Wiener-kernel analysis of neural responses in the inferior colliculus of the guinea pig

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Abstract

Wiener-kernel analysis is a technique to describe a nonlinear system in terms of a number of kernels. These kernels are obtained by cross-correlating the system’s response to a broadband noise with that broadband noise. In the auditory system, Wiener-kernel analyses have been applied to the auditory nerve and the cochlear nucleus. The current study investigated the applicability of Wiener-kernel analysis to the inferior colliculus (IC).

First- and second-order kernels were determined for hundred multi units, recorded from the IC of nine guinea pigs. The second-order kernel was decomposed, using singular-value decomposition, into a number of parallel subsystems, each of which is characterized by a filter function (an eigenvector) and a gain factor (an eigenvalue). Positive and negative eigenvalues correspond to excitation and inhibition, respectively. The results of the Wiener-kernel analyses were compared with excitatory- and inhibitory-characteristic frequencies (CFs) obtained from pure-tone responses.

We detected significant stimulus-related oscillations in the first-order kernel of twelve IC units (2.1 – 3.3 kHz). A significant second-order kernel was found in all units, where the best frequency (BF) of the highest-ranking excitatory eigenvector corresponded well with the excitatory CF. The BF of the inhibitory eigenvector did not correspond to the inhibitory CF, but often corresponded to the BF of the excitatory eigenvectors.

We showed that Wiener-kernel analysis is a powerful tool to reveal excitatory- and inhibitory-response characteristics of the IC. This can be used to study neural processes that critically depend on a balance between excitation and inhibition, such as directional hearing, hyperacusis, and tinnitus.

Keywords: wiener kernel; inferior colliculus; guinea pig; white noise; singular-value decomposition

Abbreviations: AN, auditory nerve; BF, best frequency; CF, characteristic frequency; CN, cochlear nucleus; FFT, fast-Fourier transform; IC, inferior colliculus; REVCOR, reverse correlation; RMS, root-mean-square; SEM, standard-error of the mean; SVD, singular-value decomposition
1. Introduction

Neural spike trains evoked by external acoustic stimuli can be studied in many ways. In essence, there are two general approaches (Rieke et al., 1999). The first and most common approach is to present the system with a fixed stimulus and record the average firing rate. This approach, which is used to obtain, for example, tuning curves and post-stimulus time histograms, is called the experimenter-centered approach. The experimenter defines the stimuli, and observes the responses that follow in a neuron. The second approach often uses a Gaussian-noise stimulus and studies the events preceding a spike to gain knowledge about the characteristics in the stimulus that caused the occurrence of that spike. This approach is called the subject-centered approach (Eggermont et al., 1983).

Wiener-kernel analysis is a subject-centered technique and is often used to study functional properties of the auditory system (Korenberg and Hunter, 1990; Eggermont, 1993). The analysis typically provides the researcher with three kernels: the zeroth-, the first-, and the second-order Wiener kernel that together describe the characteristics of the system up to the second-order nonlinearities. The zeroth-order kernel is simply the average firing rate evoked by the Gaussian-noise stimulus. The first-order kernel represents the linear correlation between the input signal (the noise stimulus) and the output signal (the spike train), and is identical to the commonly-used reverse-correlation (REVCOR) function. A significant stimulus-related oscillation in the first-order kernel (or REVCOR function) reflects the neuron's ability to phase lock to the fine structure of an acoustic stimulus (de Boer and de Jongh, 1978). The second-order kernel could be referred to as a second-order REVCOR function that captures second-order nonlinearities of the neuron being studied. It is significant for phase locking and for non-phase locking fibers (Recio-Spinoso and van Dijk, 2006), and consequently allows for the application of a subject-centered approach across stimulus frequencies (van Dijk et al., 1994; Temchin et al., 2005).

When digitally sampled, the second-order Wiener kernel is a symmetric matrix. Although the computation of the kernel is relatively straightforward, it may be hard to obtain an intuitive interpretation of the functional properties of the system being studied. An exception to this statement are kernels obtained from the primary auditory nerve. These kernels are often well described by a so-called sandwich model, consisting of a band-pass filter, a nonlinear element, and a low-pass filter (van Dijk et al., 1994). The filters represent tuning in the inner ear and low-pass filtering in the hair cell and hair-cell synapse. The nonlinearity presumably corresponds to the transduction process in the hair cell stereocilia. As was shown by van Dijk et al. (1994), the impulse responses of the filters can be computed from the second-order kernel, thus providing a straightforward interpretation of the Wiener-kernel analysis.

However, for locations higher in the auditory system, such a simple model cannot be applied, because the signal has been further (nonlinearly) processed in a mostly unknown way. Then, to disentangle the functional meaning from the second-order kernel, its eigenvectors and eigenvalues can be computed by, i.e., singular-value decomposition (SVD; Lewis et al.,
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2002a; Yamada and Lewis, 1999). SVD decomposes the kernel into parallel subsystems, each of which is characterized by a filter function (the eigenvector, \( u \)) and a gain function (the eigenvalue, \( w \); Figure 4.1). Basically, all the subsystems together are a different representation of the same kernel. The eigenvector is the impulse response of the corresponding filter and the eigenvalue is a gain that represents the degree by which the subsystem contributes to the total second-order kernel. A subsystem contributes either positively (for positive gain/eigenvalues) or negatively (for negative gain/eigenvalues) to the neuron's response, and thus corresponds to either excitation or inhibition, respectively. Accordingly, SVD of second-order Wiener kernels provides a method to disentangle the inhibitory and excitatory neural responses of an auditory unit evoked by Gaussian noise.

Figure 4.1 The second-order Wiener kernel. A) The second-order Wiener kernel \( h_2 \) describes the second-order nonlinear response applied to the noise stimulus \( x(t) \) in order to obtain the spike signal from the IC \( y(t) \). B) The second-order kernel is a symmetrical square matrix, that can be decomposed into eigenvectors, each of which has an eigenvalue. Correspondingly, the kernel can be regarded as a number of parallel subsystems, which all consists of an impulse response \( u \); the eigenvector) and a weight factor \( w \); the eigenvalue). Each subsystem also squares the signal \( x^2 \). The sign of the eigenvalues reflects excitation and inhibition for positive and negative values, respectively. Subsystems often occur in quadrature pairs, which are often ranked consecutively (dashed, red square). See also the study of Yamada and Lewis (1999).

Wiener-kernel analysis has been applied to study the response properties of the auditory nerve (AN) of a variety of species (e.g. 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; Henry and Heinz, 2013) and to study responses of the ventricle cochlear nucleus (CN) of the chinchilla and the cat (Wickesberg et al., 1984; Recio-Spinoso and van Dijk, 2006). Here, we applied Wiener-kernel analysis to study responses from the inferior colliculus (IC).
The IC receives and processes afferent input from the CN and the superior olivary complex, but also receives efferent auditory input and input from non-auditory centers (Ehret, 1997). This might be reflected in relative complex response properties of Wiener kernels of the IC, which might have been the reason why Wiener-kernel analysis has not yet been applied to recordings from the IC. The responses of the IC to external acoustic stimuli have been studied extensively with experimenter-centered and other subject-centered approaches. These studies revealed, amongst other things, that the IC has a distinct dorsal-to-ventral tonotopic organization and shows both excitatory and inhibitory responses to pure tones (Clopton and Winfield, 1973; Ehret, 1997; Heeringa and van Dijk, 2014). The Wiener kernels of the IC presumably reflect these diverse response patterns.

Our interest in applying Wiener-kernel analysis was coined by a more general interest in studying the physiological mechanisms involved in tinnitus and hyperacusis. Both these conditions are believed to be related to a disrupted balance of excitation and inhibition in the central auditory system (Eggermont and Roberts, 2014). With the possibility to decompose a kernel in excitatory and inhibitory subsystems (Yamada and Lewis, 1999; Figure 4.1), Wiener-kernel analysis potentially offers a useful tool to study disruptions of the balance between excitation and inhibition. The aim of the current study was to determine the applicability of Wiener-kernel analysis to IC multi units in the normal-hearing guinea pig. Zeroth-, first-, and second-order Wiener kernels were identified and classified, and the second-order kernel was decomposed with SVD in order to obtain an intuitive feeling for the contribution of the kernel to the response of neurons in the IC. Results were compared to an experimenter-centered approach, i.e. excitatory- and inhibitory-tuning curves from responses to pure tones.

2. Methods

2.1 Animal preparation

Nine normal-hearing adult albino guinea pigs (male; Dunkin Hartley; Harlan Laboratories, Horst, the Netherlands) were anesthetized with ketamine/xylazine (70 mg/kg 10% Ketamine, Alfasan, Woerden-Holland; 6 mg/kg 2% Rompun, Bayer-Healthcare, respectively; i.m.). Half the original dose was administered every hour to maintain a deep level of anesthesia throughout the experiment. Body temperature was kept constant at 38 °C using a heating pad, and heart rate and SpO₂ (blood oxygen saturation) were monitored with a pulsoximeter. A tracheotomy allowed for artificial respiration and a skull screw was placed to fixate the head of the animal. The right IC was approached by a craniotomy and partial aspiration of the overlying cortical tissue. A linear 16-channel microelectrode array (A1x16-10mm-100-413-A16; NeuroNexus) was inserted in the IC in the lateral-dorsal to medial-ventral direction. An uncoated silver wire, positioned through the craniotomy in rostral direction below the dura, served as ground. All experiments were approved by the Animal Experiment Committee of the University of Groningen (DEC # 6068B and 6068D) and were in compliance with Dutch and European law and regulations.
2.2 Stimulus presentation

All in vivo neurophysiological recordings were acquired in an anechoic sound-attenuating booth. The 16-channel neural signal was preamplified (RA16PA; Tucker Davis Technologies [TDT] Inc.) and recorded (RX5; TDT Inc.) in parallel at 16-bits AD resolution, with a 24,414 Hz sampling rate for each channel. Custom-made MatLab software (R2010b, Mathworks) was used to store the data on a PC. Acoustic stimuli were presented via a free-field electrostatic speaker (ES1; TDT) placed at ± 5 cm from the left ear (contralateral to the exposed IC), and were calibrated using a B&K microphone (type 2670) placed at the entrance of the ear canal and an amplifier (B&K; type 2610). The electrode was advanced into the IC, while successive noise bursts (duration 100 ms, 10 ms cosine ramp) were being presented via the speaker. Online inspection of the neural response signal during these presentations allowed for optimal placement of the electrode in the IC, in order to record from a maximum number of auditory units. Once sound-evoked responses were observed on at least six of the sixteen channels, the electrode position was fixed for the subsequent recordings.

For the acquisition of receptive fields, acoustic stimuli were generated using custom-made MatLab software and TDT hardware (RP2.1, PA5, and ED1). The sampling rate for stimulus generation was 97,656 Hz. Pure tones (10 ms cosine ramp, duration 300 ms) with a range of frequencies (2 – 40 kHz) and stimulus levels (0 – 80 dB SPL) were presented in random order.

To obtain recordings for Wiener-kernel analysis, Gaussian noise was digitally filtered to produce noise with a bandwidth from 2 to 40 kHz, and a flat spectrum at the entrance of the ear canal. The noise was recorded with a B&K microphone and varied within 1 dB over the 2-40 kHz bandwidth. Custom-made programs in C, Amadeus Lite (Version 2.0.7), an audio interface (Audiofire 4; Echo), and TDT hardware (PA5 and ED1) were used to design and present the noise. The stimulus was presented for 1 h with a constant stimulus level of 70 dB SPL, at 97,656 samples per second ($f_s$). The stimulus, recorded by the B&K microphone, and the 16 neural channels, recorded by the TDT equipment, were stored on a PC hard disk for offline analysis.

2.3 Data analysis

2.3.1 Tuning curves

Neural signals were filtered (300-3000 Hz, butterworth filter) and their root-mean-square (RMS) was calculated. Spikes were defined by the instants when the signal exceeded the threshold of 3 x RMS. All recordings presented in this paper 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. MatLab programs were custom-made to calculate firing rates for every presented tone. This firing rate was inserted in a matrix that organized frequency versus stimulus level in an orderly manner. Units that had a distinguishable receptive field, as determined upon visual inspection of the matrix of
pure tone responses, were included in further tuning curve and Wiener-kernel analyses. To objectively determine the tuning curves, a baseline firing rate was considered, which was calculated by averaging the firing rates evoked by pure tones with the lowest stimulus level that visually did not seem to contribute to the receptive fields. An excitatory response was defined by a firing rate that was at least twice as high as the baseline firing rate. The excitatory-tuning curve was determined by connecting, per frequency, the lowest stimulus level that evoked an excitatory response. The excitatory characteristic frequency (CF) was considered the frequency at which the lowest stimulus level resulted in an excitatory response. Similarly, the inhibitory-tuning curve was constructed by determining per stimulus frequency the lowest level that elicited an inhibitory response, i.e. a firing rate that was at least twice as low as the baseline. The inhibitory CF was considered the stimulus frequency at which the lowest level resulted in an inhibitory response. See Figure 4.2A for an example of an excitatory- and an inhibitory-receptive field and tuning curve (black and white curve, respectively).

2.3.2 Wiener-kernel analysis

To calculate Wiener kernels, the spike-detection threshold was determined by visual inspection of the filtered (300-3000 Hz) neural signals. Custom-made programs in C were used to calculate the zeroth-, first-, and second-order kernels. For an extensive description of the calculation of Wiener Kernels from auditory responses to Gaussian noise, see Eggermont (1993). Briefly, the zeroth-order Wiener kernel ($h_0$) represents the average output of the system, i.e. the average firing rate during acoustic stimulation ($N_0$), and is calculated by

$$h_0 = \frac{N}{T} = N_0,$$  \hspace{1cm} \{1\}

in which $N$ is the amount of spikes during the whole recording and $T$ is the total duration of the recording in seconds. The first-order Wiener kernel ($h_1$) equals the cross-correlation between the input of the system, i.e. the acoustic signal, and the output of the system. For neural signals, this cross correlation is proportional to the average stimulus preceding the spikes. With the appropriate normalization constants, this average is given by (van Dijk et al., 1994):

$$h_1(\tau) = \frac{1}{AT} \sum_{i=1}^{N} x(t_i - \tau),$$ \hspace{1cm} \{2\}

where $x(t)$ is the acoustic stimulus signal, $A$ is the power-spectral density of the acoustic stimulus, and $t_i$ (with $i=1,\ldots,N$) are the times at which a spike occurred. Each first-order kernel was visually inspected to check for significant stimulus-related oscillations. A fast-
Fourier transform (FFT) was performed, and the peak of this spectrum defined the best frequency (BF) of the first-order Wiener kernel. Furthermore, a group delay was determined by calculating the slope of the phase response at the BF.

To calculate the second-order Wiener kernel \( (h_2) \) the stimulus segments (length \( \tau \)) preceding each spike were again taken into account. This kernel represents the second-order cross correlation, and is calculated by

\[
h_2(\tau_1, \tau_2) = \frac{N_0}{2A^2} \left[ R_2(\tau_1, \tau_2) - \Phi_{xx}(\tau_2 - \tau_1) \right],
\]

in which \( \Phi_{xx} \) is the autocorrelation function of the noise stimulus,

\[
\Phi_{xx}(\tau) = \frac{1}{T} \int_0^T x(t)x(t - \tau) \, dt
\]

and \( R_2 \) is the second-order cross correlation function, described by

\[
R_2(\tau_1, \tau_2) = \frac{1}{N} \sum_{i=1}^{N} x(t_i - \tau_1)x(t_i - \tau_2).
\]

For digitally-sampled data, the first- and second-order Wiener kernel take the form of a vector and a symmetric square matrix, respectively. In this study, the analysis window was either \( \tau = 10.5 \) or \( 21.0 \) ms. This corresponded to a vector size equal to \( n = \tau \times f_s = 1024 \) or \( 2048 \) samples for the first-order kernel. Correspondingly, the size of the matrix that contains the second-order kernel was \( 1024 \times 1024 \) or \( 2048 \times 2048 \).

### 2.3.3 Singular-value decomposition

SVD of the second-order kernel was performed using the built-in MatLab function \( s = \text{svd}(x) \) (R2010b, Mathworks). Second-order Wiener kernels with a size \( n \times n \) were subjected to SVD, from which \( n \) subsystems, each with an eigenvector \( (u) \) of length \( n \) and a corresponding eigenvalue \( (w) \), were derived (Figure 4.1, see also Yamada and Lewis, 1999). The ranking of the eigenvectors was performed according to the absolute eigenvalues, where the first-ranked eigenvector had the highest absolute eigenvalue. Subsystems derived from decomposition of second-order kernels appear in quadrature pairs, which were often, but not always, two consecutively ranked vectors. Following Yamada and Lewis (1999), a dominance ratio was calculated by dividing the average eigenvalue of the highest-ranked quadrature pair with the average eigenvalue of the second highest-ranked quadrature pair. Note that a high dominance ratio reflects a relatively high contribution of the first two eigenvectors (or subsystems, Figure
4.1B) to the unit’s response. An FFT of the eigenvectors revealed the frequency response and the phase response, from which a BF and a group delay could be determined, respectively.

To characterize the relative contribution of excitation and inhibition to the response of the IC, we focused on the subsystems with a rank 1-10, i.e. the first five quadrature pairs. We assessed the properties of the excitatory and inhibitory eigenvectors within the first five pairs. Furthermore, we reconstructed excitatory and inhibitory subkernels, by taking the outer product of each eigenvector with itself and then computing the eigenvalue-weighted sum of the resulted matrices (Yamada and Lewis, 1999). To reconstruct the excitatory kernel, only subsystems with a positive eigenvalue were considered; for the inhibitory kernel, only subsystems with a negative eigenvalue were considered.

3. Results

From nine normal-hearing guinea pigs, 100 recordings of IC multi units were included for further analyses. These units covered the tonotopic frequency range of the IC of the guinea pig, with excitatory CFs ranging from 2 to 35 kHz. Below, results from three multi units, that show representative features, will be described in detail. The population characteristics are presented subsequently.

3.1 Results for three exemplar multi units

Figure 4.2 displays results for an IC multi unit that responds to relatively low sound frequencies. The measurements with pure-tone stimuli revealed an excitatory CF at 2.5 kHz with a threshold at 24 dB SPL. In addition, there was an inhibitory receptive field, where the pure-tone stimuli elicited a firing rate significantly lower than the unit’s baseline firing rate. The inhibitory-tuning curve had a CF at 11 kHz and a corresponding threshold at 69 dB SPL, which was well above the excitatory CF in frequency and threshold level.

The average noise-evoked firing rate, i.e. the zeroth-order Wiener kernel, was determined at 237 spikes/sec. The first-order Wiener kernel of this multi unit showed a stimulus-related oscillation with a BF at 2.1 kHz. This frequency corresponded with the excitatory CF. The first two quadrature pairs, derived from SVD of the second-order kernel, had BFs of 2.5 kHz and 2.2 kHz, respectively. These frequencies were consistent with the excitatory CF and with the BF of the first-order Wiener kernel. The fifth and sixth eigenvector, i.e. the third quadrature pair, had a negative eigenvalue and a BF at 2.6 kHz. This did not correspond to the inhibitory CF of 11 kHz, derived from responses to pure tones. However, this frequency did correspond to the excitatory CF and to the BF of the first excitatory quadrature pair. The inhibitory, third, quadrature pair had a longer response delay than the excitatory quadrature pairs: the unit’s excitatory response to the presence of 2.5 kHz in the noise occurred after around 5.1 ms, whereas the inhibitory response occurred after 9.8 ms. In the reconstructed excitatory and inhibitory kernels, this difference in response delay is
Figure 4.2 Representative example of a low-CF IC unit. A) The excitatory-tuning curve (black line) and the inhibitory-tuning curve (white line). The black arrow indicates the baseline firing rate (see Methods). B) The first-order Wiener kernel (h₁), which showed a stimulus-related oscillation. C) The amplitude spectrum of the first-order kernel. D) The second-order Wiener kernel (h₂), in which a matrix of τ₁, τ₂, and stimulus-related oscillations in color code is depicted. The original kernel of n = 2048 was zoomed in (0 – 15 ms); there were no stimulus-related oscillations outside this time window. To reduce noise, the kernel was reconstructed from the first 10 subsystems. E) The absolute eigenvalues of the ten highest-ranked subsystems. Crosses depict subsystems with a positive eigenvalue, open circles depict subsystems with a negative eigenvalue. F) Eigenvectors of the first-, second-, and third-ranked quadrature pairs, as derived from SVD analysis, depicted in the red, grey and blue lines, respectively. The first and second quadrature pair (red and grey lines) were excitatory subsystems (with positive eigenvalues), the third quadrature pair was an inhibitory subsystem (with a negative eigenvalue). The highest-ranked and second highest-ranked eigenvector of the quadrature pair is depicted with a solid and a dashed line, respectively. G) The amplitude spectrum of the eigenvectors plotted in panel F, the same legend applies. H) The excitatory second-order Wiener kernel, reconstructed from the subsystems with a positive eigenvalue (rank 1-4, 7-10). I) The inhibitory second-order kernel, reconstructed from the subsystems with a negative eigenvalue (rank 5 and 6).
Figure 4.3 Representative example of a low-CF IC unit. A) The excitatory receptive field, contoured by the excitatory-tuning curve (black line). The black arrow indicates the baseline firing rate. B) The first-order Wiener kernel. C) The second-order Wiener kernel. The original kernel of $n = 2048$ was zoomed in ($0 - 12$ ms); there were no stimulus-related oscillations outside this time window. The kernel was reconstructed from the first 20 subsystems. D) The absolute eigenvalues of the ten highest-ranked subsystems. Crosses depict subsystems with a positive eigenvalue, open circles depict subsystems with a negative eigenvalue. E) The amplitude spectrum of the eigenvectors plotted in panel F, the same legend applies. F) Eigenvectors of the first-, fourth-, and fifth-ranked quadrature pairs, depicted in the red, blue and grey lines, respectively. The first and fifth quadrature pair (red and grey lines) were excitatory subsystems (with positive eigenvalues), the fourth quadrature pair (eigenvectors 7 and 10) was an inhibitory subsystem (with a negative eigenvalue; see panel d). The highest-ranked and second highest-ranked eigenvector of the quadrature pair is depicted with a solid and a dashed line, respectively. G) The excitatory second-order Wiener kernel, reconstructed from the excitatory subsystems (rank 1-6, 8 and 9). H) The inhibitory second-order Wiener kernel, reconstructed from the inhibitory quadrature pair (rank 7 and 10).
also visible (Figure 4.2H and Figure 4.2I, respectively). The remaining quadrature pairs that were not plotted had similar characteristics as the depicted excitatory quadrature pairs.

Results of another low-frequency unit are shown in Figure 4.3. Responses to pure tones revealed an excitatory CF at 3.0 kHz with a threshold of 41 dB SPL. This multi unit had no inhibitory receptive field, indicating that none of the presented pure tones resulted in a significant reduction of the firing rate.

The zeroth-order Wiener kernel had a value of 168 spikes/sec. Even though the multi unit responded to relatively low-frequency pure tones, it did not have a significant stimulus-related component in the first-order Wiener kernel (Figure 4.3B). Nevertheless, there was a clear response visible in the second-order Wiener kernel. Decomposing this kernel with SVD revealed both positive and negative eigenvalues in the first ten highest-ranked subsystems. The eigenvectors of the first quadrature pair (rank 1 and 2) had a BF of 2.3 kHz, corresponding with the excitatory CF. Eigenvectors rank 3, 4, 5, and 6 had a similar frequency response and group delay as the first quadrature pair, and were therefore not shown. Subsystems at rank 8 and 9, both with positive eigenvalues, formed a quadrature pair responding to the same BF as the first quadrature pair. The inhibitory quadrature pair, consisting of subsystems that were not ranked consecutively (rank 7 and 10), had two different frequency responses. One frequency response (BF = 2.3 kHz) was similar to the excitatory BF and one frequency response had a BF of 4.9 kHz.

The group delay of the first quadrature pair was 4.5 ms, whereas the group delay of the fifth quadrature pair, consisting of the excitatory subsystems at rank 8 and 9, was approximately 3 ms longer, at 7.4 ms. The inhibitory frequency response of the fourth quadrature pair (rank 7 and 10) that was similar to the excitatory BF occurred even later, at 8.8 ms. The other inhibitory frequency response (BF = 4.9 kHz) of the same quadrature pair had a group delay of 5.8 ms (Figure 4.3F).

Figure 4.4 shows the results of a high-CF unit. Pure tones evoked excitatory responses with a high CF at 10.1 kHz and a corresponding threshold at 10 dB SPL. This multi unit also had a large inhibitory-receptive field at the high-frequency border of the excitatory field. The inhibitory-tuning curve had a CF of 14.7 kHz, with a threshold at 50 dB SPL.

The zeroth-order Wiener kernel was determined at 607 spikes/sec. There was no stimulus-related response visible in the first-order Wiener kernel. The second-order Wiener kernel showed a clear response to the noise stimulus. The first quadrature pair, which contributed positively to the second-order kernel, had a BF response at 9.1 kHz, with a group delay of 3.3 ms. The second quadrature pair was also excitatory and had a BF response at 11.0 kHz with a group delay of 3.1 ms. This multi unit had an inhibitory quadrature pair that depicted two stimulus-related response bands, one with a BF of 6.6 kHz and on with a BF of 11.3 kHz. The response to 6.6 kHz had a group delay of 2.9 ms, indicating that this inhibitory response
occurred slightly prior to the excitatory response and prior to the other inhibitory response of 11.3 kHz (delay 4.1 ms). This can be observed in the individual eigenvectors (Figure 4.4D) as well as in the reconstructed excitatory and inhibitory subkernels (Figure 4.4F and 4.4G, respectively).

Furthermore, the shape and width of the filter functions of the eigenvectors were not similar to the shape and width of the tuning curves. When the filter functions were inspected more closely, lobes around the peak could be observed (Figure 4.4E). For example,
the spectrum of the first quadrature pair had the highest peak at 9.1 kHz, but also smaller peaks at 8.0 kHz, 9.5 kHz, and 10.8 kHz, and the high-frequency peak of the inhibitory quadrature pair had two distinctive lobes at both sides of the peak, at 10.5 kHz and 11.6 kHz. A similar description accounts for the second excitatory quadrature pair. Those lobes could not be observed in the excitatory or inhibitory-tuning curves, representing responses to pure tones. The distance between the peaks of the lobes did not correspond to the best-modulation frequency (data not shown).

These three multi units represent the range of behaviors observed in the responses from the inferior colliculus. The first low-frequency unit (Figure 4.2) displayed a significant first-order kernel, reflecting the fact that phase information is coded by low-frequency neurons. The excitatory and inhibitory subsystems (eigenvectors of the second-order kernel) have overlapping pass bands (Figure 4.2G). The second unit (Figure 4.3) is also a low-frequency unit. Its receptive field did not show inhibition. Yet in the second-order kernel, inhibitory characteristics were clearly observed, with an inhibitory pass band well above the excitatory pass band (Figure 4.3E). The third and high-frequency unit (Figure 4.4) showed an elaborate pattern of excitation and inhibition. Inhibition was observed in the receptive field and in the second-order kernel, however, the inhibitory frequencies did not correspond. From the kernel it follows that inhibition had a shorter time delay than excitation in this unit. The subsystems obtained from the second-order kernel have partly non-overlapping frequency responses (Figure 4.4E).

3.2 Population characteristics

The mean firing rate evoked by the noise stimulus (the zeroth-order Wiener kernel) was 188 spikes/sec (± 16 spikes/sec standard error of the mean [SEM]). This was an average increase of 167 spikes/sec as compared to the mean spontaneous firing rate.

The best frequencies derived from the Wiener kernels were compared to the excitatory CF (Figure 4.5). A significant first-order Wiener kernel was observed in 12 of the 100 recorded multi units (see Figure 4.2B for an example). Eleven of these twelve multi units showed a good correspondence between the excitatory CF obtained from the receptive field, and the BF of the first-order kernel (Figure 4.5A). In one multi unit, the excitatory-tuning curve displayed a CF of 6.2 kHz, whereas the BF of the \( h_1 \) was 2.5 kHz. The BF of the significant first-order kernels ranged between 2.1 kHz and 3.3 kHz. Note that the presented noise had a low-cut off frequency at 2.0 kHz, which may have truncated low-frequency response components in these units. The best-frequency of the highest-ranked eigenvectors of the second-order Wiener kernel also showed a significant correspondence to the excitatory CF (Figure 4.5B).
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Figure 4.5 Correspondence between CFs obtained from tone responses and BFs obtained from Wiener-kernel analysis. A) The BF of the first-order Wiener kernel plotted against the excitatory CF. B) The BF of the excitatory first quadrature pair of the second-order kernel plotted against the excitatory CF.

Figure 4.6 depicts excitatory and inhibitory response characteristics revealed from the response to pure tones and the second-order kernels. Excitatory CFs were established in 98 of the 100 recorded multi units. The other two multi units did have a receptive field upon visual inspection, however, the criterion for an excitatory of inhibitory tone-evoked response was not met for any of the presented tones. Both multi units were recorded dorsal to the lowest excitatory CF measured, and, accordingly, these multi units were likely to have excitatory CFs < 2 kHz. Inhibitory receptive fields, and corresponding inhibitory CFs, were found in 36 of the 100 recorded multi units. In most of the multi units (33 of the 36), the inhibitory CF was higher than the excitatory CF (Figure 4.6A). Naturally, the excitatory CF never corresponded to the inhibitory CF, because one tone cannot both enhance and reduce the firing rate at the same time. The BFs of the inhibitory eigenvectors did not correspond to inhibitory CF, but often corresponded with the BFs of the excitatory eigenvectors (Figure 4.6B). The number of inhibitory subsystems within the ten highest-ranked subsystems was considered a measure for the amount of inhibition in the IC multi unit evoked by the noise stimulus. More than half of the multi units had no inhibitory subsystems (57%), however, there were also multi units with one inhibitory quadrature pair (i.e. two inhibitory subsystems; 26%), two inhibitory quadrature pairs (9%), or one or three inhibitory subsystems (both in 4% of the units, respectively; Figure 4.6C). Another measure for the amount of inhibition extracted from the decomposition of the second-order kernel was considered the ratio between the absolute sum of the negative eigenvalues and the sum of the positive eigenvalues. Note that the higher the absolute eigenvalue, the higher its contribution to the second-order kernel. The inhibition-to-excitation ratio ranged between 0.08 and 0.57, indicating that the contribution of excitation
to the second-order Wiener kernel was always higher than the contribution of inhibition. The height of the ratio was divided equally over the tonotopic axis of the IC (Figure 4.6D).

Group delays, as calculated from the slope of the phase response at the best frequency, are depicted in Figure 4.7. The highest-ranked eigenvectors had an average group delay of 4.74 ms (± 0.14 ms SEM). The shortest delays were found for subsystems with a high BF (Figure 4.7A). In most multi units that had both excitatory and inhibitory subsystems, the excitatory response occurred earlier, i.e. had a shorter group delay, than the inhibitory response (36 of

Figure 4.6 Excitatory and inhibitory tuning. A) The excitatory CF plotted against the inhibitory CF. Markers depicted at the top of the panel did not have an inhibitory receptive field (and CF). B) The BF of the highest-ranked excitatory eigenvector against the BF of the highest-ranked inhibitory eigenvector. Markers depicted at the top of the panel did not have inhibitory eigenvectors within the first ten subsystems. C) Distribution of the number of inhibitory subsystems within the ten highest-ranked subsystems. D) The absolute sum of the negative eigenvalues divided by the sum of positive eigenvalues (the inhibition-to-excitation ratio) is plotted against the excitatory CF.
The group delay of the highest-ranked eigenvector plotted against its best frequency. For comparison, the fit-function for group delays of AN fibers measured in the chinchilla, $r_{AN} = 1.721 + 1.863BF^{-0.771}$ is plotted (Recio-Spinoso et al., 2005; black solid line). The group delay of the highest-ranked excitatory eigenvector plotted against the group delay of the units highest-ranked inhibitory eigenvector. Markers depicted below the diagonal indicate multi units in which the highest-ranked inhibitory eigenvector has a shorter group delay than the highest-ranked excitatory eigenvector.

**Figure 4.8** Dominance ratio. Distribution of dominance ratios for the 100 recorded IC multi units in this study (red solid line). For comparison, results for 28 units in the primary auditory nerve from the frog’s basilar papilla are also shown (Yamada and Lewis, 1999; black dashed line). The numbers have been converted to show percentages of the total recorded units. A large dominance ratio indicates that the units response is dominated by the first quadrature pair of eigenvectors, as is typically observed in the primary units studies by Yamada and Lewis (1999).

Dominance ratios reflect the contribution of the first quadrature pair to the second-order Wiener kernel relative to the second quadrature pair (previously described by Yamada and
Lewis, 1999). Dominance ratios of IC multi units had an average value of 1.33 and a maximum of 2.72. The distribution of dominance ratios was skewed towards the lower values for IC units (Figure 4.8), indicating the relative important contribution of lower-ranked subsystems to the neuronal responses.

4. Discussion

The results of this study showed that Wiener-kernel analysis was applicable to characterize response patterns of multi units of the IC in normal-hearing guinea pigs. First-order Wiener kernels were found in 12% of the recorded multi units and had low-frequency oscillations between 2.1 kHz and 3.3 kHz. In all but one unit, these oscillations corresponded well with the excitatory CF, as determined from tone responses. A significant second-order kernel could be found in all neurons being studied. These kernels were decomposed in parallel subsystems, where the filter in each of the subsystems is given by an eigenvector of the kernel matrix. The best frequency of the first (highest-ranking) eigenvector corresponded well with the excitatory CF. The eigenvalue of the first eigenvector was always positive, indicating an excitatory contribution to the neuron’s response. A portion of the eigenvalues was negative. The best frequencies of these inhibitory eigenvectors did not agree well with the inhibitory CF. Rather, the inhibitory BF was often similar to the excitatory BF. Additionally, we showed that group delays were often, but not always, shorter for the excitatory responses as compared to the inhibitory responses. Dominance ratios of IC multi units were several magnitudes smaller than those measured in the AN, reflecting the higher degree of complexity in the response properties of IC neurons.

The correspondence of the BF of the highest-ranked eigenvector and the tonal excitatory CF is consistent with earlier Wiener-kernel studies in the AN and the CN (Temchin et al., 2005; Recio-Spinoso and van Dijk, 2006). Furthermore, a significant stimulus-related oscillation was found in the first-order kernels of twelve low-frequency neurons. The BF of the first-order kernel also corresponded with the excitatory CF. Oscillations in the first-order Wiener kernel reflect the neuron’s ability to phase lock to the fine structure in the acoustic stimulus (de Boer and de Jongh, 1978; Eggermont et al., 1983; Lewis et al., 2002b). Phase locking has been determined throughout the auditory system and only occurs in response to relatively low frequencies (Joris and Verschooten, 2013). In the current study, the highest first-order kernel oscillation was 3.3 kHz, which corresponds reasonably well with the upper limit of phase locking in the IC of the cat (Yin and Kuwada, 1983). Our findings indicate that the first-order Wiener kernel of an IC multi-unit reflects frequency selectivity.

Upon visual inspection, second-order Wiener kernels of the IC seemed similar to second-order kernels of the AN and the CN, with stimulus-related oscillations near the diagonal (Recio-Spinoso and van Dijk, 2006; Sneary and Lewis, 2007). SVD of the second-order kernels revealed a larger number of significant subsystems. This is also reflected by the low dominance ratios of IC units as compared to AN units (see Figure 4.8). The dominance ratio has been interpreted as a measure of the complexity of the response, in which low
dominance ratios correspond to high degrees of complexity (Yamada and Lewis, 1999). The complexity of the response in the IC probably reflects the fact that the IC is a ‘higher-order’ structure which integrates information from ‘lower’ nuclei.

Another measure that reflects this complexity is the fact that recordings of 60 minutes were required to obtain significant kernels from the IC. In the AN, recordings of a few minutes are sufficient (Recio-Spinoso et al., 2005; Temchin et al., 2005). This suggests that the spikes are not only conditioned by the stimulus, but might also be conditional to each other or be driven by other sources. At present we do not have a good explanation for the relative time-consuming nature of this analysis.

The advantage of the Wiener-kernel analysis over other methods that study stimulus-evoked spike trains, such as the spectrotemporal receptive field (STRF; Hermes et al., 1981) and the ‘zwuis’ stimulus (van der Heijden and Joris, 2003), is that Wiener-kernel analysis allows to separate excitatory and inhibitory responses of the inferior colliculus in a precise manner (Yamada and Lewis, 1999). By decomposing the second-order Wiener kernel with SVD, the excitatory and inhibitory frequencies, delays, relative weights, and pass bands can be determined. Since recent hypotheses suggest that acoustic trauma-induced pathologies, such as tinnitus and hyperacusis, are related to a disturbed balance between excitation and inhibition, gaining knowledge about the precise nature of this disturbed balance might provide additional insight in the noise-induced neurophysiological changes. Therefore, we selected the Wiener-kernel method to study properties of excitation and inhibition before and after acoustic trauma.

Separating excitatory and inhibitory responses by decomposition of the second-order Wiener kernel revealed a number of interesting findings. A large number of multi units (43%) had one or more inhibitory subsystem within the ten highest-ranked subsystems. Remarkably, the best frequency of the inhibitory eigenvectors did not correspond well with the inhibitory CF. This shows that information about reductions in the firing rate by pure tones is not readily available from Wiener-kernel analysis. However, the inhibitory BF did correspond to the excitatory BF (Figure 4.6B), suggesting that the inhibitory subsystems rather represent different processes that involve inhibition, such as adaptation or refractoriness (Sneary and Lewis, 2007). This shows that the classical methods and the Wiener kernels provide complementary information about inhibition in the IC. Group delays of the stimulus-related oscillations in the inhibitory eigenvectors were often longer than delays of the excitatory eigenvectors. However, in 7 of the 43 multi units with inhibition, the inhibitory response was faster than the excitatory response. In these multi units, adaptation or refractoriness could not have accounted for the presence of the inhibitory subsystem(s).

Another mechanism that might explain the presence of the inhibitory subsystems recorded from the IC is binaural hearing. Many neurons of the IC are sensitive to interaural time differences. Neural responses to interaural time differences consist of excitatory and inhibitory components. The inhibitory components can be both leading and lagging the excitatory components (Carney and Yin, 1989). In the current study, the stimulus was
presented by a free-field speaker, and therefore stimulated both the ipsi- and the contralateral ear. Accordingly, the spike trains recorded from the IC carried information about the interaural time difference. This might have been reflected in the response properties of the excitatory and inhibitory subsystems.

In the current study, we defined two measures for the amount of inhibition as revealed from the inhibitory subsystems of the second-order kernel: the number of inhibitory subsystems and the inhibition-to-excitation ratio. Inhibition plays a dominant role in the IC (Pollak et al., 2011) and it has previously been shown that inhibitory responses in particular are vulnerable to acoustic trauma (Heeringa and van Dijk, 2014). Both tinnitus and hyperacusis, two debilitating conditions that often result from exposure to loud sounds, are believed to be related to a disrupted balance between excitation and inhibition in the central auditory system (Eggermont and Roberts, 2014; Knipper et al., 2013). The measures of inhibition defined in the present work can be of additional use to further study the pathophysiological mechanism(s) of noise-induced tinnitus and hyperacusis.

Acknowledgements

We thank Hans Segenhout for technical support, Russ Snyder for showing us the surgical procedures involved in the IC recordings, and Fedde Tigchelaar for his assistance with the Wiener-kernel calculations.

This work was supported by the Heinsius Houbolt Foundation and the Stichting Gehoorgestoorde Kind. The study is part of the research program of our department: Healthy Aging and Communication.