rates across frequencies and attenuation, averaged across time; Figure 8) were used for analysis of characteristic frequencies (CFs) and multi-unit thresholds: the frequency with the lowest threshold — defined as a recognisable increase of spike rates in comparison to spontaneous activity — was determined visually as the CF; accordingly, the threshold was simultaneously determined as the lowest SPL where the CF response was visible (see Figure 8 for an example). To ensure that only multi-units in the ICc were included in further analysis, stimulus-dependent spiking activity was compared across all sixteen channels for each animal. If an increase of CF with increasing depth of the electrode (Figure 7) was not evident, multi-units were excluded from further analysis. Also, multi-units that did not show a CF but a similar response across presented frequencies were excluded from further analysis.

SFRs for individual multi-units were obtained by calculating and averaging spikes over the 100 repetitions of the 500 ms silent interval recordings.

Additionally, rate-level-functions (RLFs; spike rates against SPL, averaged across frequencies and time) in response to CF presentation were retrieved for each multi-unit (Figure 14).

As normal distribution could not be presumed, Mann-Whitney U tests were applied for comparing SFRs and TH between groups. Comparisons were performed for four CF bins (CF ≤8 kHz; >8 kHz and ≤16 kHz; >16 kHz and <32 kHz; >32 kHz). Moreover, due to multiple testing, Bonferroni corrections were applied. RLFs in response to CF presentation were compared between groups, using multiway analysis of variance (ANOVA). The codes for analysis were written by me and were largely based on codes written by Dr. Jannis Hildebrandt. I also performed the analysis.

2.5 Cochlear Immunhistochemistry for Synaptic Ribbon Count

In terms of evaluating potential AN fibre loss, synaptic ribbon counting was also performed. Synaptic ribbons are a presynaptic part of synapses of IHC with AN fibres (see chapter 1.2.2). As in mammals, 95% of AN fibres synapse only with one IHC the synaptic ribbon count is a good measurement for IHC afferent innervation (Kujawa and Liberman, 2009).

2.5.1 Cochlear Preparation and Immunhistochemistry

At the end of the IC recordings after animals were euthanised, both cochleae from six mice from the control group, three mice from the NE100 group and four mice from the NE105 group were taken out and fixated using 4% paraformaldehyde. For immunohistochemistry no decalcification of the temporal bone was performed to be able to identify the exact position of the cells. The bone of the apical turn, the stria vascularis and the tectorial membrane were removed to access the Organ of Corti using micropipette aspiration or fine forceps. In some cases, the bone of the basal turn was also removed. Cochleae were immunostained either with antibodies to C-terminal binding protein 2 (mouse anti-CtBP2, BD Transduction Laboratories, #612044, used at 1:200) or with antibodies to glutamate receptors (rabbit anti-GluR2/3, Millipore Bioscience, #AB1506, used at 1:50) and incubated with a standard lysine block at 4℃ overnight. As s econdary antibodies, goat anti-mouse ATTO425 (TEFLabs, at 1:200) was used to label the ribbon protein CtBP2 and biotinylated goat anti-rabbit IgG (Vector #BA-1000, at 1:200) was used to label postsynaptic terminals. Subsequent incubation was performed with either fluorescein or Alexa 488 conjugated to streptavidin (Vector, #SA-5001).

2.5.2 Synaptic Ribbon Count

Regarding counting synaptic ribbons, images were made using a laser scanning confocal microscope (Carl Zeiss LSM 510 Meta) with a 63x 1.0 NA objective, either in multiphoton or single photon confocal mode. To obtain three-dimensional reconstructions, z-axis stacks of two-dimensional images were taken in step sizes of 0.2-0.5 µm. Within the 10-30 kHz region of the cochlea, ribbons/cells were determined as averages from groups of 8-12 IHCs.

2.5.3 Data analysis

A comparison between groups was performed using one-way analysis of variance (ANOVA). Cochlea fixation was performed by me and by Warren Bakay, equally. Imaging and synaptic ribbon counts were performed by Hui Ching Ong and Raessa Qureshi.

3. Results

This study was designed to examine the effect of mild and moderate NE on the peripheral and central auditory system while asking the following questions: is mild NE — which does not cause a permanent ABR threshold shift — sufficient to cause hyperactivity in the central auditory system? If hyperactivity can be found, is it accompanied by a reduction of the amplitude of ABR wave I and histological signs of AN fibre deafferentation? Does mild acoustic trauma, i.e. trauma that only causes a temporary but no permanent ABR threshold shift, have a different effect on neuronal activity in the central auditory system than more severe acoustic trauma, which causes a permanent ABR threshold shift?

3.1 Effects of Mild and Moderate Noise Exposure on the Peripheral Auditory System

To investigate the effects of mild and moderate NE on the peripheral auditory system, ABR thresholds and wave I amplitudes in response to clicks were measured. Additionally, synaptic ribbon counts were performed on immunostained cochleas.

In summary, mild noise exposure (NE100 group) did not cause a lasting increase in ABR thresholds but nevertheless a reduction of ABR wave I amplitudes and a loss of synaptic ribbons occurred, both suggesting an AN fibre damage. After moderate trauma (NE105 group) ABR thresholds remained increased four weeks after NE. The reductions in ABR wave I amplitudes appeared more pronounced than after mild NE; synaptic ribbons were similarly decreased as after mild NE.

3.1.1 Auditory Brainstem Response Thresholds and Wave I Amplitudes

Before NE, click and tone pip ABR thresholds did not differ significantly between NE groups and the control group (Figure 9; p-values from Mann-Whitney U test in table 1). Simultaneously, before NE ABR wave I amplitudes did not differ significantly between the NE100, the NE105 and the control group (Figure 10; p-value for group effect from ANOVA: p=0.0264; Bonferroni corrected significance level alpha: p=0.05/2=0.025 due to two comparisons [amplitudes of the control group were compared with amplitudes of the NE groups before and four weeks after NE]).

One day after NE, a strong increase of ABR thresholds in response to clicks and tone pips with frequencies within and above the NE region were apparent in both NE groups with stronger increases in thresholds in the NE105 group (Figure 9). Simultaneously, one day after NE, supra-threshold ABR wave I amplitudes in response to clicks appeared decreased similarly in both NE groups in comparison to the control group (Figure 10).

Four weeks after NE on the day of IC recordings, ABR thresholds had fully recovered in the NE100 group and did not show significant differences to the control group (Figure 9, p-values from Mann-Whitney U tests in table 1). In the NE105 group, recovery was incomplete and a significant ABR threshold shift was evident in response to clicks and in response to 11 kHz and 16 kHz tone pips in comparison with the control group (Figure 9; p-values from Mann-Whitney U tests in table 1). A small increase in ABR thresholds was also seen in response to 48 kHz tone pips in the NE105 group; however, this increase did not prove significant in comparison to the control group (Figure 9; p-values from Mann-Whitney U tests in table 1).

Simultaneously, four weeks after NE, wave I amplitudes were still decreased in both NE groups and a significant effect of group was evident (p-value from ANOVA for group effect: p<0.00001). Although ABR wave I amplitudes appeared to have decreased in both NE groups in comparison with the control group four weeks after NE, a certain degree of recovery seemed to have occurred in the NE100 group compared to day one after NE, but not in the NE105 group (Figure 10).

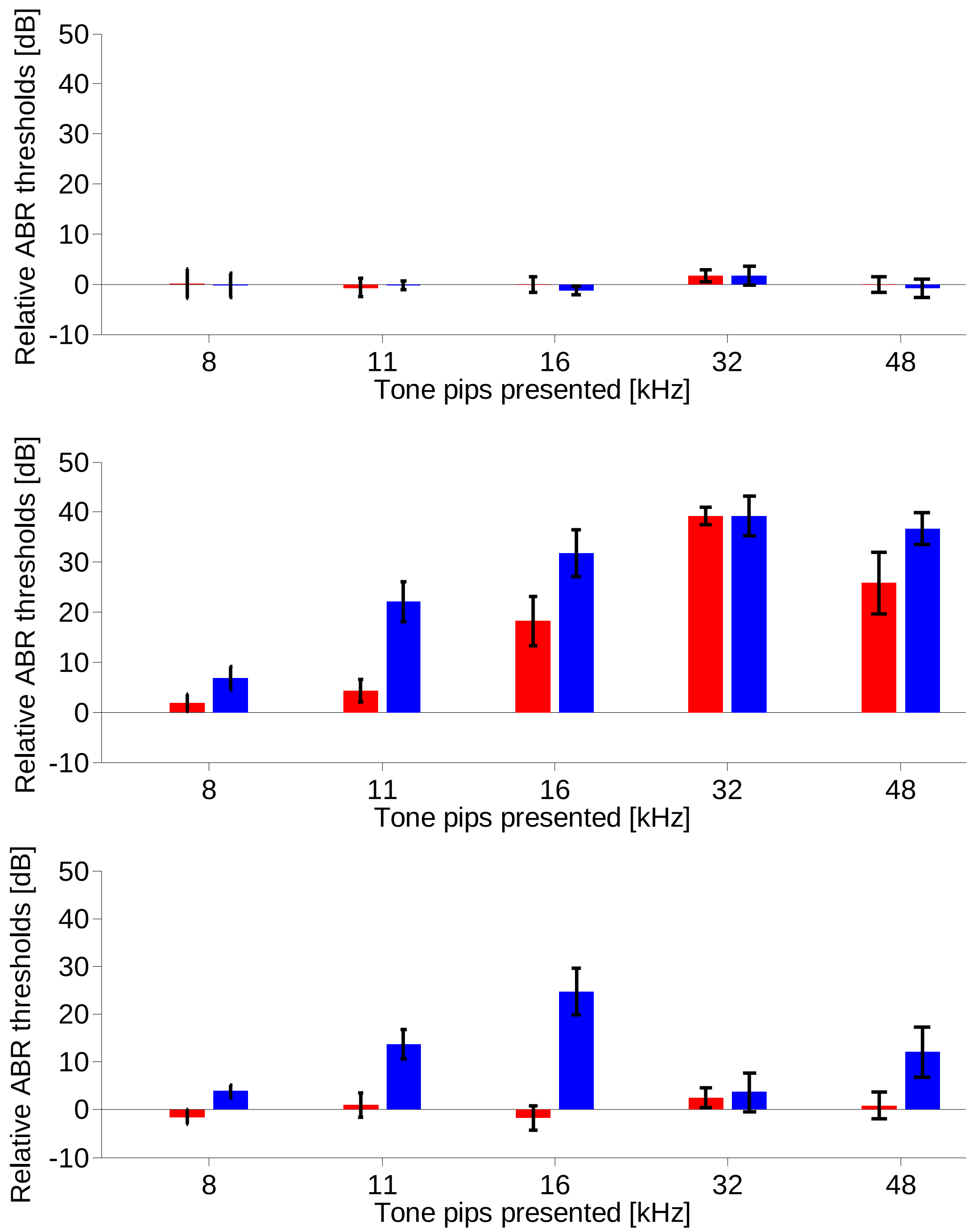


Figure 9: Mean and standard error of the mean tone pip auditory brainstem response (ABR) thresholds (THs) of the NE100 group (noise exposure: 8-16 kHz, 100 dB SPL, 2 hours; bars in red) and the NE105 group (noise exposure: 8-16 kHz, 105 dB SPL, 2 hours; bars in blue), relative to ABR THs in the control group. **Upper panel**: before noise exposure; **middle panel**: one day after noise exposure and **lower panel**: four weeks after noise exposure.

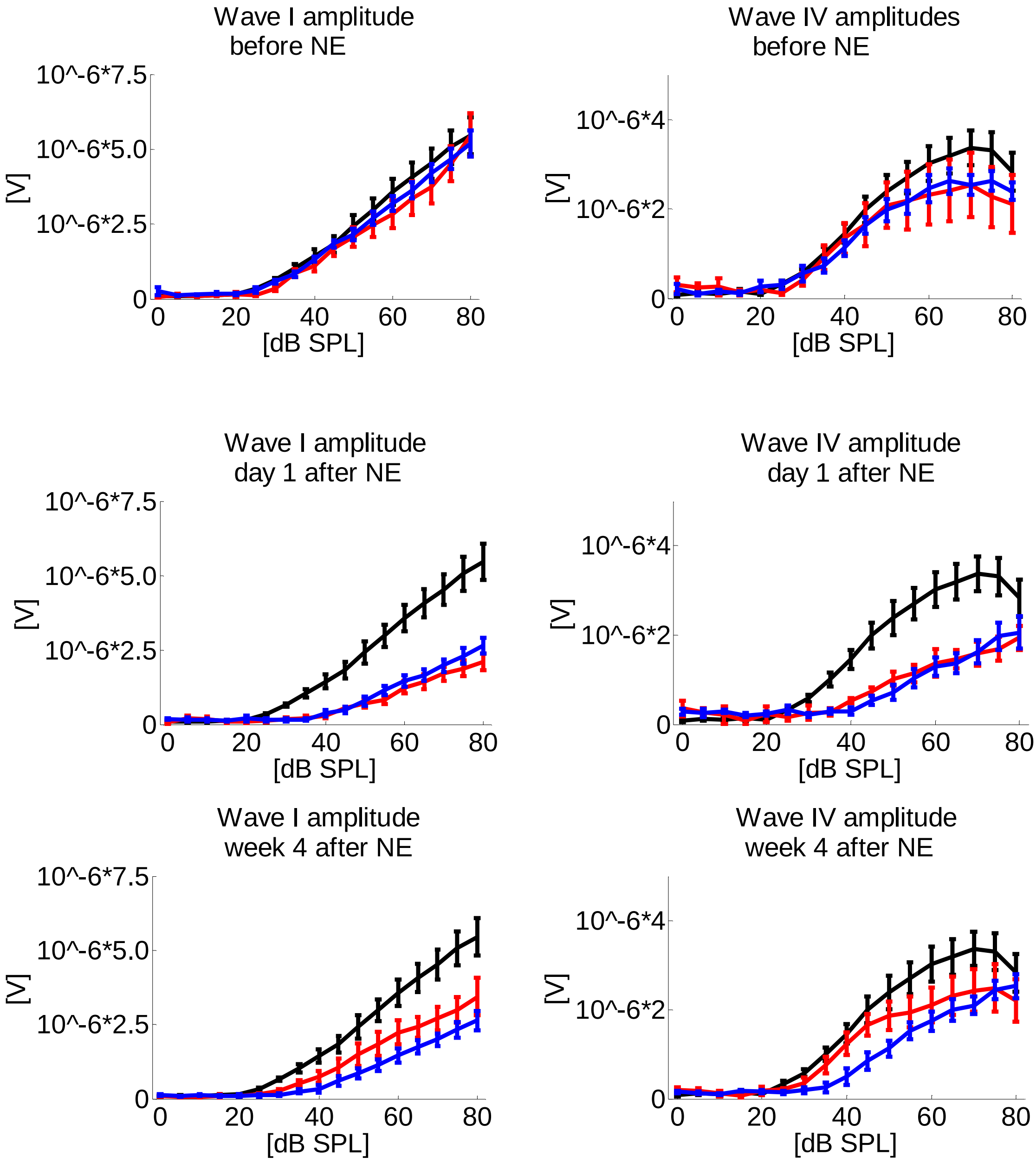


Figure 10: Auditory brainstem response wave I and wave IV amplitudes of the left ear in response to clicks, plotted against sound pressure level (in dB SPL). **Column 1:** wave I amplitudes; **column 2**: wave IV amplitudes. **Row 1:** before noise exposure (NE); **row 2:** 1 day after NE; **row 3:** 4 weeks after NE. For comparison, wave amplitudes of the control group (black) are plotted in each figure. Amplitudes of the NE100 group (8-16 kHz, 100 dB SPL noise exposure for 2 hours) are plotted in red, of the NE105 group (8-16 kHz, 105 dB SPL noise exposure for 2 hours) in blue.

		Click RE	Click LE	8 kHz	11 kHz	16 kHz	32 kHz	48 kHz
BNE	NE100 vs C	1	0.2529	0.9767	1	1	0.6364	0.8918
	NE105 vs C	0.9718	0.4593	0.8316	1	0.6429	0.5679	0.8531
ANE	NE100 vs C	0.4009	0.1469	0.7902	0.8019	0.4464	0.4719	0.6883
	NE105 vs C	0.0116	0.0015	0.2268	0.0062	0.0011	0.8034	0.1375

Table 1: P-values from Mann-Whitney U tests, comparing auditory brainstem response thresholds between noise exposure groups and the control group before noise exposure (BNE), and four weeks after noise exposure (ANE). NE100= group, exposed to an 8-16 kHz noise at 100 dB SPL for two hours; NE105= group, exposed to an 8-16 kHz noise at 105 dB SPL for two hours; C= control group. Clicks were presented to the right ear (RE) and the left ear (LE), tone pips (8 kHz, 11 kHz, 16 kHz, 32 kHz and 48 kHz) were presented to the LE. Because of four comparisons (amplitudes of the NE100 and the NE105 group were compared to the control group before and four weeks after NE), the statistical significance level was adjusted from 0.05 to 0.05/4=0.0125, using Bonferroni correction. Statistically significant p-values are marked in red.

3.1.2 Synaptic Ribbon Counts

In addition to ABR measurements, ribbon loss was quantified using immunohistochemistry. Ribbon counting was performed in three regions of the cochlea from representative animals from the control group (n=6), the NE100 group (n=3) and the NE105 group (n=4). Counts were averaged across regions. In both NE groups a reduction of synaptic ribbons was observed (Figure 11). The difference in synaptic ribbon counts per IHC between groups proved significant (p-value from one-way-ANOVA: p=0.03).

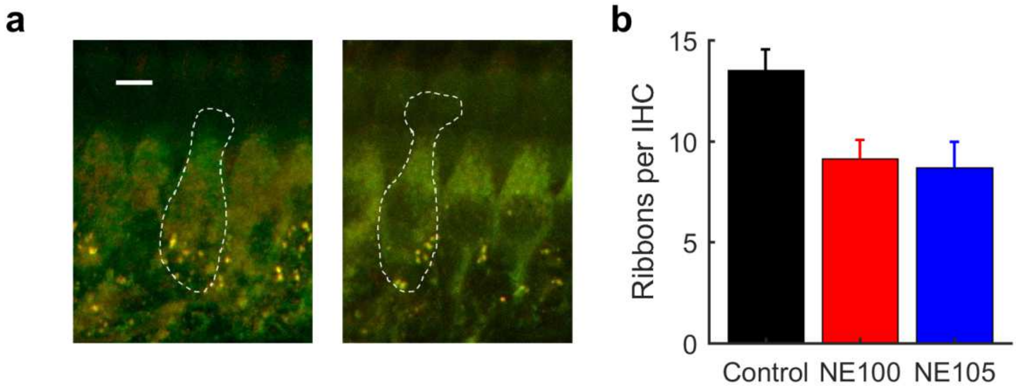


Figure 11: **a:** Example images of immunostained inner hair cells (IHCs) from a control mouse (left, scale bar = 5µm) and a mouse exposed to 105 dB SPL noise (right), with synaptic ribbons labelled green (anti-595 CtBP2) and postsynaptic terminals red (anti-GluR2/3). **b:** Bar histogram, showing mean numbers of synaptic ribbons per IHCs (black, control group [n = 6 cochleas]; red, NE100 group [8-16 kHz, 100 dB SPL noise exposure for 2 hours; n = 3 cochleas]; blue, NE105 group [8-16 kHz, 105 dB SPL noise exposure for 2 hours; n = 4 cochleas]).

3.2 Effect of Mild and Moderate Noise Exposure on the Central Auditory System

To investigate the effect of mild and moderate NE on the central auditory system, ABR wave IV amplitudes were analysed as well as the spontaneous and evoked activity of multi-units in the ICc. To sum up, in comparison to the control group, significant increases in SFRs of multi-units in the ICc were found in both NE groups which supports the hypothesis that indeed increased SFRs can be caused by cochlear damage with and without hearing loss. Interestingly, increases in SFRs were more pronounced after mild NE (NE100 group) than after more severe NE (NE105 group). Evoked responses also differed between groups: whereas mild NE (NE100 group) and more severe NE (NE105 group) appeared to have similar effects on CF distribution, there were differences in effects on response threshold and evoked firing rates. In line with ABR thresholds, mild NE (NE100 group) had no significant effect on response thresholds whereas moderate NE (NE105) significantly increased response thresholds for multi-units with CFs between 8-32 kHz. Rate level functions appeared similarly reduced in both NE groups in comparison to the control in response to high sound intensities. In response to low sound intensities, however, rate level functions were less reduced after mild NE in comparison to more severe NE.

3.2.1 Auditory Brainstem Response Wave IV Amplitudes

Interestingly, before NE, ABR wave IV amplitudes proved significantly different between NE groups and the control (Figure 10, p-value for group effect from ANOVA: $p=0.0043$; as NE groups were tested against the control group before and after NE, Bonferroni correction was applied. Bonferroni-corrected significance level alpha: $0.05/2=0.025$).

However, one day after NE, supra-threshold ABR wave IV amplitudes appeared strongly decreased in both NE groups in comparison to the control group (Figure 10). The overall strength of amplitude reduction appeared similar in both NE groups — one day after NE, the amplitude growth curve simultaneously seemed to have changed from an s-shape before NE to a linear shape in both NE groups (Figure 10).

Four weeks after NE on the day of the IC recordings, ABR wave IV amplitudes strongly differed between groups (Figure 10, p-value for group effect from ANOVA: $p<0.00001$; Bonferroni-corrected significance level alpha: 0.025). Although ABR wave IV amplitudes had not fully recovered four weeks after NE, some recovery appeared to have happened when comparisons were done between ABR wave IV amplitudes four weeks after NE and ABR wave IV amplitudes before and one day after NE (Figure 10). Recovery appeared to differ between NE groups: in the NE100 group, recovery appeared more pronounced than in NE105 (Figure 10); also the ABR wave amplitude growth function was back to being s-shaped in NE100, whereas it remained rather linear in NE105 (Figure 10). Furthermore, a difference in ABR wave IV response threshold between groups was apparent (Figure 10): whereas the mean threshold for ABR wave IV amplitude response in the NE100 group appeared similar to the threshold in the control group, the mean ABR wave IV threshold in NE105 was still increased by 10-15 dB SPL (Figure 10).

3.2.2 Evoked activity of Multi-Units in the Inferior Colliculus

In the control group, 66 multi-units from 7 mice were recorded from the ICc. In the NE100 group, 56 multi-units from 6 animals were detected, along with 111 multi-units from 10 animals in the NE105 group.

CFs ranged from 6-38 kHz in the control group. Most units had a CF between 16 kHz and 32 kHz (n=40, 60.61%). The other multi-units were distributed as follows: ≤8 kHz: n=5 (7.58%); >8 and ≤16 kHz: n=17 (25.76%) and >32 kHz: n=4 (6.6%).

In the NE groups, CF distribution appeared to be different (Figure 12): in both NE groups more units with CFs within the NE region (>8 and ≤16 kHz) were found. Simultaneously, a decrease of units was found one octave above the NE region (>16 and ≤32 kHz) in both NE groups.

In more detail, CFs ranged from 6-57 kHz in the NE100 group and from 5-48 kHz in NE105 (Figure 11). In NE100, most multi-units had CFs >8 and ≤16 kHz (n=21, 38.89%); 5 multi-units had CFs ≤8 kHz (9.26%); 12 multi-units had a CF >16 kHz and ≤32 kHz (22.22%) and 16 multi-units a CF >32 kHz (29.63%). Similarly, CFs in NE105 showed the following distribution: most multi-units had CFs >8 and ≤16 kHz (n=53, 47.75 %); 9 multi-units had CFs ≤8 kHz (8.11%); 42 had a CF between 16-32 kHz (37.84%) and 7 multi-units had CFs higher than 32 kHz (6.31%).

CF thresholds in the control group ranged from 0-45 dB SPL and had a mean ± SEM of 15.68 ± 1.30 dB SPL. The lowest CF thresholds could be found in the middle frequency regions, whereas thresholds in the high and low frequency regions were higher (Figure 13).

In the NE100 group, CF thresholds ranged from 0-60 dB SPL with a mean ± SEM of 17.41 ± 1.65 dB SPL. In the NE105 group, mean thresholds were higher (mean ± SEM: 31.62 ± 1.43 dB SPL) with thresholds ranging from 0-65 dB SPL. When separating thresholds according to CF bins, thresholds were significantly increased in the NE105 group for multi-units with CFs >8 kHz and ≤32 kHz (Figure 12; p-values from Mann Whitney U tests in table 2). In the NE100 group, threshold changes did not prove significant (p-values from Mann Whitney U tests in table 2).

To further evaluate the evoked activity of multi-units, RLFs of all multi-units in response to CF were compared between groups (Figure 13). The group effect proved to be significant (p value for group effect from ANOVA: $p<0.00001$). Also the effect of sound intensity was significant (p value for intensity effect from ANOVA, $p<0.00001$) and a significant interaction of sound intensity and group was found (p value for interaction between intensity and group from ANOVA: $p<0.00001$): evoked firing rates (EFRs) in response to low sound intensities (0-5 dB SPL sound presentation) varied between NE groups: in NE100, EFRs appeared higher than EFRs in the control group; in NE105, EFRs in response to CF presentation at very low levels appeared similar, possibly even slightly decreased in comparison to the control group and to the NE100. Increases in EFRs with increasing sound intensities were higher in NE105 than in the NE100 group, resulting in seemingly identical EFRs in both NE groups in response to CF presentation at high sound intensities (Figure 14).

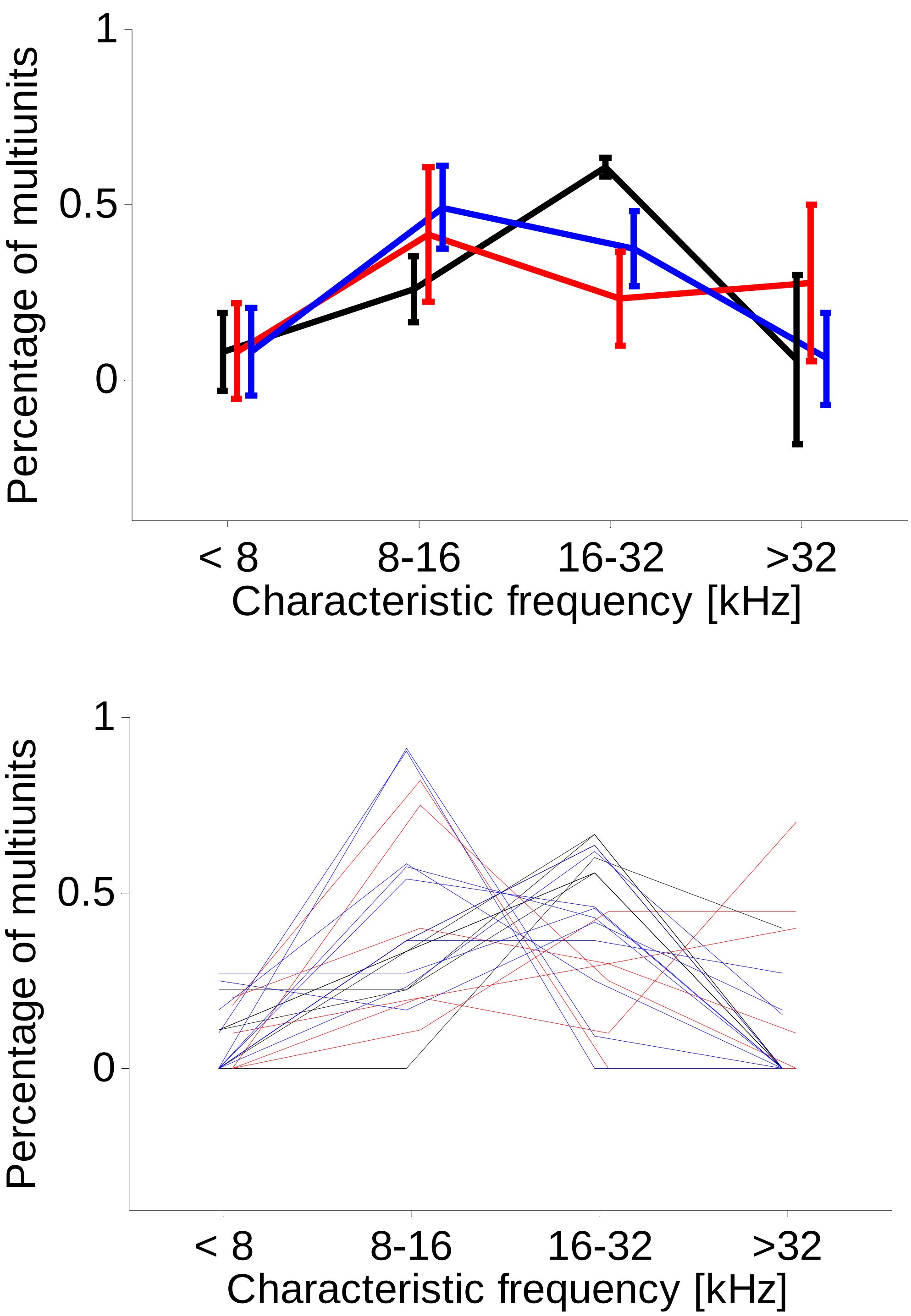


Figure 12: Effect of noise exposure (NE) on characteristic frequency (CF) distribution: **Upper figure**: plot, showing the mean ± standard error of the mean of percentages of multi-units across CF bands (≤8 kHz; >8 kHz and ≤16 kHz; >16 kHz and <32 kHz; >32 kHz); **lower figure:** plots, showing the percentages of multi-units across CF bins for each mouse individually. Black: control group; red: NE100 group (noise exposure: 8-16 kHz, 100 dB SPL, 2 hours); blue: NE105 group (noise exposure: 8-16 kHz, 100 dB SPL, 2 hours).

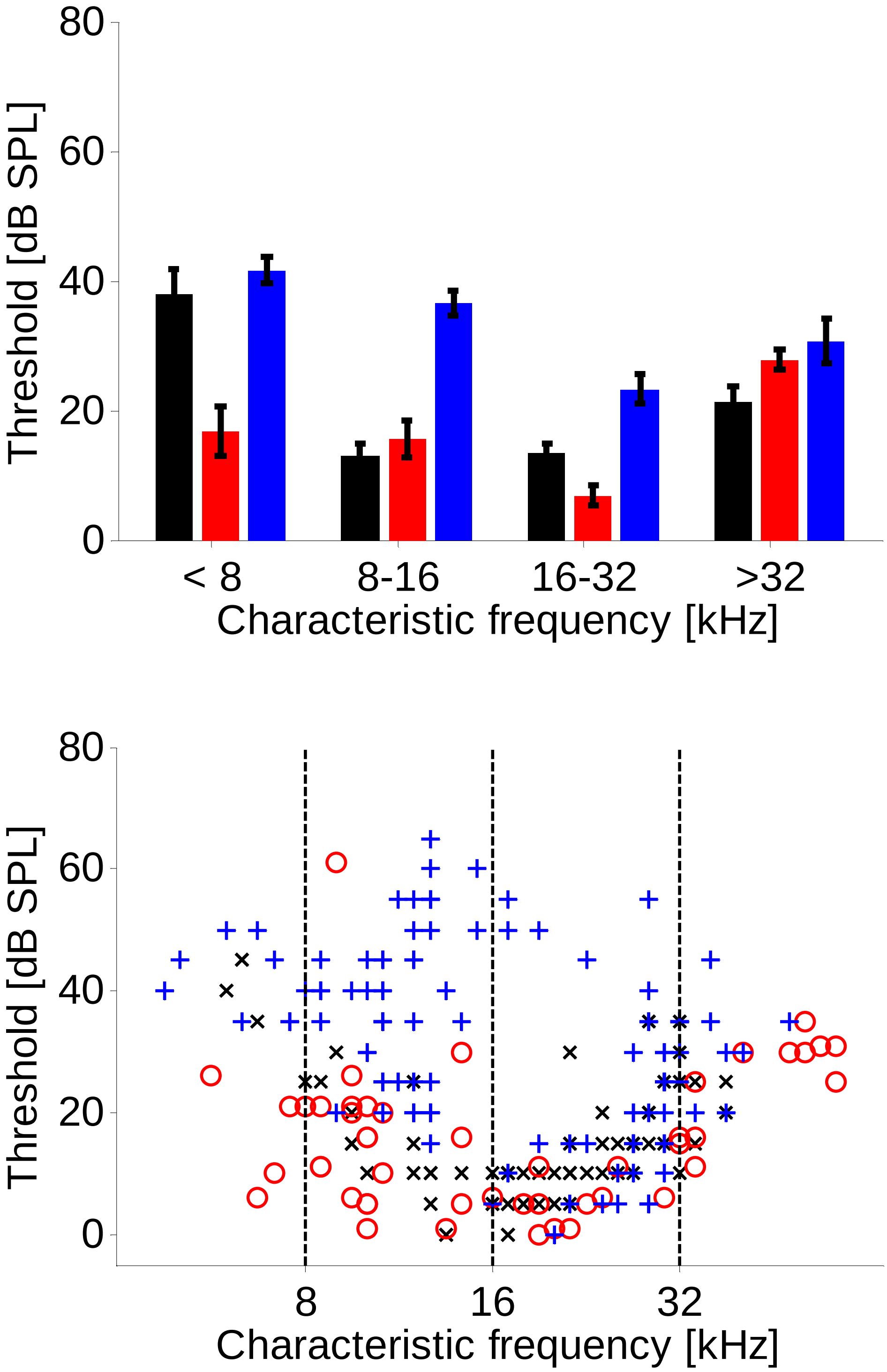


Figure 13: Effect of noise exposure (NE) on characteristic frequency (CF) thresholds (THs): **upper figure**: bar histogram, showing the distribution of mean ± standard error of the mean THs across CF bands (CF ≤8 kHz; >8 kHz and ≤16 kHz; >16 kHz and <32 kHz; >32 kHz); **lower figure**: scatter plot showing THs of individual multi-units. . Black: control group; bright red: NE100 group (noise exposure: 8-16 kHz, 100 dB SPL, 2 hours); blue: NE105 group (noise exposure: 8-16 kHz, 105 dB SPL, 2 hours).

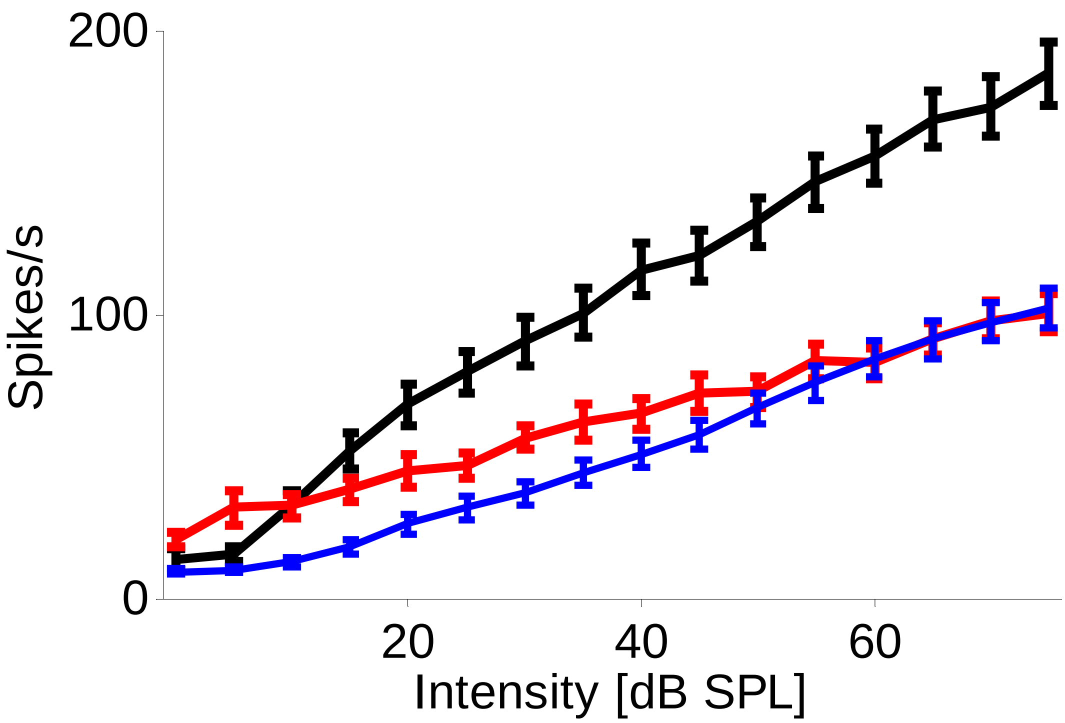


Figure 14: Rate level function: Mean ± standard error of the mean spike rates (MSR ± SEM) of multi-units in response to CF presentation from 0-75 dB SPL. Black: control group; red NE100 group (noise exposure: 8-16 kHz, 100 dB SPL, 2 hours); blue NE105 group (noise exposure: 8-16 kHz, 105 dB SPL, 2 hours)

CF		>8 kHz and ≤16 kHz	>16 kHz and ≤32 kHz	>32 kHz
SFR	NE100 vs C	<0.00001	0.0002	0.05391
	NE105 vs C	0.0073	0.0073	0.9273
TH	NE100 vs C	0.4969	0.0305	0.0272
	NE105 vs C	<0.00001	0.001	0.1091

Table 2: P-values from Mann Whitney U tests, comparing spontaneous firing rates (SFR) and thresholds (TH) in relation to characteristic frequencies (CF; >8 kHz and ≤16 kHz, >16 kHz and ≤32 kHz or >32 kHz) of multi-units in the central inferior colliculus between noise exposure groups and the control group. NE100= group exposed to a 8-16 kHz noise at 100 dB SPL for two hours; NE105= group exposed to a 8-16 kHz noise at 105 dB SPL for two hours; C= control group. Statistical analysis on multi-units with CFs <8 kHz was abandoned due to the small set of data for this CF bin (see results for evoked activity, section below). Accordingly, in total 6 comparisons were performed: both NE groups were compared to the control group for 3 CF bins and the p-value of significance was adjusted to p=0.05/6 =0.00833 using Bonferroni correction. Significant p-values are marked in red.

3.2.3 Spontaneous Activity of Multi-Units in the Inferior Colliculus

Mean SFRs in the control group were low (mean ± standard error of the mean [SEM]: 7.04 ± 0.74 spikes/s). When separating SFRs according to the determined CFs (≤8 kHz; >8 kHz and ≤16 kHz; >16 kHz and ≤32 kHz and >32 kHz), SFRs gradually increased with CFs (Figure 15).

In comparison to the control, mean SFRs in the NE groups appeared higher (NE100 group: mean ± SEM: 26.30 ± 2.35 spikes/s; NE105 group: mean ± SEM: 11.69 ± 0.93 spikes/s). Significant increases in SFRs were found for multi-units with CFs within the NE region and one octave above (≥8 kHz <32 kHz) in the NE100 group (Figure 15; p-values from Mann Whitney U tests are listed in table 2). In the NE105 group, significant increases in SFRs in comparison to the control group were found for multi-units with CFs ≥8 and <32 kHz (Figure 15; p-values from Mann Whitney U tests are listed in table 2).

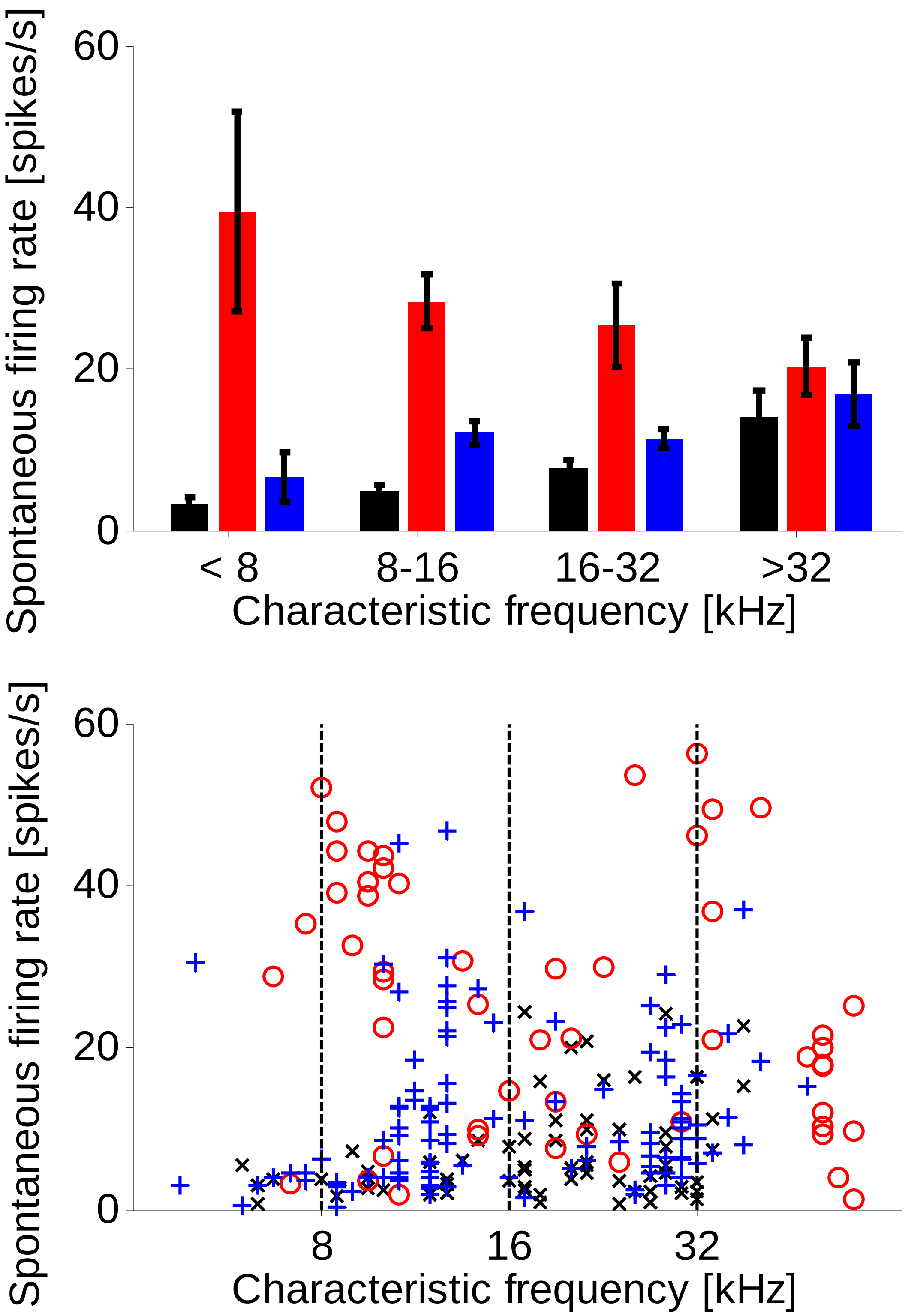


Figure 15: Effect of noise exposure (NE) on spontaneous firing rates (SFRs): **upper figure**: bar histogram, showing the distribution of mean ± standard error of the mean SFRs across characteristic frequency (CF) bands (≤8 kHz; >8 kHz and ≤16 kHz; >16 kHz and <32 kHz; >32 kHz). **Lower figure**: scatter plot, showing the distribution of SFRs of individual multi-units in relation to CF. Black and x: control group; bright red and o: NE100 group (noise exposure: 8-16 kHz, 100 dB SPL, 2 hours); blue and +: NE105 group (noise exposure: 8-16 kHz, 105 dB SPL, 2 hours).

4. Discussion

I investigated the effect of mild and moderate noise exposure on cochlear structures and on ICc multi-units in CBA/Ca mice while addressing the following questions: is mild NE that causes a temporary ABR thresholds shift (TTS) — but not a permanent ABR threshold shift (PTS) — sufficient to cause hyperactivity in the ICc? If so, is it accompanied by a reduction of ABR wave I and histological signs of AN fibre degeneration? Finally, does the effect on the AN and the ICc differ after mild NE (only TTS) in comparison to more severe NE that causes a permanent shift of ABR thresholds?

I found that mild NE (TTS only) was indeed sufficient to cause hyperactivity in the ICc. As predicted, it was accompanied by a reduction of ABR wave I and histological signs of AN fibre deafferentation. Interestingly, mild NE (TTS only) caused stronger increases in SFRs in the ICc than moderate NE (PTS), suggesting an non-monotonic relationship between the severity of noise trauma and the level of central hyperactivity.

Whether or not these central changes indeed represent neuronal correlates of tinnitus remains to be clarified in future studies.

4.1 Effects of Mild and Moderate Noise Exposure on the Peripheral Auditory System

Mild acoustic trauma (NE100 group) was induced by an 8-16 kHz noise, presented at 100 dB SPL for two hours to anaesthetised mice. This noise paradigm was based on Kujawa and Liberman (2009), who used a similar paradigm to induce HHL (TTS only) in CBA/Ca mice. Similar to findings from Kujawa and Liberman (2009), mice in the NE100 group in this study showed increased ABR thresholds one day after NE; the largest threshold increase was at 32 kHz and recovered ABR thresholds occurred four weeks after NE. Additionally, in line with findings from Kujawa and Liberman (2009), suprathreshold ABR wave I amplitudes had not fully recovered and were lower than amplitudes in the control group four weeks after NE. Also, synaptic ribbon counts were reduced, both indicating AN fibre deafferentation. Despite similar findings, it has to be noted that in contrast to Kujawa and Liberman's study (2009), mice were anaesthetised during NE in this study, thus implying that effects of NE on the auditory system might differ.

Moderate acoustic trauma (NE105 group) was caused by an 8-16 kHz octave band noise, this time presented at 105 dB SPL for two hours. In NE105, ABR thresholds in response to 11 kHz and 16 kHz had not recovered four weeks after NE, indicating more severe cochlear damage in NE105 than in NE100, presumably including OHC loss or permanent damage to IHC stereocilia next to AN fibre deafferentation (Liberman and Dodds, 1984). Simultaneously, ABR wave I amplitudes in response to clicks appeared more strongly decreased in NE105 in comparison to NE100, suggesting a more pronounced AN fibre deafferentation in NE105 than NE100; additional loss of IHCs should also be considered. In the cochlear histology, synaptic ribbon counts were similarly reduced in both NE groups in comparison to the control group.

Effects go in line with the findings of Rüttiger et al. (2013) who used NEs with durations of 1 and 1.5 hours at 120 dB SPL, and respectively found stronger increases in ABR thresholds and a more severe AN fibre deafferentation after longer NE.

In general, it is well established that the ABR wave I in both animals and humans reflects the synchronous activity of AN fibres (Buchwald and Huang, 1975; Kiang et al., 1976; Møller and Jannetta, 1981). It is also known that AN fibres vary strongly in spontaneous firing rates and thresholds for evoked activity. Commonly, AN fibres are therefore divided into three types: high SFR, medium SFR and low SFR fibres; high SFR fibres have low thresholds for evoked activity and low SFR fibres have high thresholds for evoked activity (Liberman, 1978; Winter et al., 1990). Accordingly, it has been suggested that a selective deafferentaion of AN fibres with low and medium spontaneous rates is present after mild NE (TTS only), causing reduced AN activity in response to high sound intensities but normal thresholds (Furman et al., 2013). Furman et al. (2013) therefore examined responses of single AN fibres after mild NE which did not cause a permanent ABR threshold shift but a loss of suprathreshold ABR wave I amplitudes and indeed found a change in population statistics. This goes in line with the idea of a selective deafferentation of AN fibres with low and medium SFR rates. However, it should also be considered that the change in population statistics might also be caused by changes in the activity patterns of remaining AN fibres.

Bourien et al. (2014), however, showed that the ABR wave I amplitude reflects almost exclusively activity of high and medium SFR fibres of the AN. Low SFR fibres did not contribute to N1 in CAP or to the ABR wave I amplitude respectively, because of their broadly distributed spike latencies (Kiang et al., 1976). Accordingly, a reduction in ABR wave I amplitude would not represent a loss of AN fibres with low spontaneous rates but would reflect a reduced activity of AN fibres with medium and high spontaneous rates, either due to loss or decreased synchronous activity. Besides, it should be noted that normal hearing thresholds can exist in spite of substantial AN fibre loss (Bourien et al., 2014; Chambers et al., 2016).

Considering findings from Bourien et al. (2014), reductions of wave I amplitudes indicate a loss AN fibres or a loss of synchronous firing of AN fibres with medium and high SFRs in both NE groups — and this was more pronounced in NE105. Indeed, a loss of medium and high SFR AN fibres could explain both the loss of synaptic ribbons and the reduction of ABR wave I amplitudes. However, synaptic ribbon counts do not involve a determination of affected AN fibre types, and moreover, a damage of low SFR high threshold AN fibres cannot be ruled out. The loss of synaptic ribbons could indeed reflect any combination of AN fibre loss: including the sole loss of low SFR fibres and wave I amplitudes might then have to be explained by a lack of synchrony of medium and high SFR fibres.

Considering findings from Furman et al. (2013), low SFR AN fibres are probably also affected, and accordingly a loss of all types of AN fibres seems likely.

4.2 Effects of Mild and Moderate Noise Exposure on Spontaneous Activity in the Central Nucleus of the Inferior Colliculus

Indeed, mild NE with only temporary ABR threshold shifts but deafferentation of AN fibres (NE100 group), proved to be sufficient to cause hyperactivity in the central auditory system. Increased SFRs of multi-units in the ICc were found after both mild NE (TTS only, NE100 group) and more severe NE (PTS, NE105 group). Interestingly, SFRs increased less after more severe NE (PTS, NE105 group) than after mild NE (TTS only, NE100 group). Furthermore, a more pronounced reduction of ABR wave I amplitudes (NE105 group) coincided with less increases in SFRs of multi-units in the ICc.

Results suggest that spontaneous activity in the ICc might depend on input from the AN four weeks after NE. This corresponds with findings from Mulders and Robertson (2009) who showed that four weeks after NE, ablation of the AN abolishes hyperactivity in the IC. Also, Manzoor et al. (2012) showed that multi-unit activity in the IC depends on input from DCN neurons 2-3 weeks after NE.

A strong dependency of spontaneous neuronal activity in the ICc on AN fibre activity could explain the observed coincidence of varying cochlear damage and differing increases of SFRs in the ICc: if increases in central neuronal activity are dependent on input from the AN, the pattern of AN fibres loss determines characteristics of central hyperactivity. That is, homeostatic central gain changes (increased neuronal excitability, increased excitation and reduced inhibition) after a loss of high threshold AN fibres with usually low spontaneous activity would amplify the high spontaneous activity of remaining low (and medium) threshold AN fibres and would result in high SFRs and increased evoked activity in response to low level sounds. Simultaneously, ABR thresholds would not be affected or might even be reduced and evoked activity would be increasingly impaired with increasing sound intensity. These patterns have indeed been observed here after mild NE (NE100 group). More severe noise exposure, as used in the NE105 that showed PTS, could also affect low threshold AN fibres with high SFRs and might even lead to inner or outer hair cell loss. In that case amplification of the activity of the remaining AN fibres will only lead to weaker changes in central activity

(Schaette and Kempter, 2006; Hesse et al., 2016). As a result, SFRs in the central auditory system should show a less pronounced increase, or even no increase at all; evoked activity should be impaired in response to low and high level sounds. These patterns have been observed here after moderate NE (NE105 group).

Interestingly, other studies showed that beyond four weeks, neuronal hyperactivity in the IC and in the DCN does not depend on AN activity (Zacharek et al., 2002; Mulders and Robertson, 2011), thus raising the question of if and how spontaneous activity might change at the level of ICc later in time.

In contrast to our findings, other studies have suggested a linear relationship between threshold increase and hyperactivity (Mulders and Robertson, 2009; Mulders et al., 2011). However, Mulders et al. solely used NE that caused permanent ABR threshold shifts and presumably OHC and IHC loss. They did not investigate the effects of mild NE only causing AN fibre deafferentation and TTS. Also they did not perform within study comparisons of different degrees of NE. With increasing severity of NE, different cochlear structures might be damaged — including IHC and OHC loss — and central adaption might differ in comparison to adaption after less intense NEs which does not cause IHC and OHC loss. In line with this, a nonmonotonic relationship between cochlear trauma and the development of central hyperactivity was also found for ototoxic damage caused by the administration of cisplatin, which primarily affects OHCs, with IHC loss only occurring at high doses: Kaltenbach et al. (2002) found that hyperactivity in the DCN positively correlated with the degree of OHC loss up to the point where additional IHC loss occurred, which lead to a marked reduction in the level of spontaneous neuronal activity, suggesting the IHC loss might play a role in reducing central hyperactivity.

Various studies investigating the effect of NE on spontaneous and evoked activity in the central auditory system have either focused on high intensity noise exposure, causing permanent hearing loss (Kaltenbach et al., 2004; Mulders and Robertson, 2009; Manzoor et al., 2012), or on mild noise exposure, causing only temporary hearing loss (Brozoski et al., 2002; Middleton et al., 2011; Dehmel et al., 2012). Simultaneous analysis of both kinds of noise exposures has not been done, possibly explaining why differences in effects on spontaneous activity have not been reported.

Another important difference between our study and Mulders and Robertson's above, is that they used unilateral NE whereas we used bilateral NE in this study. At the level of the IC, binaural information converges and normal input from one ear might possibly alter homeostatic changes at the level of the ICc. It should be noted that most studies on adaption in the central auditory system have used unilateral NE for induction of central adaption. Detailed comparisons between the effects of unilateral vs bilateral NE are missing thus far. As bilateral NE corresponds better to the NE of human listeners, it should particularly be studied to allow comparisons.

In both NE groups, SFRs were significantly increased for multi-units with CFs ranging from >8 to ≤32 kHz with increases being more pronounced in NE100 in comparison to NE105. Scatter plots (Figure 12) suggest that similar to other findings after intense monaural (Mulders and Robertson; 2009; Vogler, 2014) or binaural (Manzoor et al., 2012) NE, in the NE105 group, the tonotopic distribution of ICc multi-units with increased SFRs and the frequency region were ABR thresholds were increased was similar (Figure 11). In NE100, however, scatter plots indicate that multi-units with increased SFRs mainly border the region, where hearing was temporarily impaired (Figure 11). This corresponds with recent findings in the DCN: Dehmel et al. (2014) found increases in spontaneous activity being mainly located at the lower frequency border, where hearing was temporarily impaired. It should be noted though that the study of this thesis was not designed to investigate spontaneous and evoked activity of multi-units in the ICc in detail and presumed differences should only be used as indicators for further studies.

A potential cause for the suspected differences in tonotopical distribution of hyperactivity after mild NE (TTS only) and moderate NE (PTS) might lie in differences in cochlear damage and output and/or in differences of tonotopic map reorganisations at the level of the IC.

4.3 Effects of Mild and Moderate Noise Exposure on Evoked Activity in the Central Auditory System

In addition to spontaneous activity, evoked activity was studied at the level of the ICc analysing ABR wave IV amplitudes and evoked activity of multi-units in the ICc. Evoked activity also differed between groups: in line with changes in ABR thresholds, significant increases in response thresholds of multi-units in the ICc were found after moderate NE (NE105 group), but not after mild NE (TTS, NE100 group) in comparison to the control group. Interestingly, evoked firing rates in response to CF presentation appeared to be reduced in both NE groups in comparison to the control group, and not normal or increased, as might have been expected if central auditory adaption had fully counteracted reduced input from AN fibres.

4.3.1 Auditory Brainstem Response Wave IV Amplitudes

ABR wave IV amplitudes correspond to synchronous activity of neurons projecting from the SOC to the IC in mice (Kiang et al., 1976). ABR wave IV amplitudes did not prove normal four weeks after NE, as might have been expected, if central hyperactivity had fully counteracted reduced input from AN fibres. However, after mild NE (NE100 group), a strong recovery of ABR wave IV amplitudes was apparent four weeks after NE in comparison to ABR wave IV amplitudes before, and one day after, NE. Simultaneously, ABR wave I amplitudes appeared to have recovered only moderately. This finding goes in line with the assumption that central hyperactivity had developed, (incompletely) counteracting the reduced input from the AN due to deafferentation of AN fibres but also causing increased spontaneous firing rates as seen in multi-units in the ICc.

After more severe NE (NE105 group), recovery of ABR wave IV appeared to have occurred less in comparison to ABR wave IV amplitudes before and one day after NE and in comparison to recovery in the NE100 group. Simultaneously, four weeks after NE, ABR wave I amplitudes had hardly recovered. Again, this goes in line with the idea of central hyperactivity, counteracting the reduced input from the periphery. Wave IV amplitudes appear to differ between NE groups mainly in response to low sound intensities, whereas responses to high sound intensities apparently converge. The difference in ABR wave IV amplitudes in response to low sound intensities is most probably related to differences in ABR thresholds between NE groups. However additional differences in AN fibre deafferentation and hair cell damage, including hair cell loss, might also play a role.

It should be noted that ABR wave IV amplitudes already slightly differed between groups before NE; thus interpretation of results might be impaired four weeks after NE. As pre-exposure amplitudes appear slightly lower in the NE groups in comparison to the control group before NE, relative changes to pre-exposure values might be more meaningful and indeed suggest nearly full recovery of ABR wave IV amplitudes in the NE100 group (Figure 10). On the other hand, it should be considered that at the level of the SOC, evoked activity might have already differed between groups before NE. However, wave IV amplitudes are less stable than ABR wave I amplitudes and lacking stability might explain differing results before NE. If wave IV amplitudes prove not to be stable though, comparisons of ABR wave IV amplitudes have interpreted with caution.

So far, findings on wave IV (rodents) or V (humans) amplitudes in relation to NE and/or tinnitus remain ambiguous: Hickox and Liberman (2014) indeed found normal or even enhanced ABR wave IV amplitudes, but reduced wave I amplitudes in response to high sound intensities after the same mild binaural noise exposure, used here (NE100 group) but presented to non-anaesthetised CBA/Ca mice. Normal wave V amplitudes — reflecting input from the SOC to the IC in humans (Møller, 2007b) but reduced wave I amplitudes — have also been found in tinnitus subjects with a normal audiogram in response to high sound intensities (Schaette and McAlpine, 2011; Gu et al., 2012). On the other hand, reduced ABR wave IV and wave I amplitudes were found after NE in an animal model for tinnitus (Singer et al., 2013). Interestingly, animals in this study with no behavioural evidence for tinnitus had normal wave IV amplitudes despite reduced wave I amplitudes. Differences in wave IV amplitudes were not related to ABR thresholds. Singer et al. (2013) suggested stress dependent central adaption as a reason for the differences in central activity.

In summary, our results suggest that central hyperactivity can develop in response to AN fibre deafferentation, which is reflected in (partially) recovered ABR wave IV amplitudes in conjunction with reduced ABR wave I amplitudes. Our findings correspond with several studies that suggest a positive correlation of AN fibre damage and (supra-)normal ABR wave IV (rodents) or V (humans) amplitudes. The exact relation to the type and intensity of cochlear damage, to tinnitus and other auditory symptoms, or to influencing factors such as stress, however, remains unknown.

4.3.2 Evoked Activity of Multi-Units in the Central Inferior Colliculus

In line with changes in ABR wave IV amplitudes, RIFs in response to pure tones at each unit`s CF appeared similarly reduced in both NE groups compared to the control group in response to high sound intensities (Figure 14). Moreover, for low sound intensities, evoked activity appeared to differ between NE groups: in NE105, RIFs appeared decreased in comparison to the control group — also thresholds were increased (Figure 13, 14); in NE100, thresholds were normal or even subnormal (Figure 13, 14) and evoked activity in response to very low sound intensities appeared higher than evoked activity in the control group (Figure 14).

Results from RIFs also support the hypothesis of AN fibre damage dependent central neuronal changes: in NE100 where low threshold AN fibres were likely less affected but probably deafferentation of medium and high threshold AN fibres did occur, RIFs and response thresholds in responses to low sound intensities appeared normal or even enhanced and spontaneous activity appeared to be increased, whereas evoked activity in response to high intensities was reduced. In NE105 with increases in ABR thresholds and probably more pronounced deafferenation of low threshold AN fibres and presumably additional hair cell loss, RIFs showed reduced responses for low sound intensities and a threshold increase in comparison to the control group and NE105. At high sound intensities, responses were reduced in comparison to the control group, whereas differences between both NE groups were not significant.

A reduction of responses to tones in the IC was reported by Chambers et al. (2016) who used ouabain to ablate the majority of type I AN fibres. 30 days after ouabain administration, neuronal responses in the IC had partially recovered, but did not reach control levels. In contrast, other studies have reported normal or near-normal IC responses after binaural NE (e.g. Wang et al. [2002] found little or no reduction of evoked activity of single units in the ICc after intense binaural NE).

In addition to RIFs and response thresholds, CF distribution was analysed. Further analysis of frequency response areas was not performed, as recordings in the ICc were obtained from multiple types of neurons (multi-units) and did not allow detailed comparisons. In both NE groups, a substantial shift in CF distribution appeared to have happened: whereas in the control group, most multi-units had CFs with frequencies >16 and ≤32 kHz, in both NE groups the majority of multi-units had CFs >8 and ≤16 kHz (Figure 11, 12). Interestingly findings after mild NE (TTS only) and after moderate NE (PTS) go in line with previous findings of other groups (Izquierdo et al., 2008; Wang et al., 2013) who found tonotopic map reorganisations and an overrepresentation of frequencies — which bordered affected areas after NE — causing permanent increases in hearing thresholds. Thus, our results suggest that mild NE (TTS only) already causes a reorganisation of the tonotopic map at the level of the ICc. Whether the reorganisation of the tonotopic map is simply the result of residual responses (Irvine et al., 2003; Izquierdo et al., 2008) after altered input, or if mechanisms of adaption such as decreased side band inhibition (Wang et al., 2013) contribute to the observed changes, needs to be elucidated in future studies.

It should be noted that this study was not designed to investigate the origin of changes in neuronal activity. Results do not show if changes in neuronal activity originate in the ICc or if they fully derive from an earlier stage of the auditory brainstem. Detected changes could also be caused or influenced by increased spontaneous and evoked activity at other levels such as the CN (Kaltenbach et al., 1998; Kaltenbach et al., 2004; Vogler, 2011; Koehler and Shore, 2013), the SOC, the lateral lemnisci or the trapezoid bodies.

It has also to be noted that only few multi-units had CFs below 8 kHz (NE1 group: n=5, NE2 group: n=9, control: n=5) and >32 kHz (NE1 group: n=16, NE2 group: n=7, control: n=4); thus results concerning SFRs and CF thresholds in relation to CFs ≤8 kHz and >32 kHz have to be interpreted with caution.

4.4 Relation of Central Neuronal Changes to Tinnitus

It is an open question whether the observed neuronal changes are related to tinnitus, since behavioural tests were not performed in this study.

Hyperactivity in the central auditory system has indeed frequently and predominantly been interpreted as the neurophysiological correlate of tinnitus (reviewed by Eggermont and Roberts, 2015). In fact, various studies linked increases in spontaneous and evoked activity in the central auditory system after NE or the use of ototoxic drugs with behavioural signs of tinnitus (Brozoski et al., 2002; Kaltenbach et al., 2004; Bauer et al., 2008; Finlayson and Kaltenbach, 2009; Koehler and Shore, 2013).

Behavioural signs of tinnitus were found after mild NE with only temporary ABR threshold shifts (Middleton et al., 2011, Dehlmel et al., 2012, Koehler and Shore, 2013) and after more intense NE with lasting ABR threshold shifts (Kaltenbach et al., 2004; Ahlf et al, 2012; Ruttiger et al., 2013) in the majority of animals. Interestingly, Kiefer et al. (2015) found that tinnitus is more likely to occur after mild NE with only temporary increases in hearing thresholds, than after more severe NE that causes permanent increases in hearing thresholds.

Accordingly, it should be expected that in both NE groups, the majority of animals experienced tinnitus. Considering findings from Kiefer et al. (2015), more animals in NE100 (TTS only) should have experienced tinnitus than in NE105 (PTS). Thus, notably, the amount of SFR increase might positively correlate with the amount of animals experiencing tinnitus in this study.

It should be noted that few studies did not find signs of increased central gain in animals with cochlear damage and behavioural evidence for tinnitus (Singer et al., 2013; Coomber et al., 2014). Also increased SFRs were found in animals exposed to noise, regardless of the behavioural evidence of tinnitus (Coomber et al., 2014). In this context it has been considered that hyperactivity in the central auditory system might be more closely linked to hyperacusis, whereas tinnitus might be linked to a failure of central adaption (reviewed by Eggermont and Roberts, 2015). In this context, it should also be noted that behavioural tests for tinnitus are limited. While these tests may reflect tinnitus, it could be that they also refer to other neuronal pathologies. (reviewed by Eggermont and Robertson, 2015).

4.5 Conclusion and Outlook

In this study we showed that mild NE which causes only temporary but no lasting ABR threshold shifts, is sufficient to cause central neuronal adaption and increased spontaneous activity. As suspected, increased spontaneous firing rates were accompanied by a reduction of ABR wave I and histological signs of AN fibre deafferentation, supporting the hypothesis that indeed AN fibre deafferentation is sufficient to cause central hyperactivity.

Interestingly, neuronal activity in the ICc appeared to highly depend on cochlear output as increased spontaneous firing rates were more pronounced after mild NE with TTS, and AN fibre deafferentation only than after more severe NE, with PTS and apparently more severe AN fibre deafferentation and possibly additional hair cell loss. This finding suggests a non-monotonic relationship between cochlear damage and central hyperactivity. More severe NE with PTS apparently leads to less hyperactivity than mild NE with TTS only. With increasing severity of NE, different cochlear structures might be damaged — including IHC and OHC loss — and central adaption might differ in comparison to adaption after less intense NEs, thereby not causing IHC and OHC loss. What needs to be further investigated in future studies is whether this degradation of central hyperactivity is indeed caused by additional hair cell loss, by a certain type or amount of AN fibre damage or by other cochlear pathologies.

It has been suggested that central hyperactivity becomes independent of AN fibre activity later in time (Zacharek et al., 2002; Mulders et al., 2011). Accordingly, the observed changes in central neuronal activity might be altered over time; further studies need to be performed to investigate alterations in central adaption over time after different degrees of NE.

It should also be noted that bilateral NE was used in this study and several studies on NE apply unilateral NE. Effects of bilateral NE might considerably differ from effects of unilateral NE and further studies need to be performed to clarify differences in central adaption after unilateral versus bilateral NE. Also, the exact relationship between central hyperactivity and tinnitus needs to be further investigated.

Overall, an important finding of this study is the possibly non-monotonic relationship between NE and central hyperactivity. The dependency of the development of spontaneous central hyperactivity, the putative neuronal correlate for tinnitus — on a certain degree or type of cochlear damage — could possibly explain why hearing loss does not always lead to tinnitus and why tinnitus can develop without apparent hearing loss. This finding might thus offer a new approach to the pathophysiology of central adaption and the development of tinnitus.

5. Summary

So far the pathophysiology of tinnitus remains unresolved. Animal studies from several labs, however, suggest that tinnitus is related to spontaneous neuronal hyperactivity in central auditory structures following cochlear damage. Cochlear injury is commonly quantified by hearing threshold measurements. However, not all tinnitus patients suffer from increased hearing thresholds and a particular kind of cochlear damage that is not detected through threshold measurements has been suggested as a potential cause for the development of central hyperactivity and tinnitus.

In this study, I therefore investigated the effects of mild noise exposure (NE), designed to cause only temporary auditory brainstem response (ABR) threshold shifts (TTS), and more severe NE, designed to cause permanent ABR threshold shifts (PTS), on cochlear structures and on neuronal activity in the central inferior colliculus (ICc). Mice were exposed to a 8-16 kHz octave band noise at either 100 or 105 dB SPL for two hours. Cochlear damage was assessed through measurements of ABR thresholds as well as ABR wave I amplitudes and through cochlear immunohistochemistry. To evaluate changes in neuronal activity in the ICc, multi-unit recordings were performed.

We found that in the ICc, spontaneous firing rates (SFRs) of multi-units were increased in both NE groups. Interestingly, increases in SFRs were more pronounced after mild NE with only TTS, but evidence of auditory nerve (AN) fibre deafferentation than after more severe acoustic trauma with PTS and signs of more pronounced cochlear damage, possibly including more severe AN fibre deafferenation and hair cell loss.

Results indicate that indeed central hyperactivity can already develop after mild NE without permanent ABR threshold shifts. Increased SFRs of multi-units in the ICc were accompanied by signs of AN fibre deafferentation, suggesting that this type of cochlear damage might play an important role in the development of central hyperactivity. Interestingly, increases in SFRs were more pronounced after

mild (TTS only) than after more severe NE (PTS), suggesting a non-monotonic relationship between the degree of cochlear damage and the development of central neuronal hyperactivity. A dependency of the development of central hyperactivity on the type and extent of cochlear damage might explain why tinnitus can develop without increased hearing thresholds (but not necessarily after hearing loss) and might offer a new approach to the understanding of the pathophysiology of tinnitus. Additional hair cell loss might play a role in the degradation of the development of central hyperactivity.

6. Deutsche Zusammenfassung

Bislang ist die Pathophysiolgie von Tinnitus ungeklärt. Diverse Studien und Modelle weisen darauf hin, dass die Entstehung von Tinnitus mit einer neuronalen Überaktivität im zentralem Hörstystem zusammenhängen könnte. Wie diese Überaktivität durch kochlearen Schaden getriggert wird, bleibt unklar. Da nicht alle Patienten mit Tinnitus von einem Hörschwellenanstieg betroffen sind, steht möglicherweise ein Kochleaschaden mit der Entwicklung von Überaktivität im zentralen Hörsystem in Zusammenhang, der nicht durch Hörschwellenmessungen bestimmt werden kann.

Hier habe ich deshalb untersucht, ob eine Deafferenzierung von Hörnervfasern ohne Hörschwellenanstieg ausreicht, um eine zentrale Überaktivität hervorzurufen, und, wie sich ein zusätzlicher Hörschwellenanstieg auf die Aktivität im zentralen Hörsystem auswirkt: Mäuse wurden einem 8-16 kHz Oktavenbandgeräusch mit einer Lautstärke von entweder 100 dB SPL oder 105 dB SPL für 2 Stunden ausgesetzt. Die Schädigung von Hörnervfasern nach 100 dB SPL und 105 dB SPL Geräuschexposition und der Hörschwellenanstieg wurden durch frühe akustisch evozierte Potentiale (FAEP) und Immunhistochemie der Cochlea im Vergleich zu einer Kontrollgruppe untersucht. In beiden Gruppen mit Geräuschexposition fand sich eine reduzierte Amplitude der 1. Welle der FAEP und eine Verminderung der synaptische Ribbons in der kochlearen Immunhistochemie, beides Hinweise auf eine Hörnerffaserdeafferenzierung. Nach 105 dB Geräuschexposition kam es zudem zu einem Hörschwellenanstieg in den FAEP und damit mutmaßlich zu einem ausgeprägteren Verlust der Hörnervfasern mit möglicherweise zusätzlichen Haarzellverlusten.

Die neuronale Aktivität im zentralen Hörsystem wurde durch extrazelluläre Multi-Unit-Aufnahmen im zentralen inferioren Colliculus überprüft. In beiden Gruppen mit Geräuschexposition fand sich eine signifikant erhöhte Spontanaktivität der Multi-Units im zentralen inferioren Colliculus im Vergleich zur Kontrollgruppe. Interessanterweise war die Zunahme der Spontanaktivität nach milder Geräuschexposition mit Hörnervfaserverlust aber ohne Hörschwellenanhebung ausgeprägter als nach moderater Geräuschexposition mit Hörnervfaserverlust und möglicherweise zusätzlichem Verlust von Haarzellen und Hörschwellenanhebung. Gleichzeitig war die evozierte Aktivität in Antwort auf niedrige Intensitäten im inferioren Colliculus erhöht nach milder Geräuschexposition, nicht aber nach moderater Geräuschexposition.

Die Ergebnisse weisen darauf hin, dass eine Hörnervdeafferenzierung ausreicht, um die Entwicklung von Überaktivität im zentralen Hörsystem zu triggern. Zudem zeigen sie, dass ein ausgeprägter Verlust von Hörnervfasern und möglicherweise ein zusätzlicher Haarzellverlust die Entwicklung von Überaktivität im zentralen Hörsystem reduziert.

Eine mögliche Erklärung dafür ist, dass die zentrale Überaktivität das Ergebnis einer Amplifizierung der Hörnervaktivität ist: Eine zentral kompensierende Amplifikation nach Verlust von spontan wenig aktiven, hoch-schwelligen Fasern verursacht eine deutliche Zunahme der Spontanaktivität und zudem eine Erhöhung der evozierten Aktivität in Antwort auf niedrige Intensitäten, nicht aber einen Hörschwellenanstieg, Effekte die hier nach mildem akustischen Trauma beobachtet wurden. Bei zusätzlichem Verlust von spontan hochaktiven aber niedrigerschwelligen Fasern verursacht eine zentrale Amplifikation hingegen nur noch einen leichten Anstieg der Spontanaktivität und die evozierte Aktivität wird in Antwort auf alle Intensitäten nur noch teilweise kompensiert; die Hörschwellen steigen zudem an. Auch ein Verlust von Haarzellen kann zu einem Anstieg der Hörschwellen und einer Reduzierung der Hörnervaktivität führen, die alle Fasertypen betrifft und eine zentrale Amplifikation erschwert, Effekte, die hier nach moderatem akustischen Trauma beobachtet wurden.

Die Hinweise auf eine möglicherweise nicht-monotone Beziehung von kochlearem Schaden und der Entwicklung von spontaner zentraler neuronaler Hyperaktivität, dem potentiellen neuronalen Korrelat von Tinnitus, sind sehr interessant: Eine Abhängigkeit der Entwicklung von erhöhter Spontanaktivität im zentralen auditorischen System vom Typ des kochlearen Schadens, insbesondere von der Art der verbleibenden Hörnervfasern, könnte erklären, wieso Tinnitus auch ohne Hörschwellenanstieg entstehen kann, nach Hörschwellenanstieg aber nicht notwendigerweise entstehen muss und liefert damit möglicherweise neue Einblicke in die Pathophysiologie von Tinnitus.

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