Chapter 5

The immediate effects of acoustic trauma on the inferior colliculus: a Wiener-kernel analysis

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Abstract

Noise-induced tinnitus and hyperacusis are thought to correspond to a disrupted balance between excitation and inhibition in the central auditory system. We have previously shown that properties of excitation and inhibition of the inferior colliculus (IC) can be studied using Wiener-kernel analysis. The current study aimed at investigating the effects of immediate acoustic trauma on excitatory and inhibitory components of the IC, as revealed with Wiener-kernel analyses.

Neural responses were recorded from the IC of three anesthetized albino guinea pigs before and immediately after a one-hour bilateral exposure to an 11-kHz tone of 124 dB SPL. Before and after the traumatizing stimulus, neural activity was recorded during the presentation of a 1-h continuous Gaussian-noise stimulus of 70 dB SPL for Wiener-kernel analysis. Response characteristics obtained with Wiener-kernel analyses were complemented with excitatory and inhibitory responses to pure tones.

Both spontaneous and noise-evoked firing rates were significantly decreased immediately after acoustic trauma. Furthermore, multi units were tuned to lower frequencies as compared to before acoustic trauma. Wiener-kernel analysis showed that excitation and inhibition in low-CF multi units (CF < 3 kHz) was not affected, inhibition in mid-CF multi units (CF between 3 kHz and 11 kHz) disappeared whereas excitation was not affected, and both excitation and inhibition in high-CF multi units (CF > 11 kHz) disappeared after acoustic trauma. This specific differentiation could not be identified with the tone-evoked receptive-field analyses, in which inhibitory responses disappeared in all units and excitatory responses in only the high-CF units.

With this study, we showed that the effects of acoustic trauma can be identified with Wiener-kernel analysis. We confirmed an acoustic trauma-induced disrupted balance between excitation and inhibition, which was apparent in mid-CF units in particular. Our findings might give additional insight in the central pathophysiological mechanisms of noise-induced hyperacusis.

**Keywords:** Wiener kernels; inferior colliculus; acoustic trauma; singular-value decomposition; guinea pig

**Abbreviations:** ABR, auditory brainstem response; BF, best frequency; CF, characteristic frequency; IC, inferior colliculus; RMS, root-mean-square; SVD, singular-value decomposition
1. Introduction

Extensive acoustic overstimulation affects the balance between excitation and inhibition in the central auditory system (Scholl and Wehr, 2008; Dong et al., 2010a; Llano et al., 2012; Chapter 2). This disrupted balance between excitation and inhibition is thought to be an underlying mechanism of noise-induced tinnitus and hyperacusis (Noreña, 2011; Knipper et al., 2013). As there is currently no common treatment for patients with tinnitus and hyperacusis, gaining additional knowledge about this phenomenon can advance the search for such a treatment.

Recent research of our lab and others has shown that excitation and inhibition of the central auditory system can be well identified by applying Wiener-kernel analyses (Yamada and Lewis, 1999; Sneary and Lewis, 2007; Chapter 4). Wiener-kernel analysis is a technique that can be used to study functional properties of stimulus-evoked activity in the central auditory system (Korenberg and Hunter, 1990; Eggermont, 1993). To apply this analysis, neural responses from the auditory system to broadband Gaussian noise are measured. By correlating the broadband noise with the noise-evoked spike train, a set of Wiener kernels can be obtained. These kernels characterize the auditory system up to the level where the response was measured. In order to obtain functional properties of the second-order kernel, singular-value decomposition (SVD) can be applied to decompose the kernel into a number of parallel subsystems (Yamada and Lewis, 1999). Each subsystem is characterized by a filter function (an eigenvector of the kernel matrix) and a gain function (the corresponding eigenvalue; see Chapter 4, Figure 4.1). Eigenvalues can be positive or negative, reflecting excitatory and inhibitory subsystems, respectively. As such, the functional properties of excitation and inhibition in the IC can be obtained from the neural response to a single stimulus, the broadband Gaussian noise. For a more detailed description of the Wiener-kernel analysis technique and of decomposing the second-order kernel by SVD, see the Introduction of Chapter 4. Wiener-kernel analyses have previously been applied to responses of the auditory nerve and the cochlear nucleus (van Dijk et al., 1994; Yamada and Lewis, 1999; Lewis et al., 2002a, 2002b; Recio-Spinoso et al., 2005; Temchin et al., 2005; Recio-Spinoso and van Dijk, 2006; Sneary and Lewis, 2007). Recent results from our lab showed that Wiener-kernel analysis is also applicable to noise-evoked spike trains recorded from the inferior colliculus (IC; Chapter 4).

With the current study, we aimed at investigating the consequences of immediate acoustic trauma on excitation and inhibition in the IC, by studying the excitatory and inhibitory subsystems as derived from SVD of the second-order Wiener kernels. We hypothesized that inhibitory components in particular are affected by acoustic trauma. This study might further confirm earlier findings and extend our knowledge about the effects of acoustic trauma on excitation and inhibition in the central auditory system using the Wiener-kernel analysis technique. As such, this study might give additional insight in the central pathophysiological mechanisms of noise-induced tinnitus and hyperacusis.
2. Methods

Wiener-kernel analyses of noise-evoked neural responses of the IC of normal-hearing guinea pigs have previously been described in Chapter 4 of this dissertation. Details concerning the involved surgeries, stimulus presentation, neural recordings, and data analyses can be found in the Methods of Chapter 4. Here, we briefly summarize these procedures.

2.1 Animals

Three albino male guinea pigs (Dunkin Hartley; Harlan Laboratories, Horst, the Netherlands), weighing between 362 and 406 gram, were included in this study. These animals were a subgroup of the nine animals described in Chapter 4. Animals were anesthetized with intramuscular injections containing a mixture of 70 mg/kg ketamine and 6 mg/kg xylazine. Half the original dose was applied every hour to maintain a deep level of anesthesia. A tracheotomy was performed for artificial respiration during the experiment. A craniotomy, followed by partial aspiration of the overlying cortical tissue, allowed for visualization of the IC. A linear 16-channel microelectrode array (A1x16-10mm-100-413-A16; NeuroNexus) was placed in the IC in a dorsal-lateral to ventral-medial direction. Online inspection of the neural signal during presentation of successive noise bursts allowed for optimal placement of the electrode in the IC. During the entire experiment, the electrode remained at the same location in the IC. Thus, it can be assumed that neural activity was recorded from a fixed population of multi units throughout the entire experiment. The experiments were conducted in an anechoic sound-attenuating booth. The study was in agreement with Dutch and European regulations and was approved by the Animal Experiment Committee of the University of Groningen (DEC # 6068D).

2.2 Auditory brainstem response

Before and after acoustic trauma, hearing thresholds were determined by recordings of the auditory brainstem response (ABR). Thresholds for 3-ms tone pips of 3 kHz, 6 kHz, 11 kHz, and 22 kHz were determined. The threshold shifts (in dB) were calculated for every frequency to get an indication about the damage induced by the acoustic trauma. Methodological details about this procedure can be found in the Methods of Chapter 2.

2.3 Stimulus presentation

A free-field electrostatic speaker (ES1; Tucker Davis Technologies, Inc. [TDT]) was placed at approximately 5 cm from the left ear, contralateral to the exposed IC. Pure tones (duration 300 ms), with a frequency ranging from 2-40 kHz and an intensity ranging from 20-80 dB SPL, were presented to acquire receptive fields. Subsequently, a continuous Gaussian-white noise of 70 dB SPL was presented for 1 hour to acquire data for Wiener-kernel analysis. The noise had a spectrum that was flat within 1 dB between 2 kHz and 40 kHz.
For acoustic trauma, two free-field piezo tweeters (PH8; Velleman) were positioned at ± 5 cm from each ear. Animals were bilaterally exposed for 1 hour to a continuous 11-kHz tone of 124 dB SPL. To create an unobstructed path for the sounds to reach the tympanic membrane, both pinnae were folded over the head of the animal. All acoustic stimuli were presented with a sampling rate of 97,656 Hz and were calibrated using a Brüel & Kjær (B&K) microphone placed at the entrance of the ear canal and a B&K measuring amplifier (type 2670 and 2610, respectively).

2.4 Neural recordings

The 16-channel neural signal was preamplified (RA16PA; Tucker Davis Technologies [TDT] Inc.), recorded (RX5; TDT Inc.), and stored on a PC using custom-made MatLab software (R2010b, Mathworks). Neural signals were filtered (300-3000 Hz, butterworth filter) and the root-mean-square (RMS) was calculated. Spikes were defined by the instants when the signal exceeded a threshold. This threshold was determined by 3 times the RMS value for the responses to pure tones and the spontaneous-firing-rate recordings, and by visual inspection for the Wiener-kernel analyses. All recordings presented in this chapter are multi-unit recordings, indicating that no spike sorting was executed and, therefore, it is not certain whether all spikes derived from the same neuron or from more than one neuron.

2.5 Data analysis

MatLab programs were designed to analyze the firing rates evoked by pure tones and create matrices from which the excitatory and inhibitory receptive fields could be obtained. Units without a distinguishable receptive field before acoustic trauma were excluded from further analyses. The excitatory and inhibitory tuning curves contoured the excitatory and inhibitory receptive fields, respectively, and revealed a unit’s excitatory characteristic frequency (CF), inhibitory CF, and the corresponding thresholds. Furthermore, neural activity was recorded for 180 s to obtain a unit’s spontaneous firing rate. Wiener kernels and the subsequent SVD of the second-order kernels were calculated using custom-made programs in C and MatLab programs. SVD analysis of the second-order kernels revealed parallel subsystems, each consisting of a filter function (eigenvector) and a gain function (eigenvalue). The eigenvalue of a subsystem was either positive or negative, referring to an excitatory or an inhibitory subsystem. Subsystems derived from decomposition of second-order kernels appear in quadrature pairs, which are often, but not always, two consecutively ranked subsystems. From the frequency and phase response curves of the eigenvectors, the best frequency (BF) and the group delay was calculated, respectively. The results obtained with Wiener-kernel analysis were compared to and complemented with the excitatory and inhibitory CF. Detailed description of these analyses can be found in the Methods of Chapter 4.
2.6 Statistics

Statistical differences of spontaneous and noise-evoked firing rates, best frequencies, characteristic frequencies, and group delays between before and after acoustic trauma were determined with a Paired-sample T-test (IBM SPSS Statistics; Version 22). A p-value below 0.05 was considered significant and the outcomes were corrected for multiple comparisons.

3. Results

Data from 33 IC multi units were used to study the immediate effects of acoustic trauma on central excitation and inhibition, as determined by Wiener-kernel analysis. Exposure for 1h to an 11-kHz tone of 124 dB SPL resulted in elevated thresholds for all measured ABR frequencies. Auditory thresholds for 3 kHz and 6 kHz were both elevated with 13 dB on average. Thresholds for 11 kHz and 22 kHz (tones at and above the trauma frequency) were elevated with an average of 40 dB and 46 dB, respectively. A representative example of a low-, a mid-, and a high-CF multi unit are discussed below, followed by statistical analyses comparing response characteristics on the level of the population before and immediately after acoustic trauma.

3.1 Effects of acoustic trauma on a low-CF multi unit

Figure 5.1 shows results of a low-CF multi unit recorded before and immediately after acoustic trauma. Responses to pure tones revealed an excitatory receptive field with a CF of 2.0 kHz and a corresponding threshold of 35 dB SPL (Figure 5.1A). Acoustic trauma did not majorly change the excitatory receptive field (Figure 5.1B; CF = 2.1 kHz, threshold = 26 dB SPL). There was no inhibitory receptive field before and after acoustic trauma.

Both the spontaneous and the noise-evoked firing rate of this multi unit slightly decreased after acoustic trauma. The spontaneous firing rate decreased from 27 spikes/sec to 25 spikes/sec and the noise-evoked firing rate decreased from 232 spikes/sec before exposure to 205 spikes/sec after exposure. Significant stimulus-related oscillations were observed in the second-order Wiener kernel before and after acoustic trauma (Figure 5.1E and 5.1D). Excitatory and inhibitory subsystems, as obtained from decomposing the second-order kernel with SVD, were detected at both time points. The BF of the first (excitatory) quadrature pair (i.e. the first two subsystems) was not affected by acoustic trauma (BF before = 2.1 kHz; BF after = 2.1 kHz) and corresponded to the excitatory CF. The third quadrature pair (fifth and sixth subsystems) had a negative eigenvalue before as well as after acoustic trauma (Figure 5.1C). The inhibitory BF of this quadrature pair was also not majorly affected by acoustic trauma (BF before = 2.5 kHz; BF after = 2.2 kHz).
Figure 5.1 Representative example of a low-CF multi unit. The excitatory receptive field is contoured by the excitatory tuning curve (black line) before (A) and after (B) acoustic trauma. C) The absolute eigenvalues of the ten highest-ranked subsystems before (red curve) and after (blue curve) acoustic trauma. Crosses depict subsystems with a positive eigenvalue, open circles depict subsystems with a negative eigenvalue. The second-order Wiener kernel before (D) and after (E) acoustic trauma. The original kernel of n = 2048 was zoomed in (0 – 15 ms); there were no stimulus-related oscillations outside this time window. The excitatory kernel, reconstructed from the subsystems with a positive eigenvalue, before (F) and after (G) acoustic trauma. The inhibitory kernel, reconstructed from the subsystems with a negative eigenvalue, before (H) and after (I) acoustic trauma. J) Eigenvectors (EV) of the first-and third-ranked quadrature pairs, as derived from SVD analysis, depicted in the red and purple lines, respectively, before acoustic trauma (BT), and depicted in blue and orange lines, respectively, after acoustic trauma (AT). The first quadrature pair (red and blue lines) consisted of excitatory subsystems (with positive eigenvalues), the third quadrature pair (purple and orange lines) consisted of inhibitory subsystems (with negative eigenvalues). The highest-ranked and second highest-ranked eigenvector of the quadrature pair is depicted with a solid and a dashed line, respectively. K) The amplitude spectrum of the eigenvectors plotted in panel J, the same legend applies. As the frequency responses of the individual eigenvectors within a quadrature pair are assumed to be relatively similar (see also Chapter 4, Figure 4.2 – 4.4), only the frequency response of the highest-ranked EV of the quadrature pair is depicted here.
3.2 Effects of acoustic trauma on a mid-CF multi unit

Figure 5.2 shows the effects of acoustic trauma on the response characteristics of a mid-CF multi unit. Before exposure, the excitatory tuning curve had a CF of 6.2 kHz and a corresponding threshold of 50 dB SPL. After exposure, the receptive field shifted to lower frequencies and two excitatory CFs could be identified, at 2.4 kHz and at 3.7 kHz, both with a threshold of 59 dB SPL. This multi unit had no inhibitory responses to pure tones before and after acoustic trauma.

Firing rates measured in the absence and presence of the noise stimulus were both decreased after acoustic trauma. The spontaneous firing rate decreased from 24 spikes/sec to 14 spikes/sec and the noise-evoked firing rate decreased from 198 spikes/sec to 160 spikes/sec after acoustic trauma. Before acoustic trauma, this multi unit had distinct stimulus-related oscillations in the second-order kernel and showed both excitatory and inhibitory subsystems in the ten highest-ranked subsystems (Figure 5.2C). The first (excitatory) quadrature pair had a BF of 5.4 kHz. In addition, the third quadrature pair had negative eigenvalues, representing inhibition with a BF at 7.5 kHz. After exposure, the subsystems with a negative eigenvalue disappeared, i.e. acoustic trauma affected the presence of inhibition in this multi unit (see Figure 5.2, panel C and I). Furthermore, the BF of the first (excitatory) quadrature pair was lower as compared to before exposure. The excitatory BF after exposure was 2.8 kHz and corresponded to the lower excitatory CF, as revealed from the responses to pure tones (Figure 5.3, panel B and K).

3.3 Effects of acoustic trauma on a high-CF multi unit

Figure 5.3 shows the effects of acoustic trauma on a high-CF multi unit. Responses to pure tones revealed an excitatory-receptive field (CF = 16.7 kHz, threshold = 33 dB SPL) that disappeared completely after acoustic trauma. No inhibitory responses to pure tones were observed before or after acoustic trauma.

Spontaneous firing rates were decreased after acoustic trauma (from 21 spikes/sec to 14 spikes/sec), but noise-evoked firing rates of this multi unit were not affected (155 spikes/sec pre trauma, 158 spikes/sec post trauma). Before acoustic trauma, the frequency tuning to high-frequency pure tones (CF = 16.7 kHz) corresponded to the stimulus-related oscillations observed in the second-order kernel (BF = 16.1 kHz). After exposure, stimulus-related components were apparent, however, the frequency response was majorly affected (see Figure 5.3, panel E, K, and G). The first (excitatory) quadrature pair had a BF of 2.8 kHz. Both before and after exposure, no inhibitory subsystems were observed.

These three multi units exemplify that inhibition, as defined by the presence of subsystems with a negative eigenvalue, was preserved in a low-frequency multi unit, but not in a mid-frequency multi unit. Furthermore, the examples showed that frequency tuning of a low-CF multi unit was not affected by acoustic trauma, whereas in the mid-CF multi unit, a
Figure 5.2 Representative example of a mid-CF multi unit. The excitatory receptive field is contoured by the excitatory tuning curve (black line) before (A) and after (B) acoustic trauma. C) The absolute eigenvalues of the ten highest-ranked subsystems before (red curve) and after (blue curve) acoustic trauma. Crosses depict subsystems with a positive eigenvalue, open circles depict subsystems with a negative eigenvalue. The second-order Wiener kernel before (D) and after (E) acoustic trauma. The original kernel of n = 2048 was zoomed in (0 – 10 ms); there were no stimulus-related oscillations outside this time window. The excitatory kernel, reconstructed from the subsystems with a positive eigenvalue, before (F) and after (G) acoustic trauma. The inhibitory kernel, reconstructed from the subsystems with a negative eigenvalue, before (H) and after (I) acoustic trauma. Since there were no inhibitory subsystems after trauma, the matrix consists of only zeros and has, therefore, only one color. J) Eigenvectors (EV) of the first- and third-ranked quadrature pairs, as derived from SVD analysis, depicted in the red and purple lines, respectively, before acoustic trauma (BT). The eigenvectors of the first quadrature pair after acoustic trauma (AT) are depicted in blue lines. The first quadrature pair (red and blue lines) consisted of two excitatory subsystems (with positive eigenvalues), the third quadrature pair (purple lines) consisted of two inhibitory subsystems (with negative eigenvalues). The highest-ranked and second highest-ranked eigenvector of the quadrature pair is depicted with a solid and a dashed line, respectively. K) The amplitude spectrum of the eigenvectors plotted in panel J, the same legend applies. Only the frequency response of the highest-ranked eigenvector (EV1 and EV5) of the quadrature pair is depicted here.
downward shift in frequency tuning could be observed, both in the responses to pure tones (CF) and in the stimulus-related oscillations of the second-order Wiener kernel (BF). In the high-CF multi unit, noise-evoked responses showed frequency selectivity (BF) after trauma, but tone-evoked responses did not (no CF). The BF that was apparent in the second-order kernel was shifted to a lower frequency as compared to before trauma.

3.4 Population characteristics

3.4.1 Spontaneous and noise-evoked activity

Figure 5.4 shows the effects of acoustic trauma on spontaneous and noise-evoked firing rates. For illustration, the low-CF units (CF < 3 kHz; blue triangles), mid-CF units (CF between 3 and 11 kHz; white squares), and high CF units (CF > 11 kHz; red circles) have been depicted with different markers. Spontaneous firing rates were significantly decreased immediately after acoustic trauma (Figure 5.4A; paired-sample T-test: $T(32) = 5.302, p < 0.001$). Noise-evoked firing rates were also significantly decreased by acoustic trauma (Figure 5.4B; paired-sample T-test: $T(32) = 3.031, p < 0.01$). The average reduction of the spontaneous firing rates (6.2 spikes/sec) alone could not account for the average reduction in the noise-evoked firing rates (29.0 spikes/sec).

3.4.2 Frequency selectivity and frequency tuning

Figure 5.5 compares the frequency tuning between before and after acoustic trauma, as determined with responses to pure tones (CF) and responses to the noise stimulus (BF). Low-CF multi units, depicted with blue triangles, retained frequency selectivity of the CF and the BF after acoustic trauma. Frequency selectivity of mid-CF multi units, depicted with white squares, was mildly affected by acoustic trauma. Excitatory receptive fields disappeared in two mid-CF multi units which had a CF > 8.4 kHz before trauma (Figure 5.5A), whereas all mid-CF multi units showed significant stimulus-related oscillations in the second-order kernels (BF) both before and after acoustic trauma (Figure 5.5B). The frequency selectivity of most high-CF multi units (red circles) was affected by acoustic trauma. A receptive field was apparent in one high-CF multi unit (Figure 5.5A) and a stimulus-related oscillation was apparent in the second-order kernel of three high-CF multi units (Figure 5.5B). The other units lacked frequency selectivity after acoustic trauma. Moreover, frequency tuning was also affected by acoustic trauma, when examining both the CF and the BF. In the multi units in which an excitatory CF could still be determined after acoustic trauma ($n = 21$), the CF was significantly reduced (Figure 5.5A; paired-sample T-test: $T(20) = 3.801, p < 0.005$). Similarly, in the multi units in which an excitatory BF could still be determined after acoustic trauma ($n = 26$), the frequency tuning of the BF was also significantly reduced (Figure 5.5B; paired-sample T-test: $T(25) = 2.836, p < 0.01$). In other words, neurons that were sensitive to high frequencies, had an altered sensitivity to lower frequencies after acoustic trauma, both
Figure 5.3 Representative example of a high-CF multi unit. Responses to pure tones before (A) and after (B) acoustic trauma. The excitatory receptive field is contoured by the excitatory tuning curve (black line). C) The absolute eigenvalues of the ten highest-ranked subsystems before (red curve) and after (blue curve) acoustic trauma. All subsystems had a positive eigenvalue. The second-order Wiener kernel before (D) and after (E) acoustic trauma. The original kernel of n = 2048 was zoomed in (2 – 6 ms); there were no stimulus-related oscillations outside this time window. The excitatory kernel, reconstructed from the subsystems with a positive eigenvalue, before (F) and after (G) acoustic trauma. The inhibitory kernel, reconstructed from the subsystems with a negative eigenvalue, before (H) and after (I) acoustic trauma (i.e. both are zero as there are no inhibitory subsystems, see panel C). J) Eigenvectors (EV) of the highest-ranked quadrature pair, as derived from SVD analysis, depicted in the red and blue lines for before (BT) and after (AT) acoustic trauma, respectively. The quadrature pairs consisted of excitatory subsystems (with positive eigenvalues). The highest-ranked and second highest-ranked eigenvector of the quadrature pair is depicted with a solid and a dashed line, respectively. K) The amplitude spectrum of the eigenvectors plotted in panel J, the same legend applies. Only the frequency response of the highest-ranked eigenvector (EV1) of the quadrature pair is depicted here.
Figure 5.4  Spontaneous and noise-evoked firing rates. A) The spontaneous firing rate (SFR) in spikes/sec (sp/s) before acoustic trauma plotted against the SFR after acoustic trauma. A distinction has been made between low-CF units (CF < 3 kHz; depicted with blue triangles), mid-CF units (CF between 3 kHz and 11 kHz; depicted with white squares), and high-CF units (CF > 11 kHz; depicted with red circles). B) The noise-evoked firing rate (evoked FR) before acoustic trauma plotted against the evoked FR after acoustic trauma. The same legend as in panel A applies.

Figure 5.5  Frequency tuning before and after acoustic trauma. A) The excitatory CF before and after acoustic trauma. Multi units in which the excitatory receptive field disappeared after trauma are depicted at the top of the plot. The solid grey line indicates the trauma frequency (11 kHz). B) The excitatory BF of the highest-ranked eigenvector, derived from SVD of the second-order kernel, before and after acoustic trauma. Multi units in which no stimulus-related component in the second-order kernel could be observed after trauma are depicted at the top of the plot. The same legend as in panel A applies.
when observing the receptive fields (CF) and when observing the subsystems of the second-order kernels (BF).

Inhibitory responses to pure tones, and thus a corresponding inhibitory receptive field, were found in fifteen of the thirty-three multi units before acoustic trauma, all of which disappeared after acoustic trauma. Wiener-kernel analyses showed that inhibitory subsystems were also found in fifteen of the thirty-three multi units before acoustic trauma. However, after acoustic trauma, inhibitory subsystems, as revealed from responses to Gaussian noise, remained present in four multi units. In the other units (n = 11), inhibitory subsystems disappeared after acoustic trauma (see Figure 5.6, markers depicted at the top of the plot). The four multi units that retained inhibitory subsystems all were low-CF units and had a CF lower than or equal to 2.6 kHz (for an example, see Figure 5.1).

Inhibition-to-excitation ratios did not majorly change by acoustic trauma in these multi units (Figure 5.6, blue triangles). Furthermore, there was one low-CF multi unit (CF = 2 kHz) that did not have inhibitory subsystems before acoustic trauma, but had one significant inhibitory subsystem after trauma (see Figure 5.6, marker depicted at the far right of the plot). In all mid-CF and high-CF multi units, inhibitory subsystems disappeared as a result from acoustic trauma.

Together, these data show that excitation and inhibition as revealed from the Wiener kernels in low-CF multi units was not affected. Mid-CF multi units still had excitatory subsystems in response to noise, but did not have inhibitory subsystems anymore. In high-CF multi units, both excitation and inhibition was affected by acoustic trauma.

![Figure 5.6](image)

**Figure 5.6** Inhibition before and after acoustic trauma. The inhibitory subsystems disappeared in all mid- and high-CF multi units that did have inhibitory subsystems before acoustic trauma (white squares and red circles at the top of the panel). In four low-CF multi units (blue triangles), inhibitory subsystems were preserved. The inhibition-to-excitation ratio, previously defined in Chapter 4, was not affected by acoustic trauma in these units.
3.4.3 Group delays

The group delay of the unit’s highest-ranked eigenvector was not significantly changed by acoustic trauma (Figure 5.7; paired-sample T-test: $T(25) = 1.248, p = 0.223$). In the four multi units that had inhibitory subsystems before and after acoustic trauma (see Figure 5.6), the first inhibitory eigenvector had a longer group delay than the first excitatory eigenvector both before and after acoustic trauma.

![Figure 5.7 Group delays. The group delay of the highest-ranked eigenvector before acoustic trauma plotted against the group delay of the highest-ranked eigenvector after acoustic trauma.](image)

4. Discussion

With this study, we showed that the effects of acoustic trauma can be identified with Wiener-kernel analysis. This analysis demonstrated that excitation and inhibition in low-CF multi units (CF < 3 kHz) was not affected, inhibition in mid-CF multi units (CF between 3 kHz and 11 kHz) disappeared whereas excitation remained, and excitation and inhibition in high-CF multi units (CF > 11 kHz) both disappeared as a result from acoustic trauma by an 11-kHz tone. This specific differentiation could not be identified with receptive-field analysis, which showed that inhibitory responses to the presented pure tones disappeared in all units. Furthermore, we showed that multi units were tuned to lower frequencies immediately after trauma as compared to before acoustic trauma. This could be observed in the receptive-field analysis (excitatory CF) as well as in the Wiener-kernel analysis (excitatory BF). And lastly, we showed that spontaneous and noise-evoked firing rates were significantly decreased immediately after acoustic trauma.
Inhibitory responses to pure tones, and thus also inhibitory receptive fields and tuning curves, disappeared in all units immediately after acoustic trauma. Furthermore, Wiener-kernel analyses showed that inhibitory subsystems disappeared in mid-CF and high-CF multi units, but not in low-CF multi units. The mid-CF multi units are of the most interest, since they retained their excitatory components but not their inhibitory components, and thus showed a clear disruption of the balance between excitation and inhibition.

The apparent discrepancy between Wiener-kernel analysis and tone-evoked receptive-field analysis can be understood by considering a basic difference between the analyses. A tone-evoked response is always the net result of excitation and inhibition. Presumably, the excitatory receptive field (areas within the black contour in panels A of Figures 5.1 – 5.3) is the result of excitatory and inhibitory input to the IC neuron being studied. Hence, the inhibitory strength in a unit’s response can only be assessed from inhibitory receptive fields that are distinct from the excitatory receptive field. In contrast, the Wiener-kernel analysis is able to disentangle excitation and inhibition within the excitatory passband of the neuron (Yamada and Lewis, 1999; Chapter 4). In other words, it is possible to estimate the relative contribution of excitation and inhibition at stimulus frequencies that overall excite the neuron. Consequently, the tone-evoked receptive field and Wiener kernels reflect different measures of excitation and inhibition (see also Chapter 4).

Our results are consistent with molecular studies, that show a reduction in inhibitory neurotransmitters and their receptors in the IC following acoustic trauma (Szczepaniak and Möller, 1995; Milbrandt et al., 2000; Dong et al., 2010a). Furthermore, our results correspond to a disrupted balance of excitation and inhibition following acoustic trauma, and additionally showed that the disrupted balance resides specifically at multi units with a CF directly below the trauma frequency (Scholl and Wehr, 2008; Noreña, 2011; Llano et al., 2012; Chapter 2). These results may be related to the neural mechanisms involved in hyperacusis. It has been suggested that hyperacusis is related to an upregulation of central gain in the brainstem (Gu et al, 2010; Knipper et al, 2013; Hickox and Liberman, 2014). Our results indicate that tone-induced acoustic trauma affects inhibition of mid-frequency neurons tuned to frequencies below the trauma frequency. The sensitivity of these neurons remained relatively unaffected. This provides a potential substrate for hyperactivity in response to auditory stimuli of moderate levels, which might be an equivalent of hyperacusis (Zeng, 2013).

The release of inhibition, as described above, could have also been involved in the changes we observed in the excitatory CF and excitatory BF, that shifted towards lower frequencies (Wang et al., 2002). Since these results were obtained immediately following acoustic trauma, it is not likely that the downward shift in frequency tuning resulted from the generation of new pathways towards the high-CF and mid-CF multi units. This indicates that rather a damage in inhibitory pathways unmasked sensitivity to the lower frequencies. Furthermore, a downward shift in frequency tuning of auditory neurons following an acoustic trauma-induced high-frequency hearing loss is consistent with previous findings in the
auditory cortex and the IC (Noreña and Eggermont, 2003, 2005; Yang et al., 2011; Niu et al., 2013).

A reduction in spontaneous firing rate of the IC immediately following acoustic trauma has previously been shown by Niu and colleagues (2013). However, other studies, including Chapter 2 of the current thesis, have reported no change in the spontaneous firing rate immediately after exposure (Noreña and Eggermont, 2003; Chapter 2). The within-unit design of the current study allowed us to directly compare firing rates of one multi unit before and after acoustic trauma, which provided additional sensitivity for this reduction as compared to the study in Chapter 2. Furthermore, noise-evoked firing rates were also significantly reduced immediately after acoustic trauma. Our results showed that the average reduction in spontaneous firing rate alone could not account for the reduction in noise-evoked firing rate. This indicated that an additional mechanism should have accounted for the reduced noise-evoked firing rates, such as a reduced sensory input due to acoustical damage at the periphery. Our findings are consistent with previous studies in the IC, that likewise showed unchanged or reduced firing rates evoked by a number of pure tones immediately after acoustic trauma (Sun et al., 2012; Chapter 2).

In summary, we showed that Wiener-kernel analysis confirmed and complemented current knowledge about the disrupted balance between excitation and inhibition following acoustic trauma in the IC. The use of Gaussian noise as a stimulus gave an advantage over pure tones, when studying excitation and inhibition. By using Wiener-kernel analysis and a decomposition of the second-order kernel with SVD, additional information about inhibitory responses that occur within the frequency band of excitation could be unraveled. This showed that the balance between excitation and inhibition in mid-CF units, that resided at the lower edge of the exposure frequency, was particularly vulnerable for acoustic trauma. These results are of potential interest as a central neural correlate of noise-induced hyperacusis.

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