On sound and silence
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Preface

Acoustic trauma has a major impact on several aspects of hearing. In the first place, it results in an elevation of the auditory thresholds, often accompanied by difficulties in understanding speech in noisy conditions. Additionally, people exposed to loud sounds might develop tinnitus, which is defined as the perception of a sound that cannot be attributed to an external sound source. Throughout this dissertation, the term tinnitus will refer to subjective tinnitus, which involves the cases where no physiological internal sound source, such as a sound produced by a pulsating blood vessel, is causing the tinnitus. The prevalence of people that perceive noise-induced tinnitus, but are not necessarily bothered by it, is quite large. Among young adults, 89.5% report experiencing transient tinnitus after loud music exposure (Gilles et al., 2012). Another consequence of exposure to loud sounds is hyperacusis. This is a condition in which the patient has a reduced tolerance and an increased sensitivity to sound. Hyperacusis can occur alone, but is often comorbid with tinnitus. The prevalence of hyperacusis in tinnitus patients varies between 55% and 79% (Dauman and Bouscau-Faure, 2005; Schecklmann et al., 2014). Both are potentially debilitating conditions for which there are no widely accepted treatments.

There is a general consensus that the pathology of both tinnitus and hyperacusis resides, at least partly, in the central auditory system (Eggermont and Roberts, 2004; Lanting et al., 2009; Knipper et al., 2013). However, despite numerous studies and hypotheses, the exact pathophysiological mechanism(s) behind tinnitus and hyperacusis remain(s) elusive. This will be the broad subject in this dissertation. I will try to answer a few specific research questions that are aimed to fill (or to contribute to filling) small parts of the gaps in the knowledge about the mechanisms behind noise-induced tinnitus and hyperacusis.

In the following part of this introduction, I will explain the methods that I used and the reasons why I used these specific methods to study the pathophysiological mechanism(s) behind noise-induced tinnitus and hyperacusis. This will be followed by an overview of the specific aims and research questions that are addressed in the subsequent chapters of this dissertation.

Why use the guinea pig to study the effects of acoustic trauma

There are a few important reasons to study pathophysiological mechanisms of tinnitus and hyperacusis in a living animal. First, as compared to in vitro techniques, the use of laboratory animals can provide crucial information, as the whole living body is far more complex than the sum of its parts. In other words, exposing a few auditory neurons in a petri dish to a traumatizing stimulus does not give the same results as exposing a living animal to loud sounds. Second, invasive techniques, such as in vivo electrophysiology, are inappropriate for use in humans, but can be applied on animals. Furthermore, animal studies allow for the careful control of a number of factors that might otherwise influence the outcomes when studied in human subjects, such as genetic background and degree of sound exposure. And last, the effectiveness and mode of action of specific treatments for tinnitus and hyperacusis
can be studied in detail in an animal model. In the last few decades, animal studies have proven to be useful in revealing physiological, molecular, and anatomical markers of noise exposure in the central auditory system (Knipper et al., 2010; Eggermont, 2013). In order to correlate these markers with a specific consequence of noise exposure, such as tinnitus or hyperacusis, behavioral models, which aim to show whether an animal has tinnitus or hyperacusis, have been designed (Hayes et al., 2014).

Even though the rat is also often used to study the pathophysiological mechanism(s) behind noise-induced tinnitus and hyperacusis, I chose for the guinea pig. In the guinea pig, the round window is easily accessible through the bulla behind the ear. This will be important for future studies, because it allows us to investigate the mode of action and effectiveness of drug treatments that need to be applied locally on the round window (Muehlmeier et al., 2011). Furthermore, the guinea pig has frequently been used in auditory neurophysiological studies (e.g. Rees and Palmer, 1989; Dunnebier et al., 1997; Shore, 2005; Mulders and Robertson, 2009). This allows for a direct comparison of my results to the literature. However, when I started this project in 2010, there was no behavioral model yet that could test for the presence of tinnitus or hyperacusis in the guinea pig. Therefore, one of the studies I conducted addressed possible measures to assess tinnitus in guinea pigs (Chapter 6).

How to study the effects of acoustic trauma on neurophysiological measures

“Neurophysiology” is derived from the Greek words “νευρο-” (neuro-) and “ϕυσιολογια” (physiology). It refers to the study of physiological mechanisms of the (central) nervous system. Several neurophysiological methods are available, which all have varying degrees of spatial and temporal resolution. In the current study, I used the auditory brainstem response (ABR) and in vivo electrophysiology, which are described in detail below.

The auditory brainstem response

I selected the ABR as an investigative technique because it is a rapid, commonly used, and reliable method to establish hearing thresholds and integrity of the auditory system (see below). Furthermore, the ABR can also be assessed in humans, which allowed me to compare my results to human studies.

The ABR is a recording of an evoked potential that derives from the cochlea and the auditory brainstem in response to an acoustic stimulus. In laboratory animals, the ABR is obtained by placing three electrodes subcutaneously, one at the vertex, one behind the ipsilateral pinna, and one behind the contralateral pinna, for reference, recording, and grounding, respectively. Clicks (0.1 ms) and short tone pips (3 ms) are commonly used as acoustic stimuli to evoke ABRs. By subtracting the signal recorded from the electrode behind the ipsilateral pinna from the signal recorded from the electrode at the vertex, a stimulus-related waveform can be measured (see Figure 1.1). This waveform is characterized by a number of peaks, which are referred to as the ABR waves and are labeled by the Roman
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numerals I, II, III, and IV. ABRs can reveal several functional characteristics of the auditory system.

The most common application of the ABR is to determine auditory thresholds. Stimuli of decreasing sound levels are presented, which typically result in smaller amplitudes of the ABR waveforms. The lowest stimulus level at which prominent stimulus-related waveforms can still be observed is considered the auditory threshold for that particular stimulus. In the current dissertation, ABRs have been used to determine the auditory thresholds for the experiments described in Chapter 2, Chapter 5, and Chapter 6.

Furthermore, the waves of the ABR can be regarded as derived from different locations of the auditory pathway, although it is not entirely clear whether there are single sites in the brain responsible for the generation of the individual waves (Wada and Starr, 1983a, 1983b). Nevertheless, it is commonly accepted that wave I represents the summated activity of the auditory nerve and wave IV derives from a more central location, i.e. the auditory midbrain (see Figure 1.1 and Figure 1.3). When the auditory nerve integrity is compromised by moderate noise trauma, then the amplitude of wave I is reduced (Kujawa and Liberman, 2009; Lin et al., 2011). Furthermore, in tinnitus patients with normal auditory thresholds, the amplitude of wave I is also reduced but the wave IV amplitude is intact (Kehrle et al., 2008; Schaette and McAlpine, 2011; Gu et al., 2012). These findings show that studying the amplitudes of the ABR waves can be used as a tool to determine the integrity of the central auditory system. This approach has been applied in Chapter 2.

In vivo electrophysiology

The other method that I used extensively in the experiments conducted for this dissertation was in vivo electrophysiology. This method allows us to directly record neural activity from auditory neurons in the anesthetized animal. The activity of neurons consists of brief electrical

![Figure 1.1](image-url) A representative example of an auditory brainstem response of a normal-hearing guinea pig evoked by a 0.1 ms click of 70 dB SPL. The positive peaks of the waveforms are labeled with the corresponding Roman numerals.
discharges, which appear as spikes in a neural signal recorded by the electrode. Recordings of these spikes in the presence and absence of external acoustic stimuli provides a powerful tool to study the integrity and functionality of the central auditory system after acoustic trauma.

Briefly, a linear 16-channel electrode is inserted in the brain of the anesthetized animal and extracellular currents are recorded. Subsequently, the signals are filtered and algorithms are applied to remove artifacts. Spike trains can be extracted by determining the instants when the signal exceeds a predefined threshold (see Figure 1.2). On one channel, spiking activity of multiple neurons is typically recorded. Several attempts were made to sort the spikes into individual sources, however, no single units could be identified from the traces. Therefore, all the neurophysiological data reported in this dissertation is considered multi-unit activity.

Figure 1.2 Example of a filtered neural signal and the corresponding spiking threshold.

Figure 1.3 The central auditory system. On the left, a simplified, schematic illustration of the central auditory pathway is shown. The inferior colliculus is located at the level of the midbrain and connects the cochlear nucleus and superior-olivary complex to the auditory thalamus (medial geniculate body) and the auditory cortex. On the right, the inferior colliculi of the guinea pig are displayed.
All recordings reported in this dissertation derived from the auditory neurons in the inferior colliculus (IC), which is located in the midbrain and is connected to the cochlear nucleus, superior-olivary complex, and the thalamo-cortical system, among others (Figure 1.3).

I recorded both spontaneous (Chapter 2, Chapter 5) and acoustic stimulus-evoked activity (Chapter 2, Chapter 3, Chapter 4, Chapter 5). A number of analysis-techniques were used to analyze stimulus-evoked activity, which are described below.

Receptive fields

Receptive fields can be obtained by presenting pure tones that have a range of different frequencies and sound levels. Subsequently, the tone-evoked firing rates are inserted in a matrix that organizes frequency and sound level (Figure 1.4).

Figure 1.4 An example of a receptive field of an IC unit of the guinea pig. The color code indicates the firing rate in spikes/second. The excitatory receptive field is contoured by the excitatory tuning curve (black line). The characteristic frequency (CF) and threshold are defined by the lowest tip of the tuning curve (black arrow indicates the excitatory CF and threshold). The white line is the inhibitory tuning curve that contours the inhibitory receptive field, i.e. the range of tones that result in a reduction of the spontaneous firing rate.

The neurons in the inferior colliculus can have an excitatory and an inhibitory receptive field. The excitatory receptive field is defined by the range of frequencies and levels that elicit a significant increase in firing rate, whereas the inhibitory receptive field is considered to be the range of tones that elicit a significant decrease in firing rate as compared to the spontaneous
firing rate. The edge of the receptive fields are called the excitatory or inhibitory tuning curves (see Figure 1.4, black and white curves, respectively). The lowest tip of the tuning curve reveals the unit’s characteristic frequency (CF) and its corresponding threshold (black arrow in Figure 1.4). In the IC of the guinea pig, the CFs of neurons along the dorso-lateral to medial-ventral axis are organized in an ascending manner. Such a systematic progression of CFs across a brain region is called a tonotopic organization. The CF and threshold, which characterize a multi-unit, were used in the experiments of the current dissertation to determine the location and degree of damage caused by acoustic trauma (Chapter 2, Chapter 3, Chapter 5).

**Post-stimulus time histograms**

Stimulus-evoked activity can also be organized in a post-stimulus time histogram (PSTH). A PSTH is obtained by presenting a short-duration stimulus (e.g. 100 ms) for a large number of times (e.g. 300 repetitions). Subsequently, spikes are accumulated in time-interval bins relative to the timing of the stimulus. Typically, these histograms are converted to show the average firing rate before, during, and after the presentation of the repeated stimulus (Figure 1.5).

![Figure 1.5](image-url) An example of a post-stimulus time histogram of an IC unit of the guinea pig. The average firing rate in spikes/sec relative to the timing of the stimulus is plotted as a histogram (bin size 3 ms). The stimulus (blue bar) was a 22-kHz pure tone of 70 dB SPL, with a delay of 50 ms and a duration of 100 ms. This neuron shows an excitatory response to this particular stimulus.

A PSTH provides information about response characteristics, such as the magnitude, the sign (enhancement or inhibition), and the latency of the response. Previous studies showed that PSTH response characteristics are affected by acoustic overstimulation (Willott and Lu,
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1982; Wang et al., 1996). Furthermore, a PSTH of a neural response to amplitude-modulated sounds can reveal whether the spiking activity phase locks to the period of the modulation frequency (Rees and Palmer, 1989). This indicates how well the envelope of a stimulus is coded by the recorded multi-unit. In the experiments of the current dissertation, I used PSTHs to study the effects of acoustic trauma on excitatory and inhibitory responses to pure tones (Chapter 2) and on responses to amplitude-modulated noise (Chapter 3).

Wiener-kernel analysis

Another technique that I used in the current dissertation to analyze stimulus-evoked activity of the auditory system is called the Wiener-kernel analysis (Chapter 4, Chapter 5). For this analysis, spiking activity of the IC in response to a continuous Gaussian noise is recorded. Subsequently, the spike trains are cross-correlated with the broadband noise to obtain a set of Wiener kernels (Eggermont, 1993). These kernels provide information about the stimulus characteristics that evoke spikes. In other words, the technique allows us to look back at the acoustic signal preceding the spikes to investigate the stimulus characteristics that elicited the spikes. The Wiener-kernel analysis technique has also been referred to as a subject-centered approach and allows us to investigate the non-linear properties of the auditory system. Typically, the kernels up to the second-order are calculated. The first-order kernel is equal to the linear correlation between the spike train and the noise stimulus, and reflects the ability of a neuron to phase lock to the fine structure of the noise stimulus. The second-order kernel describes nonlinear characteristics of the system and can be further analyzed with singular-value decomposition (SVD). SVD decomposes the kernel into a number of parallel subsystems, each with a filter function (an eigenvector) and a gain function (the corresponding eigenvalue). The filter functions with the highest gains reveal the best frequencies of the neurons. Furthermore, the gain functions can be positive or negative, corresponding to excitation or inhibition, respectively. Thus, Wiener-kernel analysis, complemented with SVD of the second-order kernel, can reveal excitatory and inhibitory response characteristics, and, therefore, can be used as tool to study processes that critically depend on a balance between excitation and inhibition, such as tinnitus and hyperacusis. A more detailed description of the Wiener-kernel analysis and the SVD is provided in Chapter 4.

How to ask a guinea pig if he has tinnitus

In humans, the diagnosis of tinnitus is based on a declaration of the patient, which states that he or she perceives a sound that cannot be attributed to an external acoustic source. Diagnosing hyperacusis is also dependent on a declaration of the patient, who rates the sound levels according to a loudness scale ranging from quiet to painfully loud. Obviously, these diagnostic methods are not appropriate for animals. Therefore, several behavioral animal models were developed to determine whether an animal experiences tinnitus and/or hyperacusis (Turner, 2007; Hayes et al., 2014). A reliable behavioral model will allow us to assign neurophysiological correlates to tinnitus and/or hyperacusis. Therefore, I believe
that it is important to consider behavioral models when studying the pathophysiological mechanism(s) of noise-induced tinnitus and hyperacusis. The current models can be roughly divided into two categories, which are described below.

**Conditioning paradigms**

In 1988, Pawel Jastreboff and colleagues were the first to publish results of a behavioral model to determine tinnitus in animals (Jastreboff et al., 1988a). In this paradigm, the researchers trained water-deprived rats to stop licking a water dispenser at the offset of a continuous background noise. It is assumed that, if the animal is well trained, the amount of licks during the silent interval corresponds to how well the animal perceives silence. After induction of tinnitus, animals continued to lick from the water dispenser during silence, suggesting that they did not perceive silence. A number of control experiments were conducted to exclude other explanations for the increased licking behavior during silence (Jastreboff et al., 1988b). Over the last decades, several groups provided improved paradigms of the initial idea to condition an animal to show a particular behavior during silent intervals (e.g. Bauer et al., 1999; Rüttiger et al., 2003; Stolzberg et al., 2013).

**Startle-reflex paradigms**

The other widely used paradigm is the startle-reflex paradigm, which was developed by Jeremy Turner and colleagues in 2006 (Turner et al., 2006). Similar to the conditioning models, the startle-reflex paradigm assumes that tinnitus impairs the animal’s ability to perceive a silent interval. The paradigm makes use of the startle reflex, which involves a startle response to an abrupt loud sound. A small silent gap in continuous background noise prior to this loud sound acts as a cue that inhibits the startle reflex. This is called the gap pre-pulse inhibition reflex. It is assumed that animals with tinnitus have difficulties detecting the silent gap and, thus, have a smaller gap pre-pulse inhibition reflex.

**Behavioral models to detect tinnitus in guinea pigs**

More recently, it has been shown that tinnitus can be detected in guinea pigs using the startle-reflex paradigm (Dehmel et al., 2012; Berger et al., 2013). However, guinea pigs have not been subjected to the conditioning paradigms. In Chapter 6, an attempt to develop a conditioning paradigm for guinea pigs is described. The results of these experiments led to a new idea, which might be used as a potential new paradigm of detecting tinnitus in guinea pigs.

**Aims and outline of the remaining chapters**

The overarching aim of the current dissertation is to study neurophysiological and behavioral consequences of acoustic trauma in the guinea pig. The presented results may provide additional insight in the pathophysiological mechanism(s) of noise-induced tinnitus and
hyperacusis. Gaining knowledge about the pathophysiology of tinnitus and hyperacusis advances the search for a treatment. Future research in this direction might result in an animal model that allows us to test drug treatments that need to be applied on the round window of the cochlea.

In Chapter 2, the time course of neurophysiological consequences of acoustic trauma was determined. The applied acoustic trauma was associated with rapidly recovering hearing thresholds. Wave I and wave IV amplitudes of the ABR were assessed. In addition, in vivo neurophysiological recordings from the IC provided a more detailed insight into excitatory and inhibitory responses in the auditory midbrain.

In Chapter 3, the effects of immediate acoustic trauma on neural responses of the IC to amplitude-modulated noise were identified. This provided insight into the changes induced by acoustic trauma on envelope coding in the IC.

The aim of the study presented in Chapter 4 was to determine the applicability of Wiener-kernel analysis to noise-evoked spike trains of the IC. First- and second-order Wiener kernels were identified and classified, and were compared to classic excitatory and inhibitory tuning curves, obtained from responses to pure tones.

Chapter 5 describes the effects of immediate acoustic trauma on response characteristics of the IC, as revealed with Wiener-kernel analysis. We investigated whether a trauma-induced disrupted balance between excitation and inhibition (as shown in Chapter 2) could be confirmed and further defined with Wiener-kernel analysis.

In Chapter 6, possible behavioral methods to determine tinnitus in guinea pigs were studied. The initial aim of the study was to develop a conditioning model to detect tinnitus in guinea pigs. However, the first experiment showed that guinea pigs could not be trained to a silent interval in background noise. Instead, it appeared that the behavioral activity of the animals was inhibited during the silent interval. Therefore, another experiment was designed to evaluate the mobility during silence and noise in animals with and without exposure to acoustic trauma.

Chapter 7 provides a discussion about how the experiments conducted for the current dissertation add value to the current literature of this research field. Furthermore, future directions that are necessary in order to develop an animal model to test treatments for tinnitus are also described.