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On sound and silence

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Chapter 7

Discussion and Conclusion

A.N. Heeringa

Preface

The overarching aim of the current dissertation was to study the underlying pathophysiological mechanism(s) of noise-induced tinnitus and hyperacusis. Specifically, I studied the immediate and long-term effects of acoustic trauma on (1) wave amplitudes of the auditory brainstem response (ABR), (2) evoked and spontaneous activity in the inferior colliculus (IC), and (3) spontaneous behavior in noise and silence. I found that the amplitude of ABR wave I is reduced up to four weeks after bilateral acoustic trauma, whereas the amplitude of ABR wave IV is already normal one week after trauma, suggesting an increased central gain between the auditory nerve and the auditory midbrain (Chapter 2). Furthermore, inhibition in the IC is reduced by acoustic trauma, both in response to pure tones and in response to a noise stimulus, likewise implying an increase in central gain in the auditory midbrain (Chapter 2, Chapter 5). Moreover, my results showed that spontaneous activity in the IC is not majorly affected by acoustic trauma (Chapter 2). If any, it is slightly reduced shortly after trauma (Chapter 5). Envelope coding in the IC is enhanced immediately after trauma, suggesting that processing of complex sound features is also altered by acoustic trauma (Chapter 3). Moreover, my experiments showed that the Wiener-kernel analysis technique is applicable to spike trains of the IC (Chapter 4) and can be used as a tool to study the effects of acoustic trauma on response characteristics of the IC (Chapter 5). Last, the behavioral experiments showed that normal-hearing guinea pigs are active in noise, but immobile in silence. Unilateral acoustic trauma results in an altered pattern of this spontaneous behavioral activity in a subgroup of guinea pigs, which includes an increased mobility in silence and in noise (Chapter 6). This suggests that spontaneous behavior in noise and silence might potentially serve as a new measure to detect tinnitus in guinea pigs.

These individual experiments each provide answers to single, specific research questions. However, ideally, these experiments together should be combined in one comprehensive experiment. In one animal, neurophysiological measures, i.e. ABR wave amplitudes, spontaneous activity, evoked activity, and Wiener kernels, and behavioral activity could be assessed. If the behavioral paradigm can be further validated, this might provide insight into whether the animal perceives tinnitus and/or suffers from hyperacusis. Then, the neurophysiological fingerprints of acoustic trauma could be correlated to tinnitus and/or hyperacusis. Such a complete animal model would allow us to thoroughly and reliably study the effectiveness and mode of action of specific drug treatments. This is an important next step in the line of research described in this dissertation.

In the following sections, I will discuss the contributions of the neurophysiological and behavioral findings to the current literature. Subsequently, I will discuss the limitations and provide suggestions for future directions to establish a reliable animal model for tinnitus and hyperacusis. This chapter finishes with a concluding section describing the scientific and clinical implications that can be drawn from the results of this dissertation.

Neurophysiology

Increased central gain

One of the main theories regarding pathological mechanisms behind tinnitus and hyperacusis is called the increased central-gain theory (Noreña, 2011; Knipper et al., 2013; Zeng, 2013; Auerbach et al., 2014). This theory implies that a sensory system responds to a deprived external input with an abnormal homeostatic response in order to maintain a balanced state. This abnormal homeostatic response can involve an increased sensitivity to incoming signals as well as an amplification of the spontaneous activity. It has been hypothesized that the first explains the presence of hyperacusis, whereas the latter corresponds to tinnitus (e.g. Zeng, 2013). Increased hearing thresholds clearly imply a deprived sensory input to the central auditory system. However, recent studies have shown that noise-induced temporary threshold shifts can also result in a permanent deprivation of sensory input by means of the degeneration of the cochlear nerve (Kujawa and Liberman, 2009; Lin et al., 2011). This phenomenon has been termed “hidden hearing loss”. The results of the current dissertation are discussed with respect to the increased central-gain theory, both as a result of elevated hearing thresholds and as a result of “hidden hearing loss”.

In Chapter 2, I found that a sound-induced temporary threshold shift is associated with a reduction in the ABR wave I amplitude up to four weeks after acoustic trauma. This implies that the integrity of the auditory nerve is compromised by the acoustic trauma, even though the auditory thresholds fully recovered (Lin et al., 2011). Moreover, the amplitude of ABR wave IV recovered as soon as the thresholds recovered (after one week). Together, this suggests a mechanism that amplifies the reduced sensory input between the auditory nerve and the auditory midbrain. These findings are consistent with studies on ABR waves of tinnitus patients with normal audiograms that likewise demonstrate reduced wave I amplitudes but normal amplitudes of wave V (Kehrle et al., 2008; Schaette and McAlpine, 2011; Gu et al., 2012). Wave I amplitudes are also reduced in normal-hearing people with self-reported exposure to loud sounds (Stamper and Johnson, 2014). Even though the initial findings of a reduced wave I amplitude were found in tinnitus patients, it is not clear whether it is a neural marker of tinnitus and/or hyperacusis. In laboratory animals, “hidden hearing loss”, as reflected by a decreased wave I amplitude and a reduced ribbon count, is thought to correspond with the generation of hyperacusis (Hickox and Liberman, 2014). Furthermore, when equally exposed animals are classified as either “tinnitus” or “no-tinnitus”, it appears that all exposed animals, regardless of the classification, have a reduced wave I amplitude, whereas in the “tinnitus” animals, a *reduced* wave IV amplitude is found (Rüttiger et al., 2013). Since behavioral experiments were not conducted with the animals of Chapter 2, the observed ABR wave amplitude changes cannot be directly assigned to tinnitus, hyperacusis, or both. However, the altered ABR wave amplitudes are consistent with the presence of “hidden hearing loss” and with an increased central gain along the auditory pathway.

The increased central-gain theory is also related to the balance between inhibition and excitation in the central auditory system (Noreña, 2011; Zeng, 2013). Healthy homeostatic neural activity is regulated by excitatory and inhibitory interactions, among others (Turrigiano and Nelson, 2004). Therefore, a disrupted balance between excitation and inhibition in the direction of an increased excitation or a decreased inhibition could cause increased central gain. Inhibition and excitation can be studied by using molecular techniques, e.g. by investigating inhibitory and excitatory neurotransmitters and their receptors, and by using neurophysiological techniques, as performed in the current dissertation. Chapter 2 of this dissertation shows that the amount of inhibitory responses to pure tones is significantly reduced up to two weeks after acoustic trauma associated with a temporary threshold shift. On the contrary, the amount of excitatory responses is not decreased, but rather increased in response to low-frequency pure tones. Furthermore, Chapter 5 shows that inhibitory receptive fields disappear in all IC multi units immediately after acoustic trauma, whereas excitatory receptive fields remain present in the multi units with a characteristic frequency lower than the trauma frequency of 11 kHz. Inhibitory subsystems, as revealed from singular-value decomposition of the second-order Wiener kernel, are only present in multi units with a low characteristic frequency < 3 kHz. In contrast, excitatory subsystems were present in multi units with a characteristic frequency (*before* trauma) of up to 16 kHz. This indicates that in the IC, inhibition in particular is vulnerable for acoustic trauma, as revealed with receptive-field analysis and with Wiener-kernel analysis. My results indicate that acoustic trauma results in a decrease of inhibitory components in the IC, which is consistent with the increased central-gain theory (Noreña, 2011). Furthermore, my results are consistent with molecular studies, which show that acoustic trauma results in a down-regulation of inhibitory neurotransmitters and their corresponding receptors (Szczepaniak and Møller, 1995; Dong et al., 2010a, 2010b), and with neurophysiological studies, which show that noise-induced hearing loss amplifies excitatory responses in the IC (Willott and Lu, 1982; Salvi et al., 1990; Niu et al., 2013). A reduction of inhibition in the central auditory system has been suggested to be correlated to tinnitus and to hyperacusis (Knipper et al., 2010; Wang et al., 2011; Sun et al., 2014). Since the guinea pigs of Chapter 2 and Chapter 5 were not subjected to behavioral paradigms, it is not certain whether the acoustic-trauma induced neurophysiological changes presented in this dissertation reflect tinnitus, hyperacusis, or both. However, it can be speculated that they are an underlying mechanism of hyperacusis, as altered stimulus-driven activity in the IC has previously been linked to hyperacusis (Gu et al., 2010; Eggermont, 2013; Zeng, 2013).

As mentioned above, an increased central gain can also induce alterations in spontaneous spiking activity of the central auditory system. Naturally, the characteristics of spontaneous activity are specifically relevant in the absence of acoustic stimulation, making it an attractive neural correlate for the central pathophysiology of tinnitus (Kaltenbach, 2011; Noreña, 2011; Zeng, 2013; Eggermont and Roberts, 2014). Indeed, previous studies have shown that spontaneous firing rates in the IC are increased following a tinnitus-inducing noise exposure (Mulders and Robertson, 2009; Manzoor et al., 2013). However, the results of

the current dissertation show that spontaneous firing rates measured immediately, one week, two weeks, and four weeks after acoustic trauma do not significantly increase (or decrease) as compared to the control group (Chapter 2). If any, when comparing spontaneous activity in the same unit before and immediately after acoustic trauma, there is a small but significant decrease (Chapter 5). Therefore, considering the lack of hyperactivity in the inferior colliculus multi-unit recordings, it was concluded that the bilaterally exposed animals were not experiencing tinnitus, but rather hyperacusis.

In summary, my results support the theory that acoustic trauma induces an increased gain in the central auditory system. The results derived from analyses of ABR wave amplitudes, excitatory and inhibitory processes, and spontaneous activity all suggest the presence of hyperacusis, rather than tinnitus.

Enhanced envelope coding

Acoustic trauma can also alter the coding of more complex features of an acoustic stimulus, such as the envelope. Coding envelope cues properly are important when processing complex, meaningful sounds, such as speech (Shannon et al., 1995). My results showed that the neural response, phase-locked to the envelope of the acoustic stimulus, is altered immediately after acoustic trauma (Chapter 3). Modulation gain, i.e. the strength of the neural phase-locked response relative to the modulation depth of the acoustic stimulus, is increased after acoustic trauma in IC neurons. Specifically, multi units with characteristic frequencies below the trauma frequency (< 11 kHz) show enhanced envelope coding in response to modulation frequencies below 256 Hz. This finding is consistent with responses in the auditory nerve and in auditory-evoked potentials after noise-induced hearing loss (Kale and Heinz, 2010; Zhong et al., 2014), but have not been shown before in the IC.

The observed change in envelope coding at the level of the IC might be an underlying neural mechanism for the problems that hearing-impaired people encounter when listening in noisy environments (Bayat et al., 2013). Envelope information that derives from fluctuating background noises, such as competing speakers, might act as a distraction when pathologically amplified in the central auditory system. Previous studies showed that patients with sensorineural hearing loss also have normal to better-than-normal envelope detection thresholds (Moore and Glasberg, 2001; Füllgrabe et al., 2003). In addition, the increased modulation gain in phase-locked responses of the IC after acoustic trauma might be corresponding to the acoustic trauma-induced increased central gain, as discussed above.

Wiener kernels

By recording a system's response to a broadband Gaussian white noise, a set of Wiener kernels can be computed. These kernels provide a description of the nonlinear system that is being studied (Eggermont, 1993). Wiener kernels have been extensively described for noise-evoked spike trains of the auditory nerve of a variety of species (van Dijk et al., 1994, 1997; Yamada and Lewis, 1999; Lewis et al., 2002a, 2002b; Recio-Spinoso et al., 2005; Temchin et al., 2005;

Sneary and Lewis, 2007). Furthermore, there are also two studies describing Wiener kernels of noise-evoked spike trains recorded at the ventral cochlear nucleus (Wickesberg et al., 1984; Recio-Spinoso and van Dijk, 2006), only one of which reports successful recordings of the second-order kernels (Recio-Spinoso and van Dijk, 2006). In order to get an interpretation for the functional properties of the second-order kernels, they can be decomposed with singular-value decomposition into a series of subsystems, consisting of a filter function (eigenvector) and a gain value (eigenvalue; Yamada and Lewis, 1999). The eigenvectors allow us to obtain frequency responses and group delays and the eigenvalues provide a relative weight factor to the eigenvector. Furthermore, the sign of the eigenvalue indicates whether the subsystem contributes positively or negatively to the neural response, corresponding to excitation or inhibition, respectively. Singular-value decomposition has successfully been applied to recordings of the auditory nerve and the cochlear nucleus (Yamada and Lewis, 1999; Lewis et al., 2002a, 2002b; Temchin et al., 2005; Recio-Spinoso and van Dijk, 2006; Sneary and Lewis, 2007).

Since Wiener kernels, complemented with singular-value decomposition, reveal many response characteristics, including excitatory and inhibitory components, we hypothesized that it might be beneficial for studies that aim to understand the neurophysiological consequences of acoustic trauma. However, Wiener-kernel analyses had not been previously applied for noise-evoked spike trains of the IC. Therefore, the applicability of the Wiener-kernel analysis technique for noise-evoked spike trains of the IC was studied in the experiments described in Chapter 4 of the current dissertation. This chapter shows that significant first- and second-order kernels can be identified for spike trains of the IC.

In Chapter 5, the changes induced by acoustic trauma were studied using Wiener-kernel analysis. As discussed above, these changes can include altered excitation, altered inhibition, or both. Application of Wiener-kernel analysis on noise-evoked spike trains has a particular advantage over studying tone-evoked neural activity. The response to a tone is the summed result of excitation and inhibition. Hence, it is difficult to attribute any changes due to acoustic trauma to either excitation, inhibition, or both. In contrast, singular-value decomposition of Wiener kernels computed from noise-evoked spike trains allows for the explicit separation of excitation and inhibition in the neural response. In other words, a change due to acoustic trauma can be specifically linked to a change due to either excitation or inhibition. Using this analysis technique, the effects of acoustic trauma on the excitatory and inhibitory components of the IC were studied (Chapter 5). The results of this study have been discussed previously in the context of the increased central-gain theory (see section ‘increased central gain’): acoustic trauma essentially cancels inhibition, but not excitation, in neurons that are tuned to frequencies below the edge of the trauma frequency.

Spontaneous behavior

Progress in research on the pathophysiological mechanisms of tinnitus and hyperacusis critically depends on a reliable behavioral animal model that determines whether the

animal being studied has tinnitus and/or hyperacusis. Furthermore, a behavioral model will be essential when testing promising treatments for tinnitus and hyperacusis. A number of different paradigms were available when I started this project in 2010 (Turner, 2007). However, none had been tested on guinea pigs. Today, there are two studies showing that the startle reflex paradigm can be applied to determine tinnitus in guinea pigs (Dehmel et al., 2012; Berger et al., 2013). I started by developing a conditioning paradigm to determine tinnitus in guinea pigs (Jastreboff and Sasaki, 1994).

Previous studies showed that guinea pigs could be trained in a shuttle box to shuttle to the other compartment in the presence of a 15-s noise burst (Philippens et al., 1992; Agterberg et al., 2010). Therefore, we aimed at training guinea pigs in the shuttle box to respond to a silent interval in continuous noise. However, it seemed that the guinea pigs inhibited their activity during the silent intervals, demonstrating that such a conditioning paradigm with silent intervals was not readily applicable as a measure for tinnitus in guinea pigs. In our subsequent experiments, the spontaneous behavior in silence was further studied as a possible measure to detect tinnitus in guinea pigs (Chapter 6). The idea behind this measure is that normal-hearing guinea pigs show a clear distinction in spontaneous behavior during silence as compared to during noise, i.e. they are mobile during noise, whereas they do not move during silence. We hypothesized that guinea pigs with tinnitus do not perceive complete silence and, therefore, do not show this typical behavior during silence. My results showed that some guinea pigs with unilateral sound-induced hearing loss have an increased mobility during silence as well as during noise (Chapter 6). Validation experiments, as those extensively described in the discussion of Chapter 6, are essential before attributing these observations to tinnitus, hyperacusis, or both. However, the observed behavior during the silent intervals of the experiment strongly suggests that a subgroup of the traumatized animals indeed perceived tinnitus.

Limitations

Below, three methodological aspects are discussed that impacted the application or interpretation of the results reported in the current dissertation.

Anesthesia

The use of anesthetics during the neurophysiological recordings and during the exposure of the animals to acoustic trauma was, due to important ethical reasons, unavoidable. Isoflurane was used as an anesthetic during acoustic trauma for the animals that were allowed to recover from the acoustic trauma (Chapter 2, Chapter 6). During the neurophysiological procedures, a mixture of ketamine and xylazine was used to anesthetize the animals. Thus the effects of immediate acoustic trauma in Chapter 2, Chapter 3, and Chapter 5 resulted from exposure applied under ketamine/xylazine anesthesia. It has been shown that isoflurane has a protective effect on noise-induced hearing loss and noise-induced tinnitus (Kim et al., 2005; Norman et al., 2012). Furthermore, ketamine is known to be an antagonist of the *N*-methyl-

D-aspartate (NMDA) receptor, which binds glutamate, and inner ear damage from acoustic overexposure is thought to be facilitated by glutamate excitotoxicity (Puel, 1995). This suggests that, without the anesthetics, the damage could have been even more severe. Thus the noise damage as described in this dissertation may be underestimated as compared to that in real-life (unanaesthetized) situations.

Unilateral vs. bilateral acoustic trauma

In the neurophysiological experiments of the current dissertation, the animals were exposed to a free field bilateral acoustic-trauma stimulus (Chapter 2, Chapter 3, Chapter 5), as this is similar to most conditions where humans may acquire acoustic trauma. However, in the behavioral experiment of the current dissertation, a unilateral acoustic-trauma stimulus was used (Chapter 6). The reason for applying unilateral trauma was that by traumatizing only one ear, there is a smaller chance that the behavioral outcomes are confounded by hearing loss, since the unexposed ear retains normal auditory thresholds.

Even though the trauma stimulus was the same in all experiments (1-h exposure of an 11-kHz pure tone of 124 dB SPL), the mode of exposure could have induced a separate set of behavioral and neurophysiological changes. For example, spontaneous firing rates in the IC show separate characteristics in animals with bilateral acoustic trauma compared to animals with unilateral acoustic trauma (Ma et al., 2006). Furthermore, the olivocochlear system, which is activated by bilaterally presented sounds, protects the ear from damage by acoustic trauma (Liberman, 1988; Maison and Liberman, 2000). Hence, unilateral acoustic trauma likely results in more severe damage than bilateral trauma, due to the lack of protective mechanisms mediated by the efferent system. This should be considered when directly comparing the neurophysiological results with the behavioral results of the current dissertation, as well as when comparing the current results with the literature.

Contemplations about behavioral paradigms to test for tinnitus and hyperacusis

Ever since the first behavioral paradigm for tinnitus was published in the '80s (Jastreboff et al., 1988a), there has been no agreement in the current literature on the best measure to determine tinnitus in laboratory animals (Hayes et al., 2014). Both the conditioning paradigms and the startle reflex paradigms have advantages and disadvantages (Heffner and Heffner, 2012). An important concern regarding all behavioral models for tinnitus in laboratory animals is whether the behavior actually reflects tinnitus, and not something else. First of all, the assumption must be made that the animal being studied is in fact capable of experiencing a conscious percept, such as tinnitus. The next difficulty is that animals in behavioral paradigms for tinnitus can never be under stimulus control, as the stimulus that we would want them to act upon is a percept that is in their head, and thus beyond the 'control' of the scientist (Sidman, 2008). These questions, although very interesting, lie beyond the scope of this dissertation. The assumption that laboratory animals are capable of experiencing tinnitus is made in all studies on behavioral models as well as in Chapter 6 of this dissertation.

Looking forward

The next step along this line of research is to combine the electrophysiological recordings with the behavioral measure that has been suggested for tinnitus. As such, an animal model can be developed in which promising treatments that are applied at the round window of the cochlea can be tested (Muehlmeier et al., 2011). Accordingly, the effectiveness of the treatments can be evaluated by the behavioral paradigm, whereas the mode of action can be studied by the range of neurophysiological changes induced by acoustic trauma, as those described in this dissertation. The following notions are personal suggestions about the direction in which I think this research should further go.

First of all, I believe that the variability in the exposed animals is of great interest. Future studies could focus on the neurophysiological differences between the animals with normal and with abnormal behavior in noise and silence. Next, I consider reaching a consensus about the most reliable behavioral measure to determine tinnitus and/or hyperacusis in animal as an important aim for the future, if we want to directly compare results from different laboratories around the world. Furthermore, performing neuroimaging studies in laboratory animals with tinnitus would allow for a better translation between the animal studies and the human studies. Also, I believe that scientists working with animal models can move further in the direction that is taken by neuroimaging studies, namely looking at the brain as a whole instead of single locations. For example, an experiment in which *in vivo* electrophysiological recordings are made simultaneously at several locations in the auditory central system would be very interesting. Moreover, I think it is important to realize that the pathophysiological mechanism(s) of tinnitus and hyperacusis are not likely to be static, but rather dynamic in time, especially within the first hours, days, and weeks after the acoustic trauma, as is also illustrated by Chapter 2. Investigating a mechanism at only one time point is as brief as a photo, whereas we would want to know the whole movie. Therefore, in order to better understand a mechanism, one should always take multiple time points into account. And last, to understand a pathophysiological mechanism thoroughly, I think that combining neurophysiology with molecular techniques is of great importance. Molecular findings can also provide useful targets for future treatments.

The ideal experiment would combine all these aspects and different approaches, i.e. behavioral, neurophysiological, and molecular, into one animal experiment. I believe that the methodological issues that are raised by combining these approaches into one experiment are some of the most urgent issues to solve in the near future.

Conclusion

The overarching aim of the current dissertation was to study the underlying pathophysiological mechanism(s) of noise-induced tinnitus and hyperacusis. The experiments that were conducted for the current dissertation have filled some small gaps in the current literature. Together with other important scientific work, these findings might eventually help to find

the pathophysiological mechanism(s) of noise-induced tinnitus and hyperacusis. Specifically, this dissertation shows that neurophysiological correlates of acoustic trauma in the inferior colliculus include a decrease in inhibition, an increase in excitation, and enhanced envelope coding. Furthermore, this dissertation shows that Wiener kernels are applicable to study spike trains of the inferior colliculus and are an appropriate technique to further study the pathophysiological consequences of acoustic trauma. Last, a possible new measure to determine tinnitus in guinea pigs is reported.

By studying the consequences of acoustic trauma, the results of this dissertation can make a contribution to the current knowledge about underlying mechanisms of noise-induced tinnitus and hyperacusis. Therefore, these findings are of interest to auditory neurophysiologists that work on the pathophysiological mechanisms of tinnitus and hyperacusis. Showing that Wiener kernels can be measured in the inferior colliculus might provide a new tool to study these pathological mechanisms, as these analyses provide information on the balance between excitation and inhibition in a relatively straightforward way. Furthermore, my work has contributed to the literature on behavioral models for tinnitus by describing a possible new measure to assess tinnitus in guinea pigs.

The society of today expects its participants to stay active for a longer time. This underlines the importance of people not having to be hindered by hearing loss, tinnitus, or hyperacusis. Knowledge about the consequences of acoustic trauma raises more awareness in society about the traumatizing effects of overexposure. Above all, advances in research on neurophysiological correlates of acoustic trauma, tinnitus, and hyperacusis might provide new avenues to discover treatments that alleviate the burden for patients suffering from these debilitating conditions. Contributing to that goal was my motivation in conducting this research.

References

- Agterberg MJH, van den Broek M, Philippens IHCHM (2010) A less stressful animal model: a conditioned avoidance behaviour task for guineapigs. *Lab Anim* 44:206-10. doi: 10.1258/la.2009.009096.
- Agterberg MJ, Versnel H (2014) Behavioral responses of deafened guinea pigs to intracochlear electrical stimulation: a new rapid psychophysical procedure. *Hear Res* 313:67-74. doi: 10.1016/j.heares.2014.04.011.
- Auerbach BD, Rodrigues PV, Salvi RJ (2014) Central gain control in tinnitus and hyperacusis. *Front Neurol* 5:206. doi:10.3389/fneur.2014.00206.
- Bakin JS, South DA, Weinberger NM (1996) Induction of receptive field plasticity in the auditory cortex of the guinea pig during instrumental avoidance conditioning. *Behav Neurosci* 110(5):905-13.
- Bauer CA, Brozoski TJ, Rojas R, Boley J, Wyder M (1999) Behavioral model of chronic tinnitus in rats. *Otolaryngol Head Neck Surg* 121(4):457-462.
- Bauer CA, Brozoski TJ (2001) Assessing tinnitus and prospective tinnitus therapeutics using a psychophysical animal model. *J Assoc Res Otolaryngol* 2(1):54-64.
- Bayat A, Farhadi M, Pourbakht A, Sadjedi H, Emamdjomeh H, Kamali M, Mirmomeni G (2013) A comparison of auditory perception in hearing-impaired and normal-hearing listeners: an auditory scene analysis study. *Iran Red Crescent Med J* 15(11):e9477. doi:10.5812/ircmj.9477.
- Berger JI, Coomber B, Shackleton TM, Palmer AR, Wallace MN (2013) A novel behavioural approach to detecting tinnitus in the guinea pig. *J Neurosci Methods* 213(2):188-195. doi:10.1016/j.jneumeth.2012.12.023.
- Böhmer A (1988) The Preyer reflex - an easy estimate of hearing function in guinea pigs. *Acta Otolaryngol* 106:368-72.
- Bronkhorst AW, Plomp R (1992) Effect of multiple speechlike maskers on binaural speech recognition in normal and impaired hearing. *J Acoust Soc Am* 92(6):3132-3139.
- Buño W Jr (1978) Auditory nerve fiber activity influenced by contralateral ear sound stimulation. *Exp Neurol* 59(1):62-74.
- Campolo J, Lobarinas E, Salvi R (2013) Does tinnitus “fill in” the silent gaps? *Noise Health* 15(67):398-405. doi: 10.4103/1463-1741.121232.
- Carney LH, Yin TCT (1989) Responses of low-frequency cells in the inferior colliculus to interaural time differences of clicks: excitatory and inhibitory components. *J Neurophysiol* 62(1):144-161.
- Clopton BM, Winfield JA (1973) Tonotopic organization in the inferior colliculus of the rat. *Brain Res* 56:355-8.
- Coomber B, Berger JI, Kowalkowski VL, Shackleton TM, Palmer AR, Wallace MN (2014) Neural changes accompanying tinnitus following unilateral acoustic trauma in the guinea pig. *Eur J Neurosci* 40(2):2427-41. doi: 10.1111/ejn.12580.
- Crawford M, Masterson FA (1982) Species-specific defense reactions and avoidance learning. *Pav J Biol Sci* 17(4):204-14.

- Crifò S (1973) Shiver-audiometry in the conditioned guinea-pig (simplified Anderson-Wedenberg test). *Acta Otolaryngol* 75:38-44.
- Dauman R, Bouscau-Faure F (2005) Assessment and amelioration of hyperacusis in tinnitus patients. *Acta Otolaryngol* 125(5):503-509.
- de Boer E, de Jongh HR (1978) On cochlear encoding: potentialities and limitations of the reverse-correlation technique. *J Acoust Soc Am* 63(1):115-135.
- Degeest S, Corthals P, Vinck B, Keppler H (2014) Prevalence and characteristics of tinnitus after leisure noise exposure in young adults. *Noise Health* 16(68):26-33. doi: 10.4103/1463-1741.127850.
- Dehmel S, Eisinger D, Shore SE (2012) Gap prepulse inhibition and auditory brainstem-evoked potentials as objective measures for tinnitus in guinea pigs. *Front Syst Neurosci* 6:42. doi:10.3389/fnsys.2012.00042.
- Diamond IT, Neff WD (1957) Ablation of temporal cortex and discrimination of auditory patterns. *J Neurophysiol* 20(3):300-15.
- Dong S, Rodger J, Mulders WHAM, Robertson D (2010a) Tonotopic changes in GABA receptor expression in guinea pig inferior colliculus after partial unilateral hearing loss. *Brain Res* 1342:24-32. doi:10.1016/j.brainres.2010.04.067.
- Dong S, Mulders WHAM, Rodger J, Woo S, Robertson D (2010b) Acoustic trauma evokes hyperactivity and changes in gene expression in guinea-pig auditory brainstem. *Eur J Neurosci* 31(9):1616-1628. doi:10.1111/j.1460-9568.2010.07183.x.
- Duan M, Agerman K, Ernfors P, Canlon B (2000) Complementary roles of neurotrophin 3 and a N-methyl-D-aspartate antagonist in the protection of noise and aminoglycoside-induced ototoxicity. *PNAS* 97(13):7597-7602.
- Dunnebie EA, Segenhout JM, Wit HR, Albers FWJ (1997) Two-phase endolymphatic hydrops: a new dynamic guinea pig model. *Acta Otolaryngol* 117:13-19.
- Duque D, Pérez-González D, Ayala YA, Palmer AR, Malmierca MS (2012) Topographic distribution, frequency, and intensity dependence of stimulus-specific adaptation in the inferior colliculus of the rat. *J Neurosci* 32(49):17762-17774. doi:10.1523/JNEUROSCI.3190-12.2012.
- Eggermont JJ, Johannesma PIM, Aertsen AMH (1983) Reverse-correlation methods in auditory research. *Q Rev Biophys* 16(3):341-414.
- Eggermont JJ (1993) Wiener and Volterra analyses applied to the auditory system. *Hear Res* 66:177-201.
- Eggermont JJ, Roberts LE (2004) The neuroscience of tinnitus. *Trends Neurosci* 27(11):676-682.
- Eggermont JJ (2013) Hearing loss, hyperacusis, or tinnitus: what is modeled in animal research? *Hear Res* 295:140-149. doi:10.1016/j.heares.2012.01.005.
- Eggermont JJ, Roberts LE (2014) Tinnitus: animal models and findings in humans. *Cell Tissue Res* (Epub ahead of print) doi:10.1007/s00441-014-1992-8.
- Ehret G, Romand R (Eds.) (1997) *The Central Auditory System*. New York, NY: Oxford University Press.
- Ehret G (1997) The auditory midbrain, a “shunting yard” of acoustical information processing. In: Ehret G, Romand R (eds) *The Central Auditory System*. Oxford University Press, New York, pp 259-316.

- Fournier P, Hébert S (2013) Gap detection deficits in humans with tinnitus as assessed with the acoustic startle paradigm: does tinnitus fill in the gap? *Hear Res* 295:16-23. doi: 10.1016/j.heares.2012.05.011.
- Füllgrabe C, Meyer B, Lorenzi C (2003) Effect of cochlear damage on the detection of complex temporal envelopes. *Hear Res* 178:35-43.
- Furman AC, Kujawa SG, Liberman MC (2013) Noise-induced cochlear neuropathy is selective for fibers with low spontaneous rates. *J Neurophysiol* 110(3):577-586. doi:10.1152/jn.00164.2013.
- Galván VV, Weinberger NM (2002) Long-term consolidation and retention of learning-induced tuning plasticity in the auditory cortex of the guinea pig. *Neurobiol Learn Mem* 77:78-108. doi:10.1006/nlme.2001.4044.
- Gilles A, de Ridder D, van Hal G, Wouters K, Kleine Punte A, van de Heyning P (2012) Prevalence of leisure noise-induced tinnitus and the attitude toward noise in university students. *Otol Neurotol* 33(6):899-906. doi:10.1097/MAO.0b013e31825d640a.
- Grimault N, Bacon SP, Micheyl C (2002) Auditory stream segregation on the basis of amplitude-modulation rate. *J Acoust Soc Am* 111(3):1340-1348.
- Gu JW, Halpin CF, Nam EC, Levine RA, Melcher JR (2010) Tinnitus, diminished sound-level tolerance, and elevated auditory activity in humans with clinically normal hearing sensitivity. *J Neurophysiol* 104:3361-3370. doi:10.1152/jn.00226.2010.
- Gu JW, Herrmann BS, Levine RA, Melcher JR (2012) Brainstem auditory evoked potentials suggest a role for the ventral cochlear nucleus in tinnitus. *J Assoc Res Otolaryngol* 13(6):819-833. doi:10.1007/s10162-012-0344-1.
- Guillon MJ, Caston J, Ruel J, Johnson RM, Pujol R, Puel J-L (2003) Salicylate induces tinnitus through activation of cochlear NMDA receptors. *J Neurosci* 23(9):3944-52.
- Hagenaars MA, Oitzl M, Roelofs K (2014) Updating freeze: aligning animal and human research. *Neurosci Biobehav Rev* 47C:165-76. doi: 10.1016/j.neubiorev.2014.07.021.
- Hayes SH, Radziwon KE, Stolzberg DJ, Salvi RJ (2014) Behavioral models of tinnitus and hyperacusis in animals. *Front Neurol* 5:179. doi:10.3389/fneur.2014.00179.
- Heeringa AN, van Dijk P (2014) The dissimilar time course of temporary threshold shifts and reduction of inhibition in the inferior colliculus following intense sound exposure. *Hear Res* 312:38-47. doi:10.1016/j.heares.2014.03.004.
- Heffner HE, Harrington IA (2002) Tinnitus in hamsters following exposure to intense sound. *Hear Res* 170:83-95.
- Heffner HE, Koay G (2005) Tinnitus and hearing loss in hamsters (*Mesocricetus auratus*) exposed to loud sound. *Behav Neurosci* 119(3):734-42.
- Heffner HE, Heffner RS (2012) Behavioral tests for tinnitus in animals. In: Tinnitus. Eggermont JJ et al., (Eds.) New York: Springer Science+Business Media. p21-58.
- Henry KS, Heinz MG (2013) Effects of sensorineural hearing loss on temporal coding of narrowband and broadband signals in the auditory periphery. *Hear Res* 303:39-47.
- Hermes DJ, Aertsen AMHJ, Johannesma PIM, Eggermont JJ (1981) Spectro-temporal characteristics of single units in the auditory midbrain of the lightly anesthetised grass frog (*Rana temporaria* L.) investigated with noise stimuli. *Hear Res* 5:147-178.

- Herrero AI, Sandi C, Venero C (2006) Individual differences in anxiety trait are related to spatial learning abilities and hippocampal expression of mineralocorticoid receptors. *Neurobiol Learn Mem* 86(2):150-9.
- Hickox AE, Liberman MC (2014) Is noise-induced cochlear neuropathy key to the generation of hyperacusis or tinnitus? *J Neurophysiol* 111:552-564. doi:10.1152/jn.00184.2013.
- Hu B, Lin X, Huang L-S, Yang L, Feng H, Sui J-F (2009) Involvement of the ipsilateral and contralateral cerebellum in the acquisition of unilateral classical eyeblink conditioning in guinea pigs. *Acta Pharmacol Sin* 30(2):141-52. doi: 10.1038/aps.2008.18.
- Jastreboff PJ, Sasaki CT (1986) Salicylate-induced changes in spontaneous activity of single units in the inferior colliculus of the guinea pig. *J Acoust Soc Am* 80(5):1384-91.
- Jastreboff PJ, Brennan JF, Sasaki CT (1988a) An animal model for tinnitus. *Laryngoscope* 98(3):280-286.
- Jastreboff PJ, Brennan JF, Coleman JK, Sasaki CT (1988b) Phantom auditory sensation in rats: an animal model for tinnitus. *Behav Neurosci* 102(6):811-822.
- Jastreboff PJ, Sasaki CT (1994) An animal model of tinnitus: a decade of development. *Am J Otol* 15(1):19-27.
- Joris PX, Verschooten E (2013) On the limit of neural phase locking to fine structure in humans. *Adv Exp Med Biol* 787:101-108. doi:10.1007/978-1-4614-1590-9_12.
- Kale S, Heinz MG (2010) Envelope coding in auditory nerve fibers following noise-induced hearing loss. *J Assoc Res Otolaryngol* 11(4):657-673. doi:10.1007/s10162-010-0223-6.
- Kaltenbach JA (2011) Tinnitus: models and mechanisms. *Hear Res* 276:52-60. doi:10.1016/j.heares.2010.12.003.
- Kazlauskas V, Schuh J, Dall'Igna O, Pereira GS, Bonan CD, Lara DR (2005) Behavioral and cognitive profile of mice with high and low exploratory phenotypes. *Behav Brain Res* 162(2):272-8.
- Kehrle HM, Granjeiro RC, Sampaio ALL, Bezerra R, Almeida VF, Oliveira CA (2008) Comparison of auditory brainstem response results in normal-hearing patients with and without tinnitus. *Arch Otolaryngol Head Neck Surg* 134(6):647-651. doi:10.1001/archotol.134.6.647.
- Kim JU, Lee HJ, Kang HH, Shin JW, Ku SW, Ahn JH, ... Chung JW (2005) Protective effect of isoflurane anesthesia on noise-induced hearing loss in mice. *Laryngoscope* 115(11):1996-1999.
- Kitcher ED, Ocansey G, Tumpi DA (2012) Early occupational hearing loss of workers in a stone crushing industry: our experience in a developing country. *Noise Health* 14(57):68-71. doi: 10.4103/1463-1741.95134.
- Knipper M, Zimmermann U, Müller M (2010) Molecular aspects of tinnitus. *Hear Res* 266:60-69. doi:10.1016/j.heares.2009.07.013.
- Knipper M, van Dijk P, Nunes I, Rüttiger L, Zimmermann U (2013) Advances in the neurobiology of hearing disorders: recent developments regarding the basis of tinnitus and hyperacusis. *Prog Neurobiol* 111:17-33. doi:10.1016/j.pneurobio.2013.08.002.
- Korenberg MJ, Hunter IW (1990) The identification of nonlinear biological systems: Wiener kernel approaches. *Ann Biomed Eng* 18:629-654.
- Kraus KS, Mitra S, Jimenez Z, Hinduja S, Ding D, Jiang H, ... Salvi RJ (2010) Noise trauma impairs neurogenesis in the rat hippocampus. *Neurosci* 167(4):1216-26. doi: 10.1016/j.neuroscience.2010.02.071.

- Kraus KS, Canlon B (2012) Neuronal connectivity and interactions between the auditory and limbic systems. Effects of noise and tinnitus. *Hear Res* 288:34-46. doi: 10.1016/j.heares.2012.02.009.
- Kujawa SG, Liberman MC (2009) Adding insult to injury: cochlear nerve degeneration after “temporary” noise-induced hearing loss. *J Neurosci* 29(45):14077-14085. doi:10.1523/JNEUROSCI.2845-09.2009.
- Langers DRM, de Kleine E, van Dijk P (2012) Tinnitus does not require macroscopic tonotopic map reorganization. *Front Syst Neurosci* 6:2. doi:10.3389/fnsys.2012.00002.
- Lanting CP, de Kleine E, van Dijk P (2009) Neural activity underlying tinnitus generation: results from PET and fMRI. *Hear Res* 255:1-13. doi:10.1016/j.heares.2009.06.009.
- Lewis ER, Henry KR, Yamada WM (2002a) Tuning and timing of excitation and inhibition in primary auditory nerve fibers. *Hear Res* 171:13-31.
- Lewis ER, Henry KR, Yamada WM (2002b) Tuning and timing in the gerbil ear: Wiener-kernel analysis. *Hear Res* 174:206-221.
- Liberman MC (1988) Response properties of cochlear efferent neurons: monaural vs. binaural stimulation and the effects of noise. *J Neurophysiol* 60(5):1779-1798.
- Liberman MC (1989) Rapid assessment of sound-evoked olivocochlear feedback: Suppression of compound action potentials by contralateral sound. *Hear Res* 38:47-56.
- Lin HW, Furman AC, Kujawa SG, Liberman MC (2011) Primary neural degeneration in the guinea pig cochlea after reversible noise-induced threshold shift. *J Assoc Res Otolaryngol* 12(5):605-616. doi:10.1007/s10162-011-0277-0.
- Llano DA, Turner J, Caspary DM (2012) Diminished cortical inhibition in an aging mouse model of chronic tinnitus. *J Neurosci* 32(46):16141-16148. doi:10.1523/JNEUROSCI.2499-12.2012.
- Lobarinas E, Hayes SH, Allman BL (2013) The gap-startle paradigm for tinnitus screening in animal models: limitations and optimization. *Hear Res* 295:150-60. doi: 10.1016/j.heares.2012.06.001.
- Ma WLD, Hidaka H, May BJ (2006) Spontaneous activity in the inferior colliculus of CBA/J mice after manipulations that induce tinnitus. *Hear Res* 212:9-21.
- Maison SF, Liberman MC (2000) Predicting vulnerability to acoustic injury with a noninvasive assay of olivocochlear reflex strength. *J Neurosci* 20(12):4701-4707.
- Manzoor NE, Gao Y, Licari F, Kaltenbach JA (2013) Comparison and contrast of noise-induced hyperactivity in the dorsal cochlear nucleus and inferior colliculus. *Hear Res* 295:114-123. doi:10.1016/j.heares.2012.04.003.
- Milbrandt JC, Holder TM, Wilson MC, Salvi RJ, Caspary DM (2000) GAD levels and muscimol binding in rat inferior colliculus following acoustic trauma. *Hear Res* 147:251-260.
- Mills RP, Cherry JR (1984) Subjective tinnitus in children with otological disorders. *Int J Pediatr Otorhinolaryngol* 7(1):21-7.
- Moore BCJ, Glasberg BR (2001) Temporal modulation transfer functions obtained using sinusoidal carriers with normally hearing and hearing-impaired listeners. *J Acoust Soc Am* 110(2):1067-1073.
- Muehlmeier G, Biesinger E, Maier H (2011) Safety of intratympanic injection of AM-101 in patients with acute inner ear tinnitus. *Audiol Neurootol* 16(6):388-397. doi:10.1159/000322641.

- Mulders WH, Robertson D (2009) Hyperactivity in the auditory midbrain after acoustic trauma: dependence on cochlear activity. *Neuroscience* 164(2):733-746. doi:10.1016/j.neuroscience.2009.08.036.
- Mulders WHAM, Robertson D (2011) Progressive centralization of midbrain hyperactivity after acoustic trauma. *Neuroscience* 192:753-760. doi:10.1016/j.neuroscience.2011.06.046.
- Mulders WHAM, Ding D, Salvi R, Robertson D (2011) Relationship between auditory thresholds, central spontaneous activity, and hair cell loss after acoustic trauma. *J Comp Neurol* 519:2637-47. doi: 10.1002/cne.22644.
- Niu Y, Kumaraguru A, Wang R, Sun W (2013) Hyperexcitability of inferior colliculus neurons caused by acute noise exposure. *J Neurosci Res* 91(2):292-299. doi:10.1002/jnr.23152.
- Noreña AJ, Eggermont JJ (2003) Changes in spontaneous neural activity immediately after an acoustic trauma: implications for neural correlates of tinnitus. *Hear Res* 183:137-153.
- Noreña AJ, Eggermont JJ (2005) Enriched acoustic environment after noise trauma reduces hearing loss and prevents cortical map reorganization. *J Neurosci* 25(3):699-705.
- Noreña AJ, Moffat G, Blanc JL, Pezard L, Cazals Y (2010) Neural changes in the auditory cortex of awake guinea pigs after two tinnitus inducers: salicylate and acoustic trauma. *Neuroscience* 166:1194-209. doi: 10.1016/j.neuroscience.2009.12.063.
- Noreña AJ (2011) An integrative model of tinnitus based on a central gain controlling neural sensitivity. *Neurosci Biobehav Rev* 35(5):1089-1109. doi:10.1016/j.neubiorev.2010.11.003.
- Norman M, Tomscha K, Wehr M (2012) Isoflurane blocks temporary tinnitus. *Hear Res* 290:64-71. doi:10.1016/j.heares.2012.03.015.
- Philippens IHCHM, Melchers BPC, Wolthuis OL (1992) Active avoidance behavior in guinea pigs: effects of physostigmine and scopolamine. *Pharmacol Biochem Beh* 42:285-9.
- Pollak GD, Xie R, Gittelman JX, Andoni S, Li N (2011) The dominance of inhibition in the inferior colliculus. *Hear Res* 274(1-2):27-39. doi:10.1016/j.heares.2010.05.010.
- Prosen CA, Petersen MR, Moody DB, Stebbins WC (1978) Auditory thresholds and kanamycin-induced hearing loss in the guinea pig assessed by a positive reinforcement procedure. *J Acoust Soc Am* 63(2):559-66.
- Puel JL (1995) Chemical synaptic transmission in the cochlea. *Prog Neurobiol* 47(6):449-476.
- Recio-Spinoso A, Temchin AN, van Dijk P, Fan Y-H, Ruggero MA (2005) Wiener-kernel analysis of responses to noise of chinchilla auditory-nerve fibers. *J Neurophysiol* 93:3615-3634. doi:10.1152/jn.00882.2004.
- Recio-Spinoso A, van Dijk P (2006) Analysis of responses to noise in the ventral cochlear nucleus using Wiener kernels. *Hear Res* 216-217:7-18.
- Rees A, Palmer AR (1989) Neuronal responses to amplitude-modulated and pure-tone stimuli in the guinea pig inferior colliculus, and their modification by broadband noise. *J Acoust Soc Am* 85(5):1978-1994.
- Rieke F, Warland D, de Ruyter van Steveninck R, Bialek W (1999) *Spikes, exploring the neural code*. MIT press, London.
- Robertson D, Bester C, Vogler D, Mulders WHAM (2013) Spontaneous hyperactivity in the auditory midbrain: relationship to afferent input. *Hear Res* 295:124-9. doi: 10.1016/j.heares.2012.02.002

- Rodgers RJ (1997) Animal models of 'anxiety': where next? *Behav Pharmacol* 8(6-7):477-96.
- Rüttiger L, Ciuffani J, Zenner HP, Knipper M (2003) A behavioral paradigm to judge acute sodium salicylate-induced sound experience in rats: a new approach for an animal model on tinnitus. *Hear Res* 180:39-50.
- Rüttiger L, Singer W, Panford-Walsh R, Matsumoto M, Lee SC, Zucotti A, ... Knipper M (2013) The reduced cochlear output and the failure to adapt the central auditory response causes tinnitus in noise exposed rats. *PLoS One* 8(3):e57247. doi:10.1371/journal.pone.0057247.
- Salvi RJ, Hamernik RP, Henderson D (1978) Discharge patterns in the cochlear nucleus of the chinchilla following noise induced asymptotic threshold shift. *Exp Brain Res* 32(3):301-320.
- Salvi RJ, Saunders SS, Gratton MA, Arehole S, Powers N (1990) Enhanced evoked response amplitudes in the inferior colliculus of the chinchilla following acoustic trauma. *Hear Res* 50:245-258.
- Salvi RJ, Wang J, Ding D (2000) Auditory plasticity and hyperactivity following cochlear damage. *Hear Res* 147:261-274.
- Sansone M, Bovet D (1970) Avoidance learning by Guinea pigs. *Q J Exp Psychol* 22(3):458-61.
- Schaette R, McAlpine D (2011) Tinnitus with a normal audiogram: physiological evidence for hidden hearing loss and computational model. *J Neurosci* 31(38):13452-13457. doi:10.1523/JNEUROSCI.2156-11.2011.
- Schecklmann M, Landgrebe M, Langguth B, TRI Database Study Group (2014) Phenotypic characteristics of hyperacusis in tinnitus. *PLoS One* 9(1):e86944. doi:10.1371/journal.pone.0086944.
- Scholl B, Wehr M (2008) Disruption of balanced cortical excitation and inhibition by acoustic trauma. *J Neurophysiol* 100:646-656. doi:10.1152/jn.90406.2008.
- Shannon RV, Zeng FG, Kamath V, Wygonski J, Ekelid M (1995) Speech recognition with primarily temporal cues. *Science* 270(5234):303-304.
- Shore SE (2005) Multisensory integration in the dorsal cochlear nucleus: unit responses to acoustic and trigeminal ganglion stimulation. *Eur J Neurosci* 21(12):3334-3348.
- Shore SE, Koehler S, Oldakowski M, Hughes LF, Syed S (2008) Dorsal cochlear nucleus responses to somatosensory stimulation are enhanced after noise-induced hearing loss. *Eur J Neurosci* 27:155-68. doi: 10.1111/j.1460-9568.2007.05983.x
- Sidman M (2008) Reflections on stimulus control. *Behav Analyst* 31(2):127-35.
- Sneary MG, Lewis ER (2007) Tuning properties of turtle auditory nerve fibers: evidence for suppression and adaptation. *Hear Res* 228:22-30.
- Stamper GC, Johnson TA (2014) Auditory function in normal-hearing, noise-exposed human ears. *Ear Hear* (Epub ahead of print).
- Stolzberg D, Hayes SH, Kashanian N, Radziwon K, Salvi RJ, Allman BL (2013) A novel behavioral assay for the assessment of acute tinnitus in rats optimized for simultaneous recording of oscillatory neural activity. *J Neurosci Methods* 219(2):224-232. doi:10.1016/j.jneumeth.2013.07.021.
- Sun W, Deng A, Jayaram A, Gibson B (2012) Noise exposure enhances auditory cortex responses related to hyperacusis behavior. *Brain Res* 1485:108-116. doi:10.1016/j.brainres.2012.02.008.
- Sun W, Fu Q, Zhang C, Manohar S, Kumaraguru A, Li J (2014) Loudness perception affected by early age hearing loss. *Hear Res* 313:18-25. doi:10.1016/j.heares.2014.04.002.

- Szczepaniak WS, Møller AR (1995) Evidence of decreased GABAergic influence on temporal integration in the inferior colliculus following acute noise exposure: a study of evoked potentials in the rat. *Neurosci Lett* 196:77-80.
- Takemura K, Komeda M, Yagi M, Himeno C, Izumikawa M, Doi T, ... Yamashita T (2004) Direct inner ear infusion of dexamethasone attenuates noise-induced trauma in guinea pig. *Hear Res* 196:58-68.
- Temchin AN, Recio-Spinoso A, van Dijk P, Ruggero MA (2005) Wiener kernels of chinchilla auditory-nerve fibers: verification using responses to tones, clicks, and noise and comparison with basilar-membrane vibrations. *J Neurophysiol* 93:3635-3648. doi:10.1152/jn.00885.2004.
- Turner JG, Brozoski TJ, Bauer CA, Parrish JL, Myers K, Hughes LF, Caspary DM (2006) Gap detection deficits in rats with tinnitus: a potential novel screening tool. *Behav Neurosci* 120(1):188-195.
- Turner JG (2007) Behavioral measures of tinnitus in laboratory animals. *Prog Brain Res* 166:147-156.
- Turrigiano GG, Nelson SB (2004) Homeostatic plasticity in the developing nervous system. *Nat Rev Neurosci* 5(2):97-107.
- van der Heijden M, Joris PX (2003) Cochlear phase and amplitude retrieved from the auditory nerve at arbitrary frequencies. *J Neurosci* 23(27):9194-9198.
- van Dijk P, Wit HP, Segenhout JM, Tubis A (1994) Wiener kernel analysis of inner ear function in the American bullfrog. *J Acoust Soc Am* 95(2):904-919.
- van Dijk P, Wit HP, Segenhout JM (1997) Dissecting the frog inner ear with Gaussian noise. I. Application of high-order Wiener-kernel analysis. *Hear Res* 114:229-242.
- von der Behrens W (2014) Animal models of subjective tinnitus. *Neural Plast* 2014:741452. doi:10.1155/2014/741452.
- Wada SI, Starr A (1983a) Generation of auditory brain stem responses (ABRs). II. Effects of surgical section of the trapezoid body on the ABR in guinea pigs and cat. *Electroencephalogr Clin Neurophysiol* 56(4):340-351.
- Wada SI, Starr A (1983b) Generation of auditory brain stem responses (ABRs). III. Effects of lesions of the superior olive, lateral lemniscus and inferior colliculus on the ABR of the guinea pig. *Electroencephalogr Clin Neurophysiol* 56(4):352-366.
- Walton JP, Frisina RD, O'Neill WE (1998) Age-related alteration in processing of temporal sound features in the auditory midbrain of the CBA mouse. *J Neurosci* 18(7):2764-2776.
- Wang J, Salvi RJ, Powers N (1996) Plasticity of response properties of inferior colliculus neurons following acute cochlear damage. *J Neurophysiol* 75(1):171-183.
- Wang J, Ding D, Salvi RJ (2002) Functional reorganization in chinchilla inferior colliculus associated with chronic and acute cochlear damage. *Hear Res* 168:238-249.
- Wang H, Brozoski TJ, Caspary DM (2011) Inhibitory neurotransmission in animal models of tinnitus: maladaptive plasticity. *Hear Res* 279:111-117. doi:10.1016/j.heares.2011.04.004.
- Wickesberg RE, Dickson JW, Gibson MM, Geisler CD (1984) Wiener kernel analysis of responses from anteroventral cochlear nucleus neurons. *Hear Res* 14:155-174.
- Willott JF, Lu SM (1982) Noise-induced hearing loss can alter neural coding and increase excitability in the central nervous system. *Science* 216(4552):1331-1334.

- Yamada WM, Lewis ER (1999) Predicting the temporal responses of non-phase-locking bullfrog auditory units to complex acoustic waveforms. *Hear Res* 130:155-170.
- Yang S, Weiner BD, Zhang LS, Cho SJ, Bao S (2011) Homeostatic plasticity drives tinnitus perception in an animal model. *Proc Natl Acad Sci USA* 108(36):14974-14979. doi:10.1073/pnas.1107998108.
- Yin TCT, Kuwada S (1983) Binaural interaction in low-frequency neurons in inferior colliculus of the cat. II. Effects of changing rate and direction of interaural phase. *J Neurophysiol* 50(4):1000-1019.
- Zeng FG (2013) An active loudness model suggesting tinnitus as increased central noise and hyperacusis as increased nonlinear gain. *Hear Res* 295:172-179. doi:10.1016/j.heares.2012.05.009.
- Zheng XY, Henderson D, Hu BH, Ding DL, McFadden SL (1997) The influence of the cochlear efferent system on chronic acoustic trauma. *Hear Res* 107:147-159.
- Zheng Y, Hamilton E, Stiles L, McNamara E, de Waele C, Smith PF, Darlington CL (2011a) Acoustic trauma that can cause tinnitus impairs impulsive control but not performance accuracy in the 5-choice serial reaction time task in rats. *Neurosci* 180:75-84. doi: 10.1016/j.neuroscience.2011.02.040.
- Zheng Y, Hamilton E, McNamara E, Smith PF, Darlington CL (2011b) The effects of chronic tinnitus caused by acoustic trauma on social behaviour and anxiety in rats. *Neurosci* 193:143-53. doi: 10.1016/j.neuroscience.2011.07.026.
- Zhong Z, Henry KS, Heinz MG (2014) Sensorineural hearing loss amplifies neural coding of envelope information in the central auditory system of chinchillas. *Hear Res* 309:55-62. doi:10.1016/j.heares.2013.11.006.
- Zuccotti A, Kuhn S, Johnson SL, Franz C, Singer W, Hecker D, ... Knipper M (2012) Lack of brain-derived neurotrophic factor hampers inner hair cell synapse physiology, but protects against noise-induced hearing loss. *J Neurosci* 32(25):8545-8553. doi:10.1523/JNEUROSCI.1247-12.2012.

Summary

Acoustic Trauma

Damage caused by prolonged exposure to loud noise is called acoustic trauma. The most well-known consequence of acoustic trauma is an increase in hearing thresholds, i.e. hearing impairment. However, acoustic trauma can also cause ringing in the ears, which is called tinnitus in medical terms. Tinnitus is a common phenomenon: nearly 90% of adolescents report having had a tinnitus at least once. However, there are also people who constantly have tinnitus. This can be very disabling. Additional symptoms that are common with tinnitus are difficulties with concentrating, insomnia, and stress, which can sometimes turn into depression and even attempted suicides. Furthermore, acoustic trauma can also cause hyperacusis, a condition in which the patient is hypersensitive to sounds of normal volume. Unfortunately, at present there are no treatments available that are able to consistently cure tinnitus and hyperacusis.

One reason for the lack of a treatment is the fact that we do not completely understand the underlying mechanisms of tinnitus and hyperacusis. It is known that, in addition to damage in the ear, the brain also plays an important role in the development of tinnitus and hyperacusis. Therefore most of the research described in this dissertation is focused on the brain. More specifically, I studied the consequences of acoustic trauma on the function of the brain: in other words, the neurophysiological consequences of acoustic trauma. Because neurophysiology in humans can only be studied indirectly, the current studies were conducted in laboratory animals. The last chapter is devoted to the question of how we can determine if animals have tinnitus or hyperacusis. The overarching purpose of this dissertation is to contribute to the development of an animal model to test treatments for tinnitus and hyperacusis.

Recording brain activity in a laboratory animal

The central auditory system is defined as the brain areas that are processing acoustic information (see the figure on the front page of this dissertation). The brain cells (neurons) in these regions communicate with each other by means of electrical signals, or action potentials. By analyzing the patterns of these action potentials, the processing of sound can be studied. Action potentials of neurons can be recorded by implanting an electrode in the brain of the experimental animal. The rate at which a neuron fires action potentials is also called the neural activity. By presenting specific sounds, different auditory neurons increase or decrease their spontaneous neural activity, which is referred to as excitation and inhibition, respectively. **Chapter 2** shows that acoustic trauma results in a disturbed balance

between excitation and inhibition. In particular, the number of neurons showing inhibition is decreased after acoustic trauma.

This result was subsequently confirmed and further investigated by analyzing neural activity using the Wiener kernel technique. Wiener kernels are determined by cross-correlating the presented sound with the evoked action potentials. The sound frequencies to which the neuron responds can be extracted from these kernels. It can also be determined whether this response is excitatory or inhibitory. In **Chapter 4**, the application of the Wiener kernel technique is described for neural activity of the inferior colliculus. Next, in **Chapter 5**, Wiener kernels are applied on neural activity measured before and immediately after acoustic trauma. By calculating the Wiener kernels we have gained more insight into the balance between excitation and inhibition in neurons of normal-hearing animals and how this balance is disturbed due to acoustic trauma. It turns out that mainly in mid-frequency neurons, which respond to frequencies just below the trauma frequency, no inhibition and only excitation is present after acoustic trauma.

Decreased inhibition in auditory neurons will generally lead to higher firing rates in response to sound. It can be assumed that this will cause sounds to be perceived louder than normal. Thus, reduced inhibition might lead to hyperacusis. This is part of the reason why this dissertation asserts that an acoustic trauma-induced disturbed balance between excitation and inhibition could be an underlying mechanism of hyperacusis.

Neurons of the auditory system also code for the slower fluctuations of the amplitude of a sound (the amplitude modulations). These are encoded for by timing the action potentials at a specific phase of the amplitude modulation, also called phase locking. **Chapter 3** describes the effects of acoustic trauma on the degree of phase locking in the inferior colliculus. It appears that neurons respond more strongly to amplitude modulations after acoustic trauma. This may underlie the problems that people with hearing loss encounter with understand speech in fluctuating (amplitude modulated) background noise.

How can we ask a guinea pig if he has tinnitus or hyperacusis?

In order to determine whether acoustic trauma-induced abnormalities in neural activity correlate with tinnitus or hyperacusis, behavioral models have been developed. A behavioral model is defined as a method which can determine from the behavior of the animal whether it has tinnitus or hyperacusis. Models that determine tinnitus are based on the assumption that the animal with tinnitus does not perceive silence. This dissertation describes a potential new method to determine tinnitus (and perhaps hyperacusis) in a laboratory animal, in this case, the guinea pig. This method is based on the finding that guinea pigs show different natural behavior during noise and in silence. In silence, they sit still and in noise, guinea pigs will move and walk around. **Chapter 6** shows that guinea pigs that have been exposed to acoustic trauma are also active during the silent intervals. This suggests that they may not have perceived silence and have tinnitus. When it has been determined whether the degree

of activity during sound correlates with sound intensity, this method could possibly also be used to determine hyperacusis in laboratory animals.

Conclusions

In summary, this dissertation shows that acoustic trauma is not limited to hearing loss in the ear, but also has several consequences for the processing of sound in the central auditory system. Acoustic trauma disturbs the balance between excitation and inhibition in the mid-frequency neurons and enhances the response to amplitude modulations in the inferior colliculus. These findings, in combination with the proposed new behavior model, will potentially contribute to the development of an animal model in which a direct relationship can be established between the neurophysiological effects of acoustic trauma and the presence of tinnitus or hyperacusis. Such an animal model opens new pathways for further investigation of the mechanisms of tinnitus and hyperacusis, and for the development of treatments.

Samenvatting

Geluidstrauma

De schade die je op kan lopen door langdurige blootstelling aan hard geluid wordt geluidstrauma genoemd. Het meest bekende gevolg van geluidstrauma is een verhoging van de gehoordrempels, oftewel slechthorendheid. Echter, geluidstrauma kan ook leiden tot oorsuizen, ook wel tinnitus genoemd in medische termen. Tinnitus komt veel voor; bijna 90% van de adolescenten zegt wel eens tinnitus te hebben gehad. Echter, er is ook een groep mensen die constant een tinnitus heeft. Hier kan men veel last van krijgen. Bijkomende klachten die vaak voorkomen met langdurige tinnitus zijn concentratieproblemen, slapeloosheid en stress, welke soms over kunnen gaan in depressie en zelfs zelfmoordneiging. Verder kan geluidstrauma ook leiden tot hyperacusis, een conditie waarin de patiënt overgevoelig is voor geluiden met normale geluidssterkte. Helaas zijn er tot op heden geen behandelingen beschikbaar die tinnitus en hyperacusis kunnen doen verdwijnen.

Een van de redenen voor het ontbreken van een behandeling is het feit dat we de onderliggende mechanismes van tinnitus en hyperacusis nog niet precies begrijpen. Wel is bekend dat, naast gehoorschade in het oor, de hersenen ook een belangrijke rol spelen bij het ontstaan van tinnitus en hyperacusis. Het grootste deel van het onderzoek dat is beschreven in dit proefschrift is daarom gericht op de hersenen. In deze studies worden de consequenties van geluidstrauma op de functie van de hersenen, oftewel de neurofysiologische consequenties van geluidstrauma, bestudeerd. Omdat neurofysiologie in de mens alleen indirect bestudeerd kan worden is het huidige onderzoek uitgevoerd in proefdieren. Het laatste hoofdstuk is gewijd aan de vraag of we kunnen vaststellen of het proefdier tinnitus of hyperacusis heeft. Het doel van dit proefschrift is om bij te dragen aan de ontwikkeling van een proefdiermodel voor het testen van behandelingen voor tinnitus en hyperacusis.

Metten van hersenactiviteit in een proefdier

Een deel van de hersenen is voornamelijk gericht op het verwerken van geluid. Deze gebieden worden samen ook wel het centraal auditief systeem genoemd (zie de figuur op de voorpagina van dit proefschrift). De hersencellen (neuronen) in deze gebieden communiceren met elkaar door middel van elektrische signalen, de actiepotentialen. Door de patronen van deze actiepotentialen te analyseren kan de verwerking van geluid bestudeerd worden. Actiepotentialen van neuronenvan kunnen gemeten worden door een elektrode te implanteren in de hersenen van het proefdier. De vuurfrequentie waarmee een neuron actiepotentialen afgeeft wordt ook wel de neurale activiteit genoemd. Door het aanbieden van specifieke geluiden wordt de spontane neurale activiteit van verschillende neuronenvan dan wel verhoogd (excitatie) of verlaagd (inhibitie). **Hoofdstuk 2** laat zien dat geluidstrauma resulteert in een

verstoorde balans tussen excitatie en inhibitie. Met name het aantal neuronen dat inhibeert is verminderd na geluidstrauma.

Dit resultaat is vervolgens bevestigd en verder onderzocht door de neurale activiteit te analyseren met de Wiener kernel techniek. Wiener kernels worden uitgerekend door het gepresenteerde geluid te correleren met de actiepotentialen. De geluidsfrequenties waarop het neuron reageert kunnen vervolgens afgeleid worden uit de kernels. Ook kan bepaald worden of deze reactie exciterend of inhiberend is. In **hoofdstuk 4** wordt de toepassing van de Wiener kernel techniek op neurale activiteit van de inferior colliculus beschreven. Vervolgens worden in **hoofdstuk 5** Wiener kernels toegepast op neurale activiteit gemeten voor geluidstrauma en direct na geluidstrauma. Door het uitrekenen van de Wiener kernels hebben we meer inzicht gekregen in hoe de balans tussen excitatie en inhibitie is opgebouwd in neuronen van normaalhorende proefdieren en hoe deze balans is verstoord als gevolg van geluidstrauma. Het blijkt dat er voornamelijk in de mid-frequente neuronen, welke reageren op frequenties net onder de trauma frequentie, wel excitatie maar geen inhibitie meer aanwezig is na geluidstrauma.

De verminderde inhibitie zal in het algemeen leiden tot een hogere vuurfrequentie van auditieve neuronen in response op geluid. We nemen aan dat dit er toe zal leiden dat geluiden over het algemeen als luider worden waargenomen. De verminderde inhibitie zou daarmee kunnen leiden tot hyperacusis. Mede daardoor wordt er in dit proefschrift gesteld dat de verstoorde balans van excitatie en inhibitie als gevolg van geluidstrauma een onderliggende pathologie is van hyperacusis.

Neuronen in het auditief systeem coderen ook voor de langzamere veranderingen van de sterkte van het geluid (de amplitude modulaties). Neuronen coderen hiervoor door hun actiepotentialen precies te timen met een bepaalde fase van de amplitude modulatie, ook wel phase locking genoemd. **Hoofdstuk 3** beschrijft de consequenties van geluidstrauma op de mate van phase locking in de inferior colliculus. Het blijkt dat neuronen sterker reageren op de amplitude modulaties na geluidstrauma. Dit kan ten grondslag liggen aan de problemen die mensen met gehoorverlies ervaren met het verstaan van spraak in fluctuerende (amplitude gemoduleerde) achtergrond geluiden.

Hoe kunnen we aan een cavia vragen of hij tinnitus of hyperacusis heeft

Om te bepalen of de afwijkingen in hersenactiviteit correleren met tinnitus of hyperacusis, zijn er gedragsmodellen ontwikkeld. Onder een gedragsmodel wordt een methode verstaan waarbij uit het gedrag van een proefdier kan worden opgemaakt of hij tinnitus of hyperacusis heeft. De methodes voor het bepalen van tinnitus zijn gebaseerd op de aanname dat een proefdier geen stilte kan ervaren wanneer hij tinnitus heeft. Dit proefschrift beschrijft een mogelijk nieuwe methode om tinnitus (en wellicht hyperacusis) te bepalen in een proefdier, in dit geval de cavia. Deze methode is gebaseerd op de bevinding dat cavia's verschillend

natuurlijk gedrag laten zien in ruis en in stilte. In stilte zitten cavia's stil en in ruis zullen ze bewegen en rondlopen. **Hoofdstuk 6** laat zien dat cavia's die zijn blootgesteld aan geluidstrauma ook actief worden tijdens de stilte intervallen. Dit suggereert dat ze de stilte niet ervaren en mogelijk tinnitus hebben. Wanneer er is vastgesteld of de mate van activiteit tijdens geluid correleert met de luidheid van de ruis zou dit wellicht gebruikt kunnen worden voor het vaststellen van hyperacusis in het proefdier.

Conclusies

Samenvattend toont dit proefschrift dat geluidstrauma niet beperkt blijft tot gehoorschade in het oor, maar ook diverse consequenties heeft voor de verwerking van geluid in het centraal auditief systeem. Geluidstrauma verstoort de balans tussen excitatie en inhibitie in midfrequente neuronen en versterkt de reactie op amplitude modulaties in de inferior colliculus. Deze bevindingen, in combinatie met het voorgestelde nieuwe gedragsmodel, zullen mogelijk bijdragen aan de ontwikkeling van een proefdiermodel waarin een directe relatie gelegd kan worden tussen de neurofysiologische consequenties van geluidstrauma en de aanwezigheid van tinnitus of hyperacusis. Een dergelijk proefdiermodel opent nieuwe paden voor het verder onderzoeken van de mechanismes van tinnitus en hyperacusis en voor het ontwikkelen van behandelingen.

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Curriculum Vitae

Amarins N. Heeringa was born in 1986 in Deinum, the Netherlands. From 1998 until 2004, she went to the Atheneum at OSG Piter Jelles Âldlân. She started with Life Science & Technology at the University of Groningen in 2004, where she specialized in Molecular Physiology and Pharmacology.

After receiving her Bachelor's degree, she travelled for a year in South-East Asia and the United States, after which she started in 2008 with the Research Master Behavioural and Cognitive Neurosciences (BCN) at the University of Groningen. Within this master, she specialized in Molecular and Clinical Neuroscience and she performed two research projects. Her minor thesis was supervised by Dr. P. Meerlo at the University of Groningen and was titled: 'Does sleep deprivation affect hippocampal functioning?'. For her major thesis, she studied the role of the immune system in bone cancer-derived pain at the Université de Bordeaux 2, which was supervised by Dr. J.P. Konsman.

After graduating cum laude in 2010, she started working on a PhD project at the ENT department of the University Medical Center Groningen, which resulted in the current dissertation. Her project was supervised by Dr. P. van Dijk and was connected to the graduate school of Behavioural and Cognitive Neurosciences (BCN).

After finishing her PhD project, she moved to the US to do a postdoc at the Kresge Hearing Research Institute at the University of Michigan in the lab of Dr. S. Shore, studying non-auditory connections to the ventral cochlear nucleus.