ELECTROPHYSIOLOGICAL EVIDENCE

FOR TINNITUS IN THE CENTRAL NUCLEUS OF

THE INFERIOR COLLICULUS

BY

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Abstract

In previous work, little attention has been paid to the differences between neural responses to a sound exposure that did or did not produce a tinnitus percept. Here, neurons in rat central nucleus of the inferior colliculus were studied in noise-exposed rats characterized for the presence of a tonal tinnitus percept using a gap inhibition acoustic startle response paradigm. Recordings were done in three groups of animals: unexposed, exposed with tinnitus, and exposed without tinnitus. Presumably the neural correlates of tinnitus can be isolated from the effects of sound exposure in this way. A single electrode recording method was utilized to isolate and record from single units with monaural free field stimulation of the ear contralateral to the inferior colliculus. There is a loss of inhibition in tinnitus positive animals, specifically a loss of narrowly tuned type I units in the regions of threshold shift. This is accompanied by an edge effect, hyperactivity flanked by hypoactivity in the frequency regions affected by the sound exposure, and highly regularly firing units in the hyperactive region.

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Preface and Acknowledgments

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List of Abbreviations

ABR   Auditory brainstem response

ANF(s)   Auditory nerve fiber(s)

ANOVA   Analysis of variation

BF   best frequency

CDF   Cumulative density function

CIC   central nucleus of the inferior colliculus

CV   Coefficient of variation

DcIC   dorsal nucleus of the inferior colliculus

DCN   dorsal cochlear nucleus

GIASR   Gap inhibition of the acoustic startle reflex

IC   Inferior Colliculus

ICX   external nucleus of the inferior colliculus

ISI   Interspike interval

LSO   lateral superios olivary complex

MSO   medial superior olivary complex

nBTC   normal Best Threshold curve

no tinn   tinnitus negative

tinn   tinnitus positive

SR   Spontaneous rate

VCN   ventral cochlear nucleus

Chapter 1

Introduction

Tinnitus has been broadly defined as the perception of an acoustic stimulus where there is no corresponding external acoustic stimulation. By this definition there are a plethora of conditions or causes that produce tinnitus symptoms. The complexity of determining the type, severity and quality of the tinnitus percept, as well as uncertainties about its cause, makes tinnitus difficult to characterize and treat.

Tinnitus can be categorized as subjective or objective. Objective tinnitus refers to tinnitus induced by body sounds that are detected by the peripheral auditory system, often sounds connected to blood flow. Subjective tinnitus is associated with dysfunction of the central auditory system, and is not the result of peripheral auditory stimulation. Studies suggest that the perception of subjective tinnitus is not the same as the perception of physical auditory stimulation (Jastreboff, 1990). Subjective tinnitus is commonly associated with certain diseases, such as Ménière's disease (Minor et al., 2004) and vestibular schwannoma (Levo et al., 2000). It is also a side effect of a plethora of drugs, including common drugs like aspirin and antibiotics (Seligmann et al, 1996). It is also associated with a variety of head traumas--a current example is the increasing prevalence of tinnitus in veterans with blast-related head trauma (Magnuson et al., 2012). Tinnitus is the most common complaint of war veterans (Cave et al., 2007), usually associated with a hearing loss due to noise overexposure. Most commonly, tinnitus is associated with a hearing loss. However, tinnitus has also been seen in patients without any obvious

damage to the peripheral auditory system, in that the patients have normal audiograms and present no other sign of auditory deficit (Roberts et al., 2010).   Recent animal studies suggest that the audiogram may not be sensitive to loss or malfunction of auditory nerve fibers (Lin et al., 2011), so that there may be undiagnosed damage in tinnitus patients with apparently normal hearing based on threshold testing. Thus the current working model of tinnitus is that the percept arises when there are changes in the central auditory nervous system in response to a decrease in input from the auditory periphery (Norena and Farley, 2013).

Studying Tinnitus in an animal model

Acoustic trauma is a common method of tinnitus induction in laboratory animals (Kaltenbach, 2011) and is used here. In previous work, little attention has been paid to the differences between cases in which a sound exposure did or did not produce a tinnitus percept. However, it is essential that we parse out the effects of noise-induced hearing loss from the mechanism of tinnitus generation. Konig et al. (2006) showed the variability in a controlled human population sample with only noise-induced hearing loss patients.   The paper suggests that there is variability, not only in whether or not overexposure induces tinnitus, but also in the type of tinnitus that is perceived.   This suggests that close attention needs to be paid to whether or not a sound exposure produces tinnitus.

Here, we use monaural acoustic trauma to produce a hearing loss and then test the animals for both hearing loss (with auditory brainstem potentials, ABRs) and for the presence of tinnitus. For the latter, we use a Gap Inhibition Acoustic Startle (GIASR)

paradigm, modified from the paradigm established by Turner et al. (2006), to differentiate animals with a noise-induced loss only from animals with tinnitus. This paradigm is effective in testing for a tonal tinnitus in noise-exposed animals.

After characterizing the animals as above, we study single neurons in the inferior colliculus (IC) using extracellular recording techniques. The properties of IC neurons are compared between control animals, animals with hearing loss but no tinnitus, and animals with hearing loss and tinnitus.

Why the Inferior Colliculus?

Studies of acoustic trauma have shown anomalies in the auditory system from the cochlea (Liberman and Dodds, 1984) to the cortex (Rajan, Irvine et al., 1993).   The dorsal cochlear nucleus (DCN) has been the focus of study as the primary site of tinnitus generation. Somatic tinnitus, i.e. tinnitus induced or modulated by manipulations of the jaw, head, neck or shoulders (Levine, 1999; 2003), gives clinical evidence of DCN involvement since the DCN is the first site of auditory and non-auditory integration in the auditory system (Young et al 1995). Large effects can be seen in units in the DCN in response to electrical or proprioceptive stimulation of the somatosensory system (Young et al 1995, Kanold and Young 2001, Shore 2005). However, Brozoski et al. (2012) showed that severing the output of the DCN early after sound exposure prevents the development of tinnitus, but severing the DCN outputs in an animal with tinnitus does not get rid of the tinnitus percept. This suggests that while the DCN may play an important role in the development of tinnitus in the central auditory system, it does not play a role in persistent tinnitus perception after acoustic trauma.   Our working

hypotheses are as follows:

(1) Tinnitus generation reflects changes in the circuitry of the brainstem auditory system, at the level of the IC or below, and is expressed in the responses of IC neurons.

(2) If elevated spontaneous activity is a correlate of tinnitus, as is usually assumed (Kaltenbach, 1998), then the spontaneous activity of at least one group of IC neurons should be elevated.

(3) If increased synchrony among neurons is a correlate of tinnitus, as suggested by Seki and Eggermont (2003), it should be evident by irregularities in the spontaneous activity in IC neurons in tinnitus positive animals.

The IC is a central processing center in the auditory system. The IC can be partitioned into three parts- the central nucleus (CIC), the external nucleus (ICX) and the dorsal nucleus (DcIC) based on anatomical differences in the structures (Morest and Oliver, 1984). Like the DCN, IC receives cross-modal inputs from multiple sensory nuclei, mainly in the ICX (Zhou and Shore, 2006; Aitkin et al., 1978). Descending input to the IC, from the auditory thalamus and cortex, terminates mainly in the ICX and DcIC. The CIC receives primarily ascending auditory input, direct and indirect, from all of the auditory nuclei of the brainstem, including the dorsal and ventral cochlear nuclei (DCN and VCN), the medial and lateral superior olivary complexes (MSO and LSO) and the lemniscal structures. The CIC projects to higher auditory nuclei, including the auditory thalamic structures and the auditory cortex. CIC is a laminar structure, described as having functional zones (Oliver, 2005), and has a dorsal to ventral tonotopy. The CIC has been studied for its role in sound localization and the processing of inter-time difference (ITD), inter-level difference (ITD) and spectrotemporal cues. A variety of

brainstem circuits have been demonstrated to support binaural response sensitivity in CIC (Pollak, 2012). CIC monaural response types are similar to the response types seen in VCN and DCN units, with the effects of excitatory and inhibitory inputs evidenced in the level dependent responses in the frequency response maps (Ramachandran et al. 1999). In some cases, direct connections between neurons in brainstem nuclei and specific response types in CIC have been demonstrated. More relevant to tinnitus, Davis (2002) showed that there is a direct connection between type IV units in the DCN and Type O units in CIC. Therefore if an increase in spontaneous activity in DCN is indeed a neural correlate of tinnitus, as prior studies suggest (Manzoor et al. 2013; Kaltenbach 1998; Brozoski et al. 2002), it ought to be evident in type O units of the CIC.

Finally, in rats (our model animal) CIC is fairly superficial and therefore is an easy target for electrophysiological study. While activity in the CIC has been examined in some studies (Bauer et al. 2008; Mulders and Robertson, 2009; Dong et al. 2010), none have used behavioral testing to dissociate the effects of sound overexposure from a tinnitus percept in the inferior colliculus.

Research Synopsis

Several theories of the neural correlate of tinnitus have been published. The most common theory is hyperactivity in the nuclei of the ascending auditory pathway (Kaltenbach, 2011; Eggermont, 2013). Tinnitus has also been associated with an increase in spike synchrony and bursting and a loss of inhibition in the central nervous system nuclei (Roberts, 2011; Seki and Eggermont, 2003). Letham (MSE thesis, 2007) suggests that there is irregularity of single unit spontaneous rates in sound exposed cat DCN. Note

that many of these studies fail to test animals for tinnitus, and may erroneously characterize the effects of noise exposure, not tinnitus. The goal of this study is to look at the changes that occur in the CIC after sound exposure, using the behavioral characterization to tease out the traits that are likely tinnitus related from the effects of hearing loss.

Studies in the DCN and VCN of sound-exposed animals suggest changes in the types of single unit responses, increases in the unit thresholds and broadening of tuning (Ma and Young, 2006; Cai et al. 2008). Chapter 3 explores these types of changes as they occur in the CIC after exposure and identifies changes that are unique to animals that test positive for tinnitus. While in both groups of animals, we see shifted thresholds in the frequency region affected by the sound exposure, we find that in tinnitus positive animals there is a loss in inhibitory response type units, relative to the unexposed animals.

Chapter 4 discusses changes in spontaneous activity after noise exposure and the possibility of hyperactivity as a neural correlate of tinnitus. Since the unexposed CIC has a range of spontaneous rates, it is necessary to establish a method of comparing spontaneous rates between the three animal groups (unexposed, exposed without tinnitus, exposed with tinnitus). We found that the proportion of hyperactive units in both exposed groups of animals increases relative to the unexposed animal group, however in the tinnitus positive animals there is a narrowband frequency dependence to this shift, while in tinnitus negative animals, the increase is broadband.

Chapter 5 describes the regularity of neuronal spiking in single units in unexposed vs. exposed rats. Salinas and Sejnowski, 2000, demonstrate an increase in spike

variability as a correlate of increased input synchrony, so increased irregularity may imply an increase in input synchrony to neurons due to reorganization of the nucleus after sound exposure. Seki and Eggermont, 2003, suggest that there is increased synchrony in the auditory cortex after sound exposure, and suggest increased synchrony as a correlate of tinnitus. Here we use standard methods for looking at regularity in spike timing and spike rate of the spontaneous activity (Gabbiani and Koch, 1998). We find that in the tinnitus positive animals there is increased regularity in the hyperactive units compared to the regularity of units of similar rates in the unexposed animals.

Chapter 2

Methods

The goal of this study is to use a behavioral paradigm that identifies animals with a tonal tinnitus, along with physiological recording of single unit neuronal activity to pinpoint a neural correlate of tinnitus on the single unit level. All procedures were conducted in accordance with protocols approved by the Johns Hopkins University Animal Care and Use Committee.
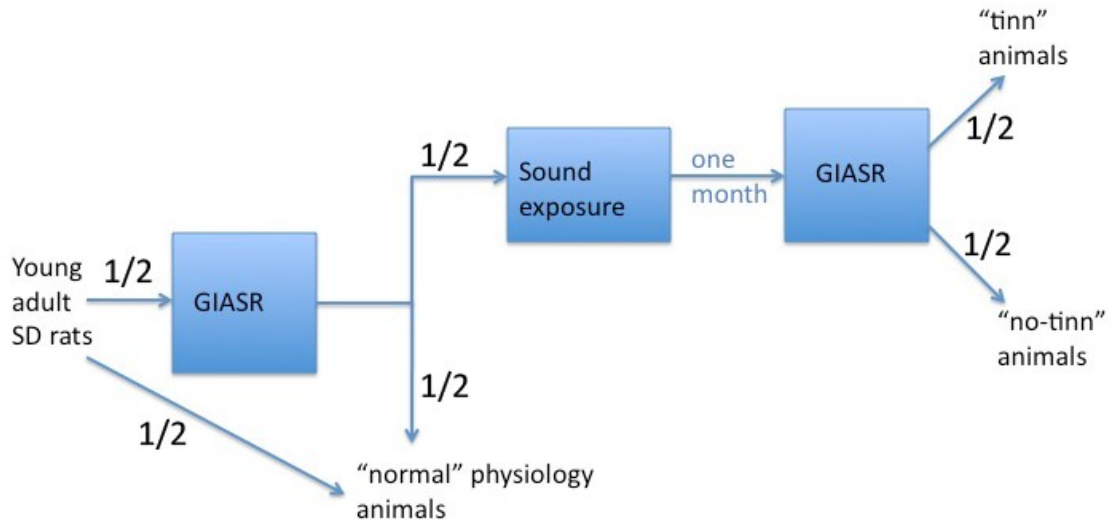
Figure 2.1- Animal classification scheme.

Animals are housed in the university provided animal housing upon arrival at the institution. Fifty percent of these animals are used as unexposed (control) animals for physiology, the other fifty percent become behaviorally tested animals and are prescreened for sufficient, measureable inhibition of the acoustic startle. Animals that fail the behavioral pre-screening, also become unexposed animals. Animals that pass the prescreening measures are sound exposed as described below. Post-exposure screening begins two weeks post-exposure and may extend to 11 or 12 weeks post-exposure. Animals that lose inhibition of the acoustic startle are classified as tinnitus positive and animals that continue to exhibit startle suppression are classified as tinnitus negative animals.

An overview of the classification of animals as unexposed, tinnitus positive and tinnitus negative are shown in figure 2.1. Briefly, young adult male Sprague Dawley rats

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(Harlan Laboratories) are used for physiological study or are prescreened for sufficient inhibition of startle in the behavioral paradigm, as explained later. The animals that pass behavioral prescreening (roughly half) are sound exposed and assessed for tinnitus at two to twelve weeks post-exposure. Those that fail the behavioral screening are added to the normal physiology group.

Behavioral screening:

The behavioral screening method is an altered version the gap inhibition acoustic startle paradigm as described by Turner et al (2006). The method employs the tendency of rats to startle in response to an unexpected acoustic stimulus to test for tinnitus (see figure 2.2). Basically, a rat startles in response to a loud stimulus (Figure 2.2A). If a gap is placed a fixed time before the loud stimulus, the startle diminishes (Figure 2.2B). If the tinnitus percept were to match the background stimulus, then the tinnitus would fill the gap, and the startle would not be suppressed (Figure 2.2D).
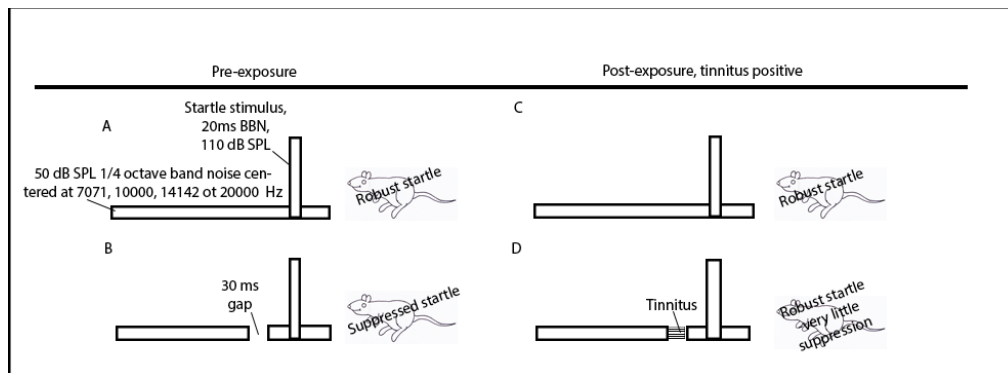
Figure 2.2- Schematic Diagram of the behavioral test for tinnitus.

This figure, modified from a figure in Kraus et. al. 2010, is a depiction of the gap inhibition paradigm theory. Briefly, a detectable gap prior to a startle stimulus (B) leads to inhibition of the startle (A). In an animal with tinnitus, the ability to detect the gap masks the Tinnitus, and there is less inhibition of the startle (D). See the text for the parameters used in this study.

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The animal is placed in a cage with a piezoelectric base on a platform, which detects motion in the cage, in a sound attenuation chamber. Once an animal acclimates to the setup, behavioral screening begins with a test of the acoustic startle and startle inhibition. Startle inhibition is tested with a single session of gap-inhibition of the acoustic startle reflex (GIASR), with a fixed background (50 dB SPL broad band noise) and variable gap-to-startle delays (10, 20, 30, 40 and 50 ms), followed by a single session with a fixed gap (30-50 ms) and variable background (1/4 octave band noise centered at 7071 Hz, 10000 Hz, 14142 Hz or 20000 Hz at 50 dB SPL). The startle stimulus is a 20ms burst of noise at 110dB SPL. These are the parameters to assess whether or not an animal has a robust startle response with inhibition of the startle modulated by the introduction of a gap before the startle stimulus.
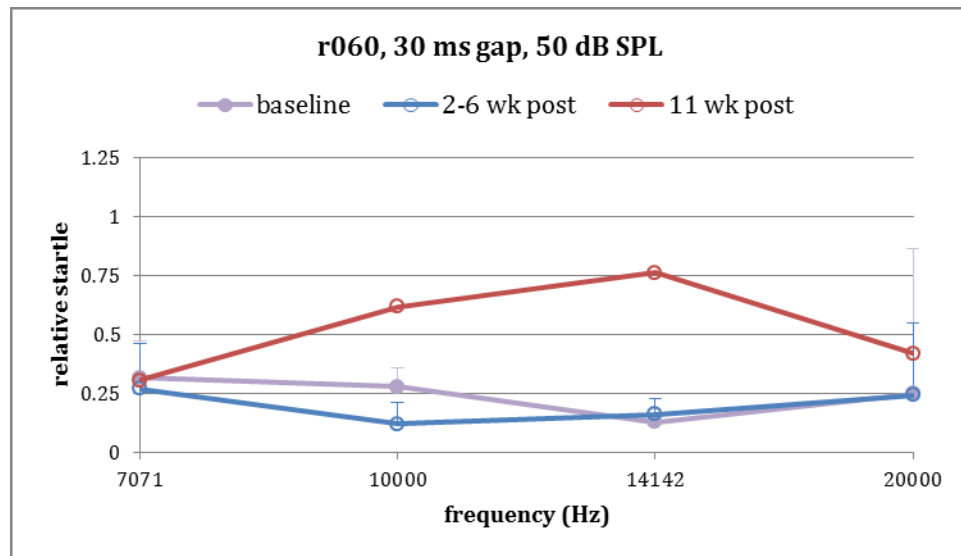
Figure 2.3- GIASR data for a single animal (r060).

This animal had a relatively flat baseline, which was maintained at 2-6 weeks post exposure. However, at 11 weeks post-exposure, the animal exhibited a loss of inhibition of the acoustic startle at 14kHz.

Behavioral measurements are assessed pre- and post- sound exposure using a fixed gap (30 ms) and a variable background (1/4 octave band noise centered at the frequencies stated above). This is the "baseline" startle response data. Pre-exposure data is collected 1 to 2 weeks prior to sound exposure and the post-exposure data is collected 2 or more weeks after the sound exposure.

Animals are classified as tinnitus positive (post-exposure) if there is a consistent loss of inhibition at a single frequency, or at adjacent frequencies, while inhibition at all other frequencies is maintained (see figure 2.3).

Sound Exposure

Awake animals are unilaterally sound exposed to a 16kHz pure tone for 2 hours as follows. The animals are placed in a cage mounted on a rotating platform. Two Pyramid TW57 tweeter speakers are mounted to the ceiling of a sound acoustic chamber, sitting 20 cm from the chamber ceiling. The walls of the chamber are lined with acoustical foam. The cage sits at a distance below the speakers to maintain a sound level between 105 and 125 dB SPL (as measured by a Larson-Davis sound level meter, z-scale) and rotates one degree every 30 seconds.

The animals are housed in the university's vivarium prior to exposure and are moved to a low ambient noise chamber ("quiet room") 1 day to 3 weeks post-exposure. The noise levels in the quiet room is on average 32 dB SPL in the audible range for rats,

compared to 36 dB SPL with levels up to 85 dB SPL in the university's vivarium due to traffic and machinery noise.

Two to twelve weeks post-exposure, animals are screened for tinnitus using GIASR paradigm described above. Electrophysiological experiments are from one to four months post-exposure.

Auditory brainstem responses (ABR) are used to assess the degree of hearing loss post-exposure. ABRs are taken one day pre-exposure and 7 to 10 days post-exposure under light anesthesia, using a procedure similar to the procedure described in Lina and Lauer (2013). Animals are anesthetized with Ketamine (40 mg/kg) and Xylazine (10 mg/kg) and placed on a heating pad (body temperature maintained at 37°C) inside a sound-attenuating chamber lined with acoustic form. Three platinum electrodes are inserted subcutaneously over the bulla of the respective ear (recording electrode), in the vertex of the skull (reference electrode), and in the ipsilateral leg (ground electrode). The responses are amplified using a World Precision Instruments ISO-80 amplifier and bandpass filtered between 30 and 3000 Hz using a Krohn-Hite filter bank. Acoustic stimulus generation is as described below, with the exception that a Crown Amplifier is used in the ABR setup. A Fostex dome tweeter speaker (FT28D) is placed in alignment with the respective ear (approximately 18" from the animal's ear). ABR thresholds are measured on-line for clicks and at 2.5, 6, 8, 10, 11.2, 16, 22.4, 32 and 40kHz.

All behavioral screening and characterization procedures, as well as sound exposures and ABRs, are done by Kerrie Tiedemann. Physiological recordings are done blind to the ABR data or the behavioral characterization of the animal as tinnitus positive or tinnitus negative.

Physiological Recording

Male Sprague Dawley rats weighing between 248 and 609 grams (mean 416; median 409) are anesthetized with isoflurane in an inhalation chamber. Upon sedation, the animal's head is shaved and a mixture of Ketamine (75 mg/kg im) and Acepromazine (2.5 mg/kg im) is administered to achieve an areflexic state of anesthesia. Atropine sulfate (0.1 mL) is administered intraperitoneally to reduce secretions. Saline (1-3 mL) is administered subcutaneously to keep the animal hydrated. Lidocaine (1 mL, 100mg/mL) is applied subcutaneously to the top of the skull to relieve any local pain inflicted by stereotaxic mounting on ear bars, or the incision. Supplemental doses of Ketamine (25-40 mg/kg im) are given as necessary to maintain an areflexic state. Throughout the experiment the animals are on a regulated heating pad and the animal's body temperature is maintained at 37°C. Animals are euthanized at the end of the experiment with a lethal intraperitoneal dose of a barbiturate-based euthanasia solution.

The animals are stereotaxically mounted using non-rupture 45° tip rat ear bars and a rat adapter so that lambda and bregma are in a horizontal plane, aligned with measurements in a standard rat brain atlas (Paxinos and Watson 2004). An incision is made along the midline and the skin and underlying tissue is retracted to expose the skull. Holes are drilled to fit two small machine screws (size 0, 1/8" long) in the skull anterior to Bregma. A captive stud (size 8, 1/2" long), imbedded in dental acrylic, serves as a head bar to fix the head to the stereotaxic frame while allowing the removal of one of earbar for free-field acoustic stimulation (see figure 2.4).

A fenestration is made anterior to lambda, contra-lateral to the stimulated ear to expose occipital cortex. An A-M Systems tungsten microelectrode (5 MOhms) is advanced stereotaxically through the occipital cortex to IC below at an angle five degrees anterior to the sagittal plane. Typically, the electrode is advanced ~1mm through cortex before undifferentiated neural activity ("hash") can be detected in response to a broadband noise stimulus.
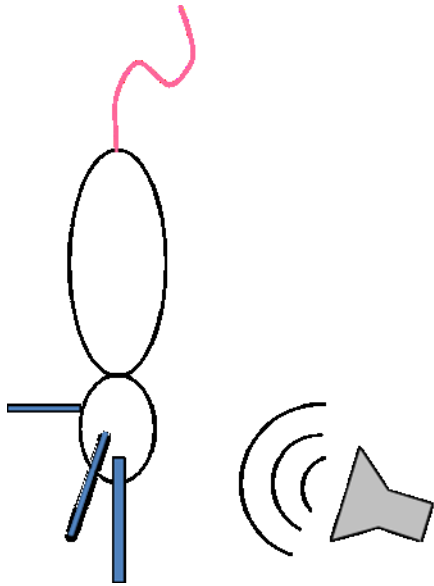
Figure 2.4- Schematic drawing of the electrophysiological recording setup.

The head post, earbar and recording electrode are mounted to the stereotax on the side of the head contralateral to acoustic stimulation. The stereotax is dismantled ont he side of the animal where the speaker is placed, allowing for free field acoustic stimulation.

The electrical signal from the electrode is amplified using an AM systems amplifier (model 1800, AM Systems). The signal is bandpass filtered from 200Hz to 4 kHz using a Krohn Hite filter (model 3202). A Schmitt trigger is used to detect spikes and spike times are digitalized and recorded using a National Instruments digital interface board (model PCI6602).

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The ear contralateral to the exposure (fenestration) is stimulated using a free-field speaker with broadband noise and tones (1 to 50kHz).

Acoustic Stimulation and neuron classification

Signals were generated using TDT-RP2 real time processors and TDT-PA5 programmable attenuators (Tucker-Davis Technologies). The signal is amplified using a Parasound Zamp v3 amplifier. A Fostex dome tweeter (model FT28D) is placed six inches from the animal's open ear. The sound levels in the chamber are calibrated using a B&K 1/4" probe microphone, placed near the animal's ear before recording commences, connected to a B&K amplifier.

Broadband noise at -50 dB attenuation is used as the search stimulus while advancing the electrode through cortex. The inferior colliculus is recognized by the onset of hash in response to noise. Once the inferior colliculus is located, the search stimulus is switched to tones (20 to 50 dB SPL). The best frequency (the frequency that elicits the most robust response at low sound levels, BF) is tuned audio-visually. Location of neurons in the central nucleus of the IC was assumed when recordings at a series of locations showed a steady increase in the best frequency with increasing depth. Best frequencies typically ranged from 1 to 50 kHz. Because of limitations in the acoustic system, only units up to about 45kHz could be studied. Once a unit is isolated and best frequency and threshold determined, a series of response maps and rate level functions are recorded to classify the neuron. Response maps show the responses to 200ms tone pips, beginning at the best frequency and alternating above and below BF at 25 stimuli per octave at a fixed attenuation for two octaves. At least three response maps, at varying

levels, are taken per unit, usually at threshold, threshold+20 and threshold+40 dB. Rate

level functions are taken using 200 ms tones at a fixed frequency at increasing level-

usually 5 or 10 dB below threshold to 40 to 60 dB above threshold to determine the

driven response of the neuron. Noise rate level functions are also taken in some units.

In noise rate level functions, a 200-ms broadband noise is used instead of a fixed tone.

Responses in monaural rat CIC are similar to those documented in cat

(Ramchandran and May 1999).   The units are characterized as type V, I or O, based on

the response maps and rate level functions.   If the unit had spontaneous activity, a ten-

minute recording is taken with no acoustic stimulation. In units with no spontaneous

activity or very low spontaneous activity, a one to two-minute recording is taken to

document no or very low spontaneous firing.

Data Analysis

All data analysis uses MATLAB 7.0 (Mathworks). A database of recorded and

analyzed data is kept in Microsoft Excel (Office 2008).

Determining the edge frequency

The data analysis is done relative to the edge frequency of the damage resulting

from the sound exposure. The edge frequency is defined as the midpoint of the steepest

slope of the threshold function for the auditory brainstem response (ABR) recorded seven

to ten days post exposure, and is computed as follows. The ABR threshold is plotted on a

logarithmic frequency scale and a linear (dB) threshold scale. The edge is defined as the

midpoint of the segment of the ABR threshold function with the steepest slope. To calculate the slope. linear interpolation of the points on the ABR was done; that is, given two points on the ABR (f_1, t_1) and (f_2, t_2), the slope of the line is given by the equation,

$$m = \frac{t_2 - t_1}{\log(f_2) - \log(f_1)}.$$

The midpoint of the line is defined as

$$(m_x, m_y) = (10^{\frac{1}{2}(\log f_1 + \log f_2)}, \frac{1}{2}(t_1 + t_2))$$
. See figure 2.5.
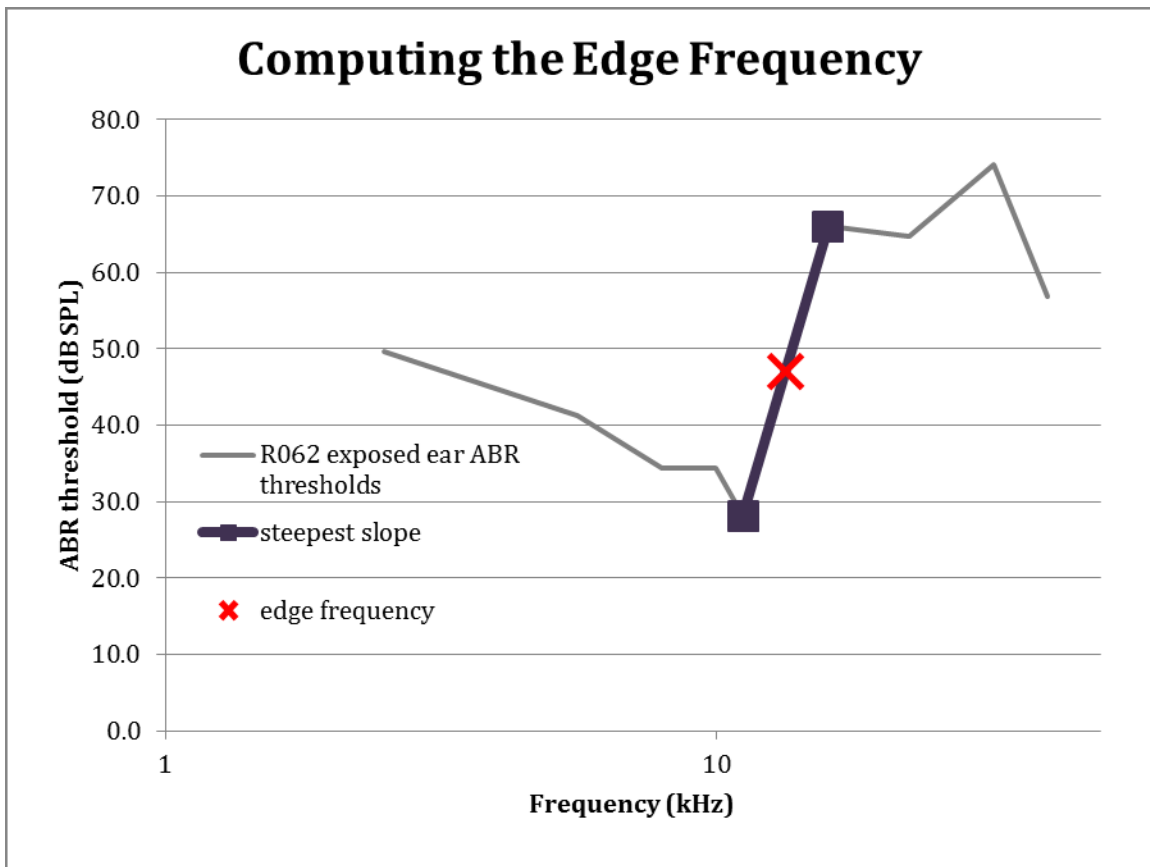
Figure 2.5-Determining the edge frequency.

A schematic diagram showing how the edge frequency is calculated on the ABR of an exposed animal. The slope between consecutive frequency points on a semi-log scale is calculated. The midpoint of the steepest slope is defined as the edge frequency.

Generating a Best Threshold Curve for physiological rat data

The normal Best Threshold Curve (nBTC) is a contour of the lowest unit thresholds determined using all available data from normal and sound-exposed animals. Figure 2.6 shows the data and the generated nBTC. Threshold shifts of single units reported in this thesis are determined with respect to the nBTC.
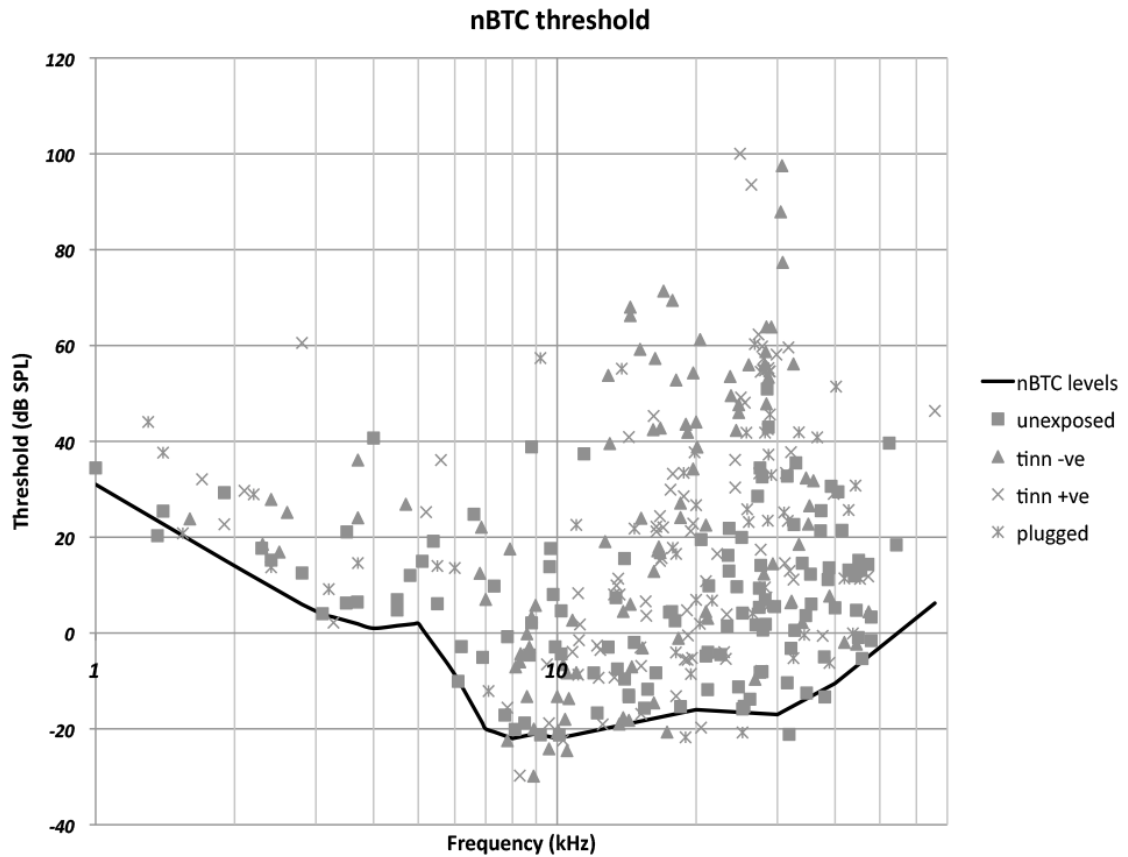
Figure 2.6- - The normal Best Frequency Curve (nBTC).

The nBTC is based on the lowest recorded thresholds in all recorded single units. All single unit thresholds were plotted from unexposed, exposed and plugged (recordings from CIC contralateral to the ear plugged during the sound exposure). The median pre-exposure ABR threshold curve was shifted and transformed to create the nBTC curve.

Measures of Regularity

The coefficient of variation (CV) is a standard measure used in neurophysiological studies to assess the regularity of spike trains (Nawrot et al., 2007). Usually it is used to assess how a spike train deviates from being Poisson in nature. Studies show that the CV increases, i.e.- spiking becomes more irregular, with increased input synchrony to a neuron (Salinas and Sejnowski, 2000). Studies also show an increase in variability of spiking in neurons from peripheral sensory neurons to the associated cortical neurons (Kara et al, 2000).
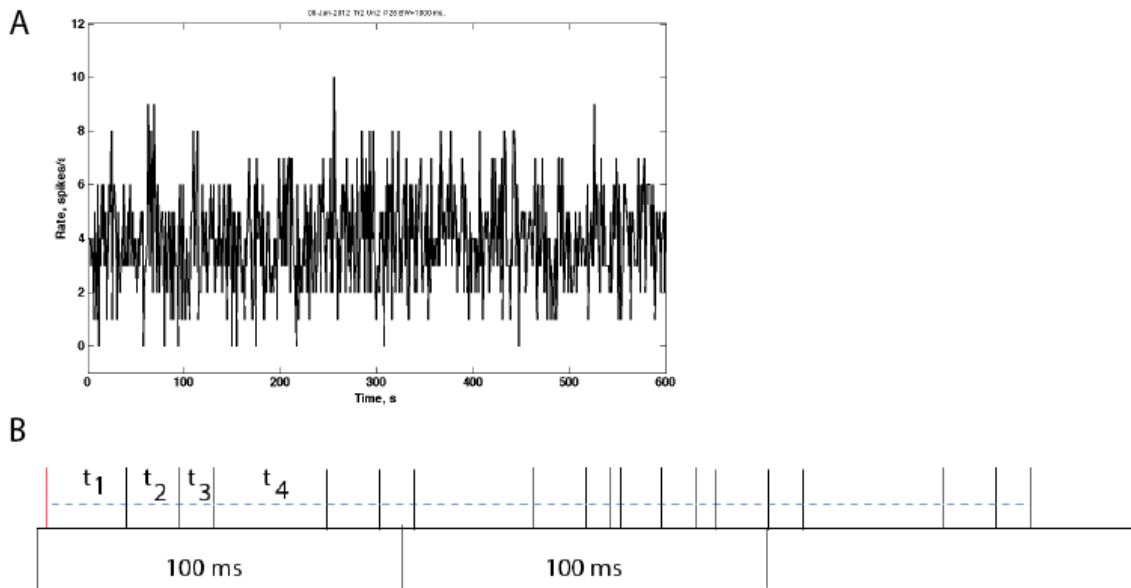
Figure 2.7- A schematic drawing of regularity measures.

(A) The spontaneous rate of an unexposed single neuron in rat CIC, binned in 1 s bins to generate a plot of the spike rate versus time. (B) The difference between spike times of successive spikes is taken to determine the coefficient of variation. The Fano factor is based on statistics of spike counts. The Fano factor at 100ms, divides the total recording time into 100ms bins, indicated schematically by the boxes below the spike train, and then computes statistics of the spike counts in each bin.

While the CV gives a regularity estimate on short time scales, the Fano factor is the appropriate measure for looking at longer time scale interactions (Nawrot et al., 2007). In auditory periphery studies using the Fano factor, Teich and Khanna (1985) showed very un-Poisson like behavior at large counting windows. This was attributed to a very small long-term time dependence in the spontaneous spike train. This effect has also been shown in LSO neurons (Teich, 1990), with more pronounced deviations from Poisson behavior. We expect to see this type of behavior in CIC neurons as well.

The two measures we use for regularity are the coefficient of variation of the interspike intervals, CV, and the Fano factor of the spike counts binned in time intervals binwidths of varying size (see figure 6).

The CV is a simple measure of regularity of a spike train. It is computed by taking the interspike times for the duration of the spontaneous recording and calculating the standard deviation over the mean, or

$$CV = \frac{\sigma}{\mu}$$

For a Poisson process, the CV is one, since the standard deviation of the interspike intervals (ISI) is equal to the mean. A CV value less than one, suggests regularity in the spike train, while a value greater than one suggests increased variability in the spike train. The sample mean,

$$\hat{t} = \frac{1}{k} \sum_{i=1}^{k} t_i$$

where $t_1, t_2, \ldots t_k$ are successive interspike intervals of a spontaneous rate recording, and variance,

$$\hat{\sigma}_t^2 = \frac{1}{k} \square_{i=1}^{k} (t_i - \hat{t})^2$$

of the ISI distribution are a reliable estimate of the empirical variance and mean if there are sufficient spikes recorded. For this reason, we calculate CV for medium and high spontaneous rate neurons only. The underlying assumption in CV analysis is that there are no correlations in successive interspike intervals (Gabbiani and Koch, 1998).

The Fano factor of the spike counts gives an assessment of changes in regularity of rate. This is computed by dividing the length of the spontaneous recording, T, into intervals of length L. We vary L to be 100 ms, 3 s and 10s, we also look at the slope of the Fano factor between L = 3 s and L = 10 s as an estimate of the long-range behavior of the spike train. An estimate of the mean and variance of the spike counts in each bin are taken using in a window of length L. The Fano factor is computed as

$F(T) = \frac{\hat{V}(T)}{\hat{N}(T)}$, where $\hat{N}(T)$ and $\hat{V}(T)$ are the estimated mean and variance of the spike counts respectively. Like the CV, the Fano factor is one for a Poisson process. The Fano factor is not a time-independent measure like the CV, so the Fano factor at higher window lengths gives an estimate of time dependent fluctuation in the spontaneous rate recording. Calculations of the CV and Fano Factor are compared across the three groups of animals.

Significance Testing

Significance tests between groups are done using ANOVA, two-sample Kolmogorov-Smirnov tests and Willcox-ranksum tests. Significance testing across paired groups and across frequency intervals is done using Fisher contingency tables.

Chapter 3

Physiological Changes

Studies in the DCN and VCN suggest changes in the types of single unit responses, as well as characteristic changes such as increases in the unit thresholds and broadening of tuning (Ma and Young, 2006; Cai et al., 2008). This chapter explores these changes in the contralateral CIC, the major target of DCN and VCN projections from the exposed ear. The goal is to identify any changes that may be unique to animals that test positive for tinnitus.

Results

There is a great deal of variability in the rat audiogram after sound exposure. The Auditory Brainstem Response (ABR) gives a good indication of the magnitude and the locus of threshold shifts (figures 3.1 and 3.2). Because of the variability in the frequency at which the threshold shift occurs, shown in the ABR threshold curves in figure 3.1, the analysis proceeds by lining up the ABR curves by the edge frequency of the threshold shift, see figure 3.3.
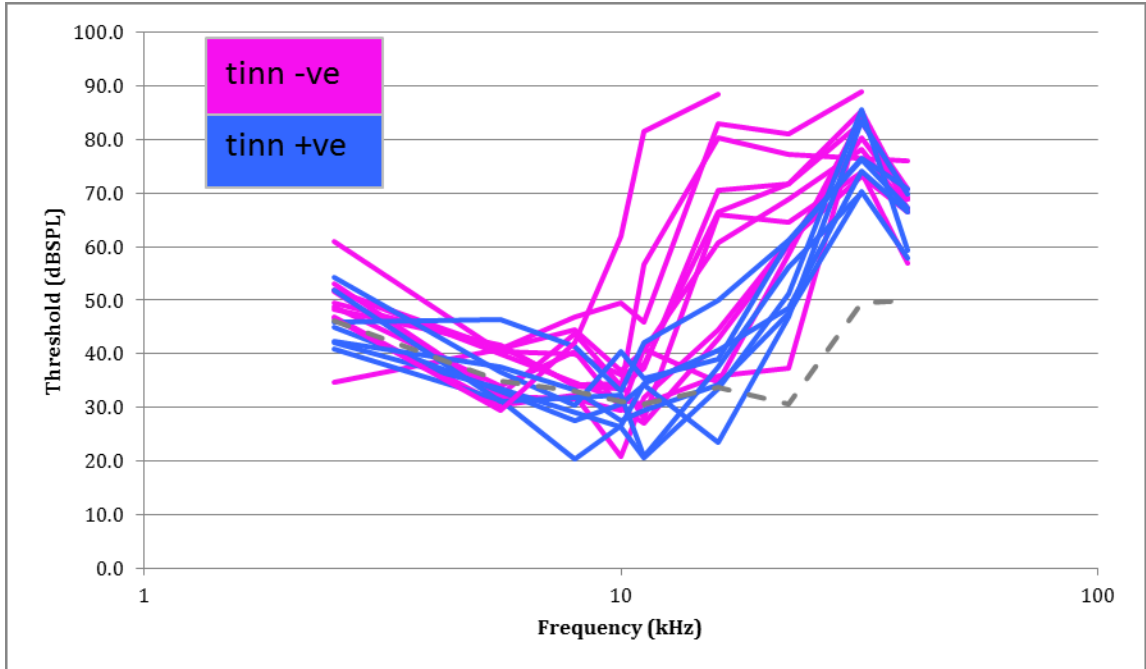
Figure 3.1- Auditory Brainstem Responses for exposed animals, 7-9 days post exposure.

In this figure, the blue lines are ABRs from tinnitus positive animals, the magenta lines are ABRs from tinnitus negative animals, and the dashed grey line is the mean pre-exposure ABR for all the animals. There is a wide degree of variability across exposed animals after the sound exposure, in the locus of threshold shift.
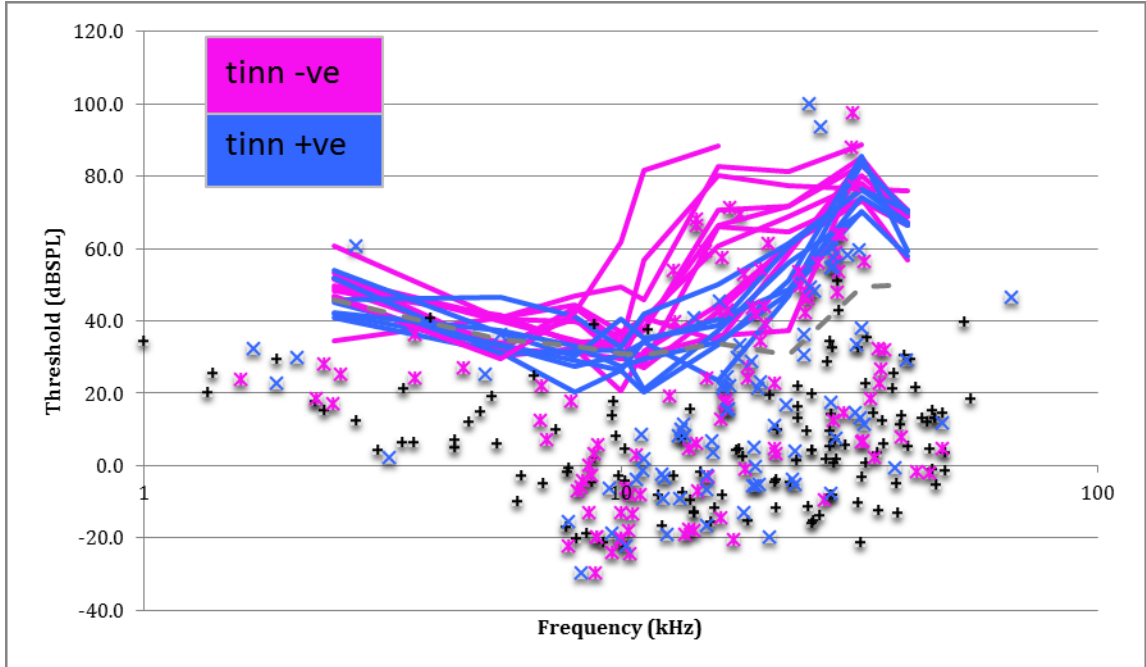
Figure 3.2- ABR thresholds and single unit physiological thresholds for all exposed animals.

This figure shows the ABRs from figure (3.1) with the single unit thresholds overlaid. Magenta asterisks (*) indicate tinnitus negative unit thresholds, blue x's indicate tinnitus positive units, black pluses indicate unexposed units.
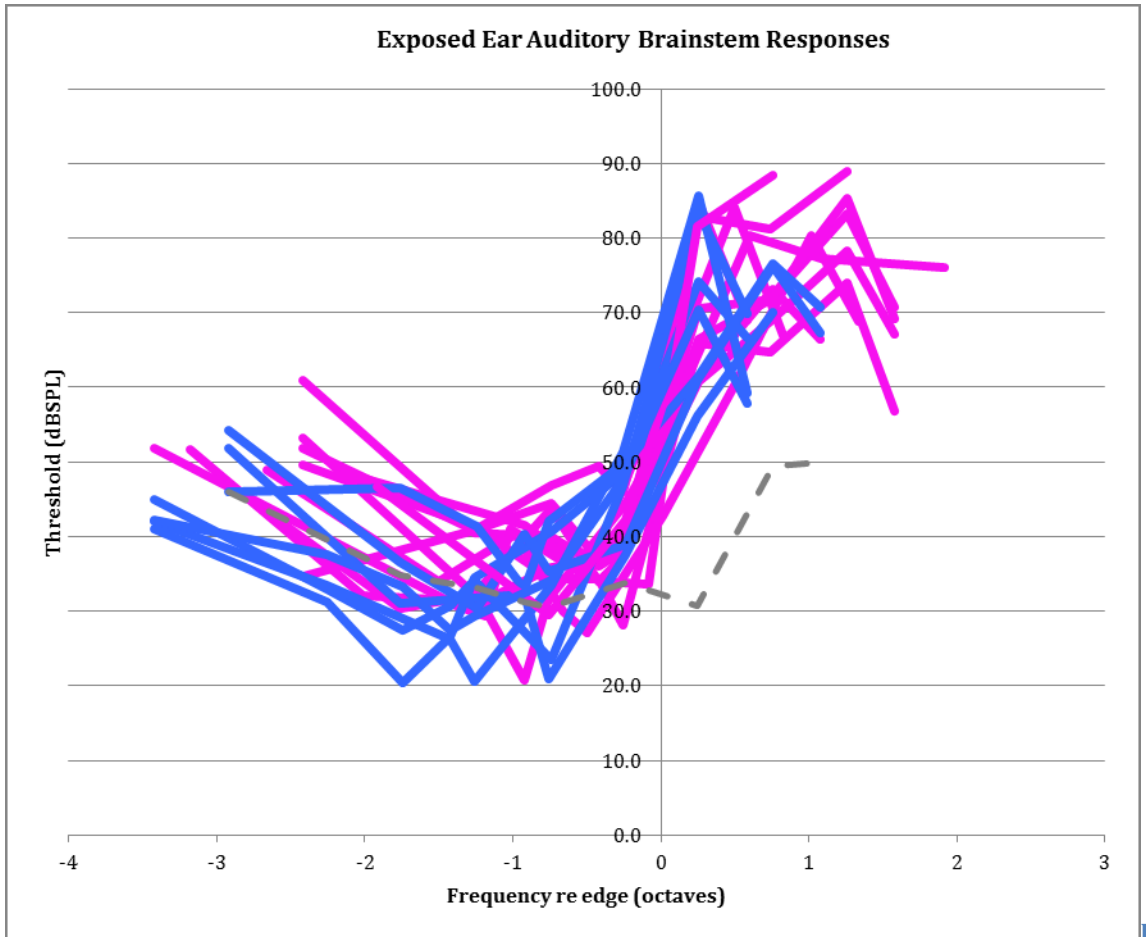
Figure 3.3. - Auditory Brainstem Responses relative to the edge frequency.

The ABRs are shifted with respect to the edge frequency, i.e.- the midpoint of the steepest slope of the exposed ABR. As in figure 1, the blue lines are the tinnitus positive animals, the magenta lines indicate the tinnitus negative animals and the grey line are the pre-exposure mean ABR levels. The median edge frequency of the exposed animals, 18.931 kHz, is used as the edge frequency for the pre-exposure ABR. The median edge frequency for the positive animals was 13.387 kHz, and 26.773 kHz for the negative animals.

Edge Frequency

The edge frequency for all of the exposed animals was computed as described in the methods section. The median edge frequency of tinnitus negative animals (median = 13.387 kHz) is notably less than that of tinnitus positive animals (median = 26.773 kHz). It is unclear why a high frequency hearing loss might be associated with a tonal (median = 14 kHz) tinnitus as shown in the behavioral results.
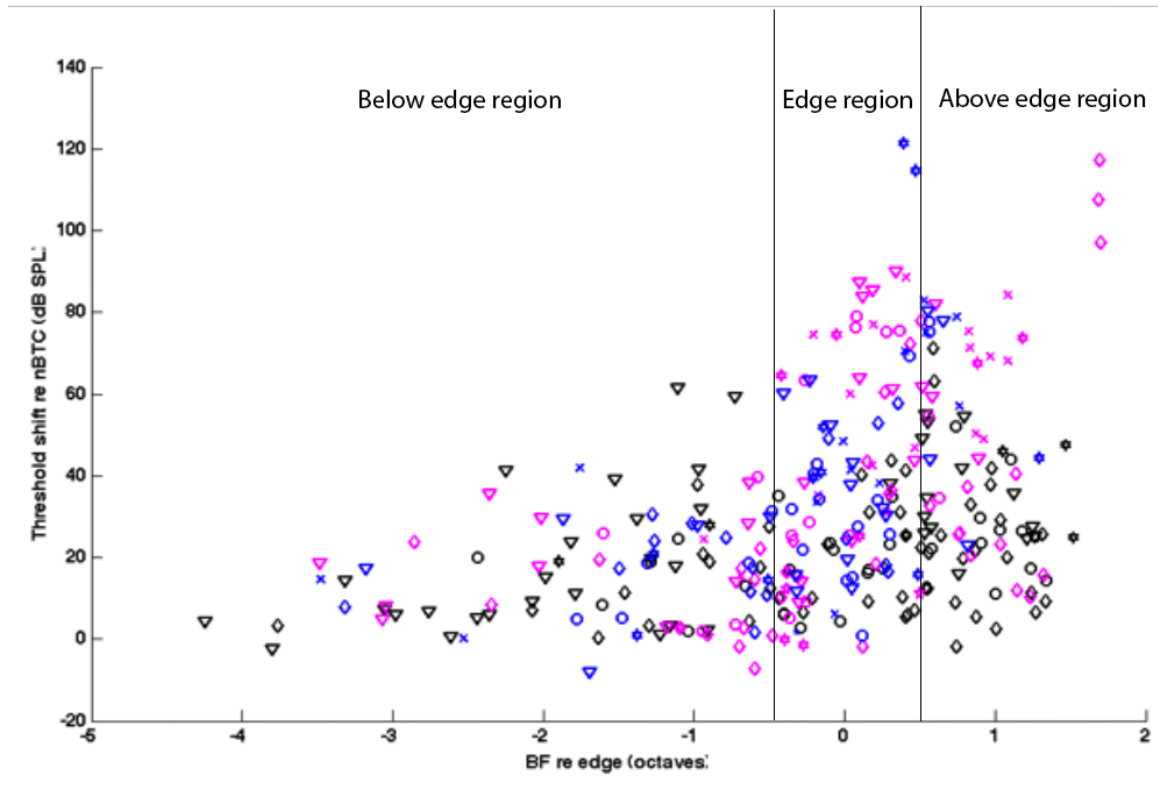
Figure 3.4 - Thresholds re. nBTC curve.

The gray vertical lines indicate BF = -0.5 and BF = 0.5 octave w.r.t. the edge frequency. The frequency regions re edge are defined as shown. Black markers denote unexposed single units, magenta markers denote single units from tinnitus negative animals, blue markers denote single units from tinnitus positive animals. Diamonds denote type I units, triangles denote type V units, circles denote type O units and 6-point stars denote unclassified units. x's denote class A tail units and 5-point stars denote class C tail units.

Threshold Shift

Figure 3.4 shows threshold shift relative to the normal best frequency curve (re nBTC) versus the BF relative to the edge frequency (BF re edge) for all recorded data. The degree of threshold shift is identical in the tinnitus positive and tinnitus negative groups (p=0.1885, two-sample Kolmogorov-Smirnov test). In the above-edge region, the average threshold shift is 25 dB SPL larger than unexposed threshold values (p<0.01,

two-sample Kolmogorov-Smirnov test) in tinnitus negative animals, and 37 dB SPL larger than normal threshold values (p<<0.001, two-sample Kolmogorov-Smirnov test) in tinnitus positive animals. In the edge region, there is a 23 dB SPL average threshold shift over unexposed thresholds in tinnitus negative animals, and 18 dB SPL threshold shift over unexposed threshold values in tinnitus positive animals (p<0.01, two-sample Kolmogorov-Smirnov test). Mean thresholds in the region below the edge are not significantly different from the unexposed single units.
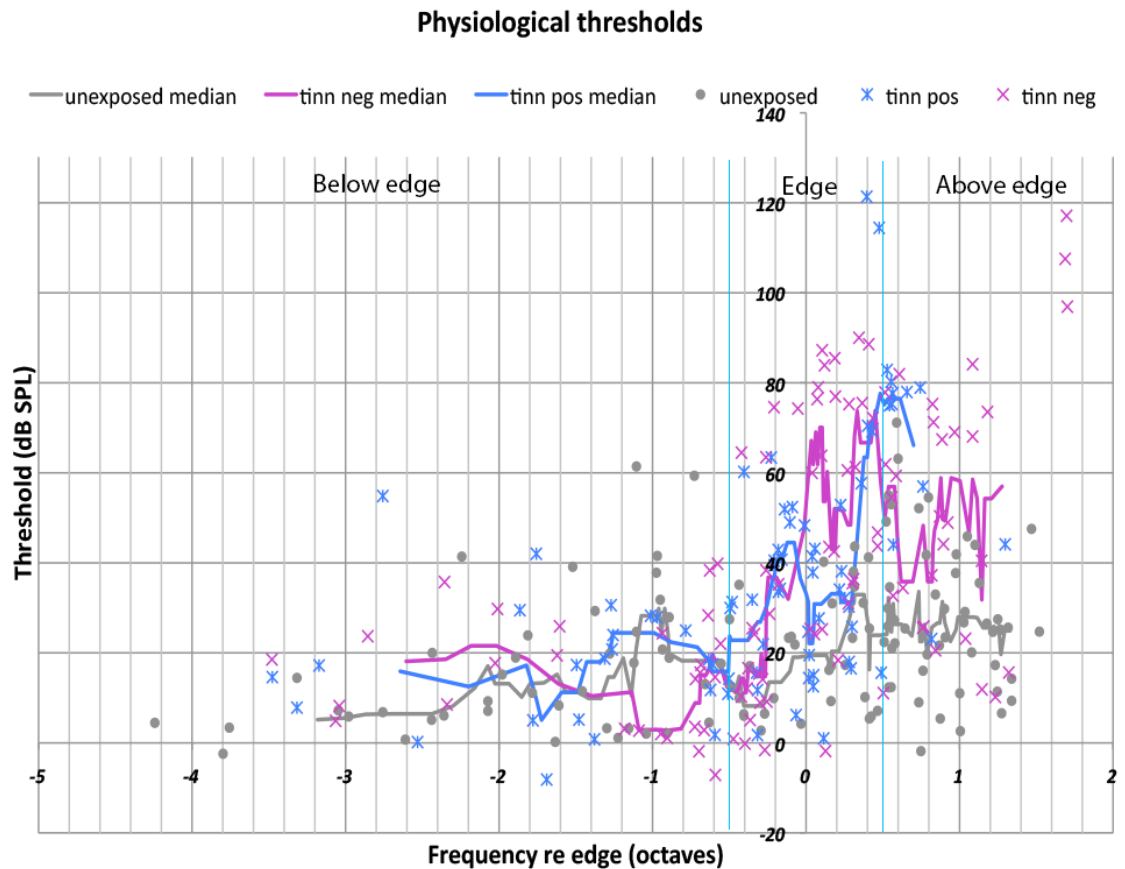
Figure 3.5- Median threshold shifts.

This is another representation of the threshold data shown in figure 3.4, including an 8 point moving median curve to show the differences in thresholds across the three animal groups. Here the unexposed group is gray, the tinnitus negative group is magenta and the tinnitus positive group is blue. The cyan vertical bars are at BF = -0.5 and BF = 0.5 octaves, demarking the regions relative to the edge region.

This suggests that in both exposed animal groups there is a significant shift in single unit thresholds at both edge region and above-edge region single unit best frequencies. Figure 3.5 adds median threshold shift lines to the data points that show the affected regions are the regions about the edge frequency and the region above the edge frequency.

Unit Type Classification

Responses in monaural rat CIC were similar to those documented in cat (Ramachandran and May 1996). The units are characterized as type V, I or O, based on the response maps and rate-level functions. Type V units have a widening band of excitation with increased sound level and a monotonic tone rate level function (figure 3.6, rate level function not shown). As in cat, these units are typically at lower frequencies and commonly are low spontaneous rate neurons.

Type I units have much narrower, level tolerant tuning (figure 3.7). Often Type I units have inhibitory sidebands flanking the excitatory region in the response maps. Type I units have non-monotonic tone rate level functions, but at high levels the discharge rate does not drop to below the spontaneous rate. Type I units are typically spontaneously active.

Type O units have a closed region of excitation, i.e.- there may be a region of excitation, which is overcome by inhibition at higher levels (figure 3.8). These units are sometimes called closed units since there is a closed excitatory region, but otherwise the neuron is characteristically inhibited. Type O units typically have spontaneous activity, but several type O units were recorded with no spontaneous activity. Tone rate level

functions for type O units are non-monotonic with the driven rate dropping to the

spontaneous rate or below the spontaneous rate at high sound levels.
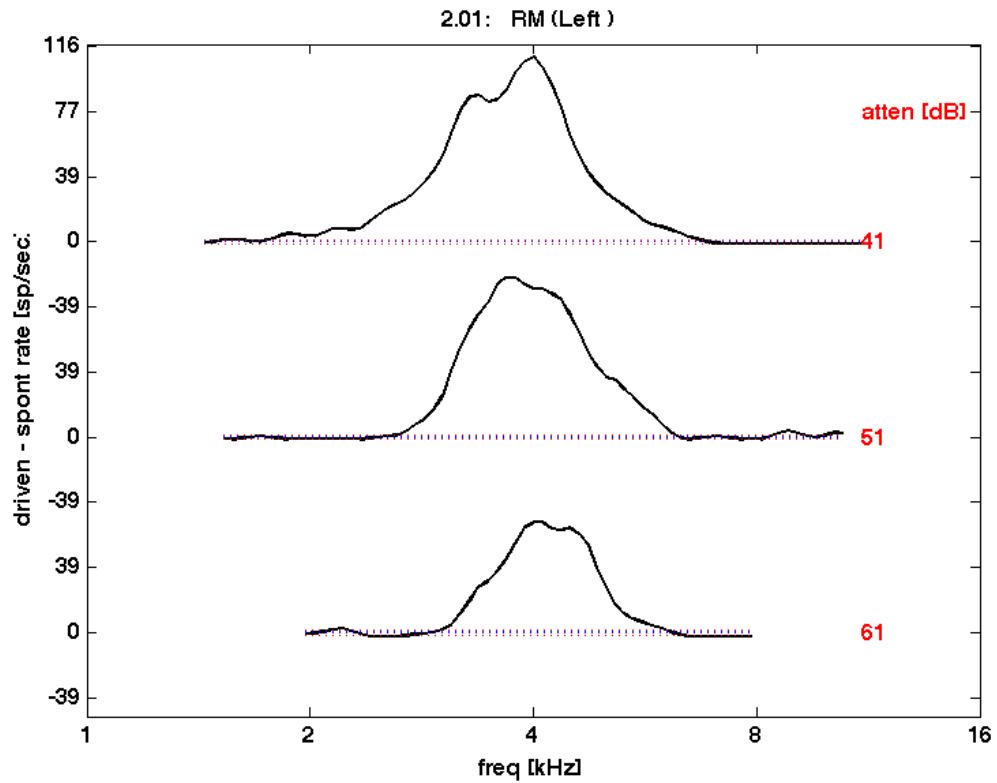
Figure 3.6- Response maps of a type V unit.

Type V units characteristically have an increasing band of excitation with increasing level. For this type V unit, the threshold was confirmed by the rate level function.
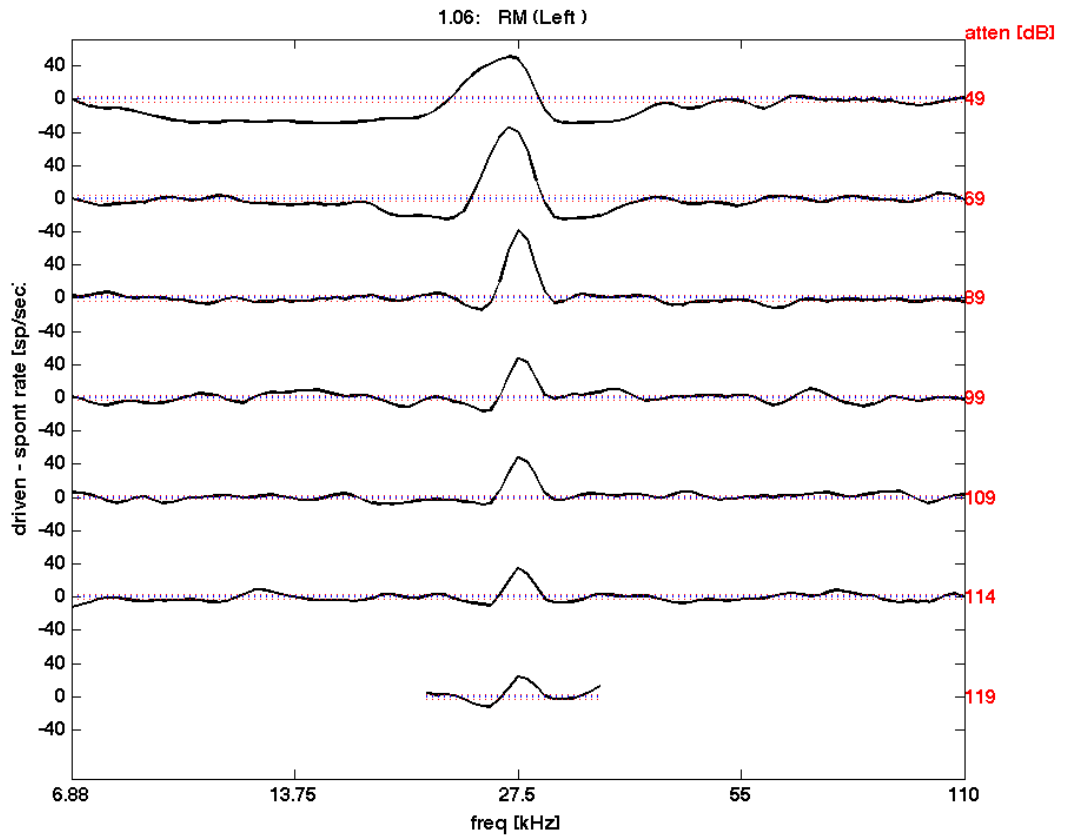
Figure 3.7- Response map for a Type I unit from an unexposed animal.

Type I units are characterized by level tolerant sharp tuning. Type I units often have inhibitory sidebands, and the rate level function for these functions (not shown) are non-monotonic, but driven rates do not decrease below spontaneous.
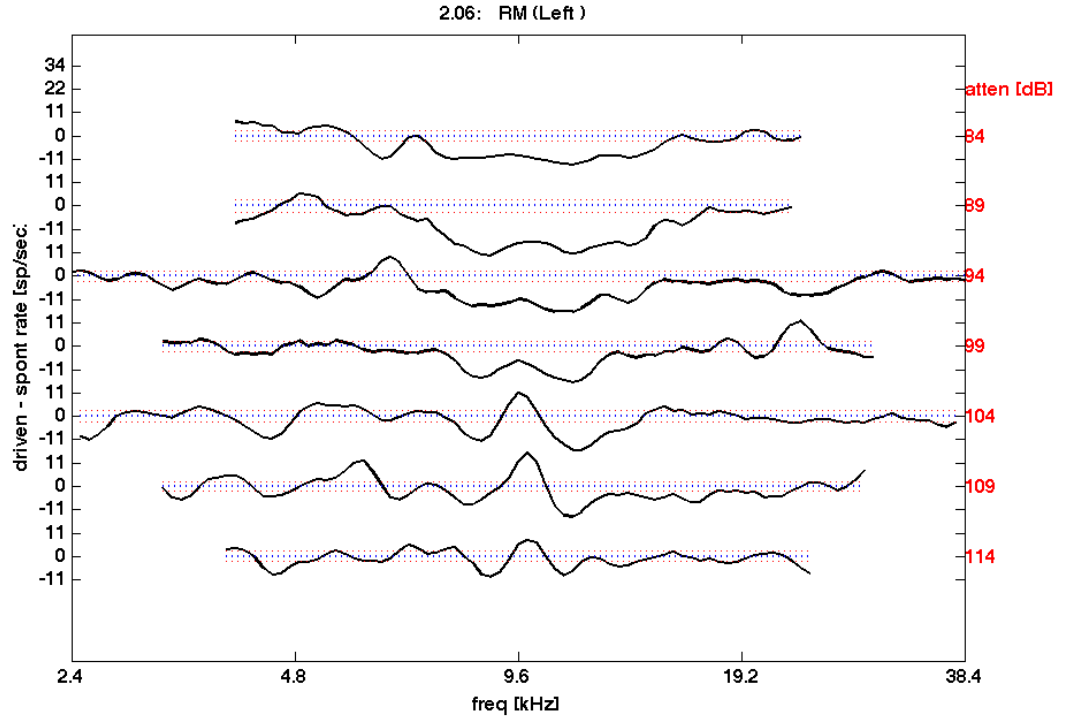
Figure3.8- Response maps for a Type O unit from an unexposed animal.

Type O units are characterized by broad inhibition. This unit has a small region of excitation at the lowest level, but has an increasing region of inhibition as the sound level increases.
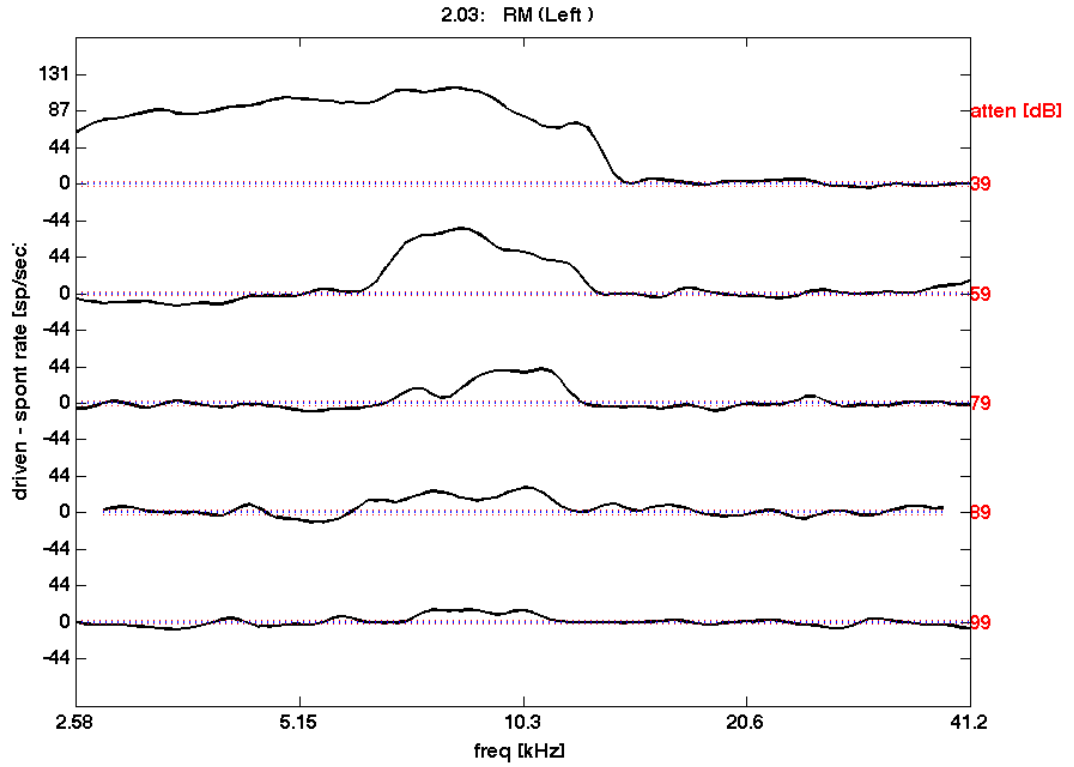
Figure 3.9- Response maps for a Class A tail unit from an exposed animal.

This tail neuron is from a Tinnitus negative animal. Class A tail units are characterized by a broadband left tail response at high levels.
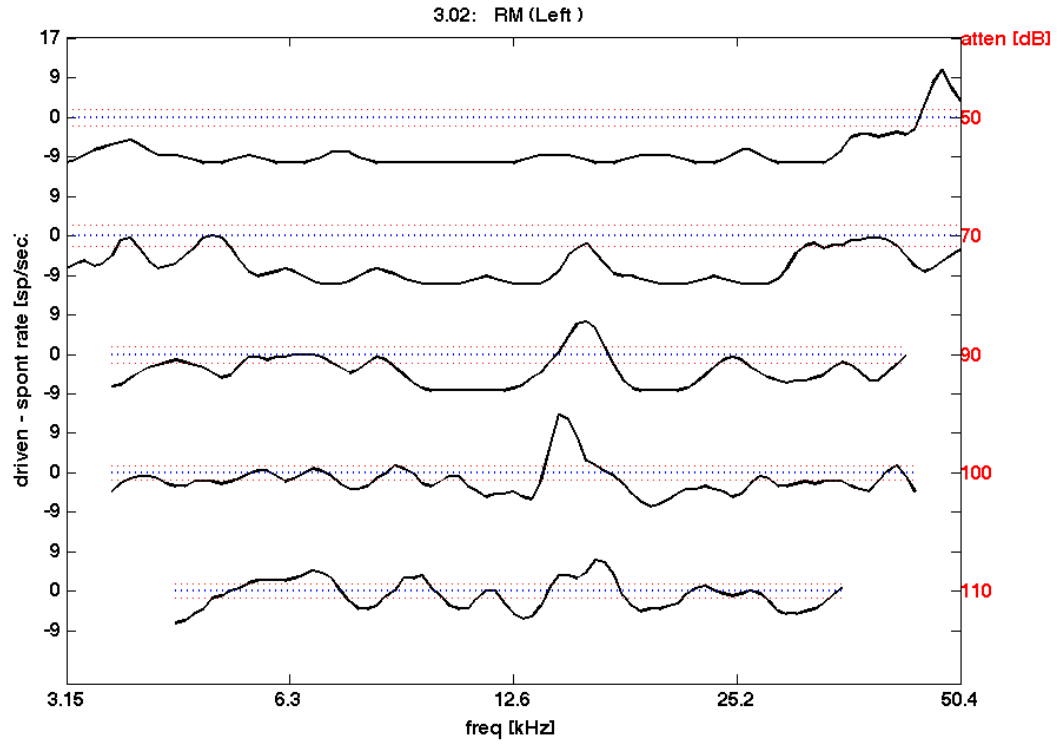
Figure 3.10- Response maps for a class C tail unit from an exposed animal.

This figure shows the RM for a class C tail unit from a Tinnitus negative animal. These units show broadband inhibition at high levels. This class C tail unit showed strong inhibition at lower sound levels, but is completely over come by inhibition as the sound level increases.

Units that are recorded, but have insufficient data to classify their response type or whose response type did not fit one of the types described above were characterized as unclassified.

In the exposed animals, most of the units met the classification criteria as stated above for types V, I, and O. Some units fit the description of tail units as in Ma and Young (2006), and are classified as such (figures 3.9 and 3.10). Mostly these units are class A or class C tail units, meaning strong and broad excitation or inhibition respectively. Note that type class C tail units are similar to type O units, except that the response is broadband.

Changes in prevalence of response types

Table 3.1 shows the counts of all units by type in the unexposed, tinnitus negative and tinnitus positive animals. Table 3.2 condenses this information into excitatory versus inhibitory unit types, where excitatory units are type V units and inhibitory units are type I and type O units. The tail units are also classified as excitatory tail units (A) and inhibitory tail units (C) and incorporated into excitatory or inhibitory unit counts.

In unexposed animals (top part of Table 3.1), low frequency units (BFs less than 13.4kHz) tend to be type V units, which do not show inhibition. Ramachandran et al. (1999) and Oliver (2005) suggest that these neurons were derived from low frequency inputs from the MSO. At medium and high frequencies, inhibition dominates the unit types, so there are proportionally more type I and type O neurons. This suggests narrower tuning, perhaps due to the inhibitory inputs.

	below-edge	edge	above-edge
		unexposed	
V	29	5	21
I	13	23	25
O	7	19	16
unclassified	2	1	5
total # units	51	47	67
		tinnitus negative	
V	13	11	4
I	12	12	14
O	4	10	2
unclassified	2	7	2
Class A tail	2	10	7
Class C tail	0	1	1
total # units	33	51	30
		tinnitus positive	
V	4	12	4
I	10	6	0
O	4	13	2
unclassified	3	6	1
Class A tail	3	6	4
Class C tail	0	1	0
total # units	24	44	11

Table 3.1- Type distribution across animal types.

In unexposed animals, like in cat, type V units are prevalent at low frequencies, type I and type O units are prevalent in the higher frequency range (counts are significantly different, $p \ll 0.01$, chi-squared). In exposed animals there is an increase in the number of type V units, and a loss of type I and type O units in the edge region (counts significantly different, $p < 0.05$, chi squared). In the tinnitus positive animals, though, there is an increase in type V units, and a decrease in type I units(counts significantly different, $p \ll 0.01$, chi squared).. The distribution of units in the above-edge region is not significantly different from the unexposed distribution for either exposed group. However, the tinnitus negative group is statistically different from the tinnitus positive group ($p < 0.05$).

	below edge	edge	above edge
		unexposed	
inhibitory	20 (39.2%)	42 (89.4%)	41 (61.2%)
excitatory	29 (58.9%)	5 (10.6%)	21 (31.3%)
		tinn neg	
inhibitory	16 (48.5%)	23 (45.1%)	17 (56.7%)
excitatory	15 (45.5%)	21 (41.2%)	11 (36.7%)
		tinn pos	
inhibitory	14 (58.3%)	20 (45.5%)	2 (18.2%)
excitatory	7 (29.2%)	18 (40.9%)	8 (72.7%)

Table 3.2- Excitatory vs. inhibitory response types.

Condensing the units types into excitatory and inhibitory response types makes it clear that there is a loss of inhibitory response types in post exposure animals, particularly in the edge frequency region (significant on both edge and above-edge regions, $p<0.05$, chi-squared). The columns don't add to 100% because of the exclusion of unclassified units.

In the acoustic trauma animals (bottom 2/3 of Table 3.1), there is a statistically significant decrease in the fraction of type I neurons in the edge or above-edge frequency region, in favor of type V and type A tail neurons. This means that there are fewer inhibited (I and O) neurons and more excitatory (V and class A tail) units in the frequency region with threshold shift (Table 3.2).

mean thresholds (re nBTC)	unexposed	tinn -ve	tinn +ve
type I	21.5	37.5	36.5
type O	21.6	46.0[*] ($p<0.01$)	36.2
type V	35.9	56.5	39.6
class A tail	-	58.7[*] ($p<<0.01$)	53.2[*] ($p<<0.01$)
class C tail	-	11.6	1.7

Table 3.3- Mean thresholds by unit type in the edge and above edge regions.

Type O units had significantly higher thresholds in tinnitus negative animals. Class A tail units had statistically higher thresholds compared to the unexposed unit type thresholds in both exposed animal groups. Statistical significance of the median thresholds was tested by ranksum across the rows.
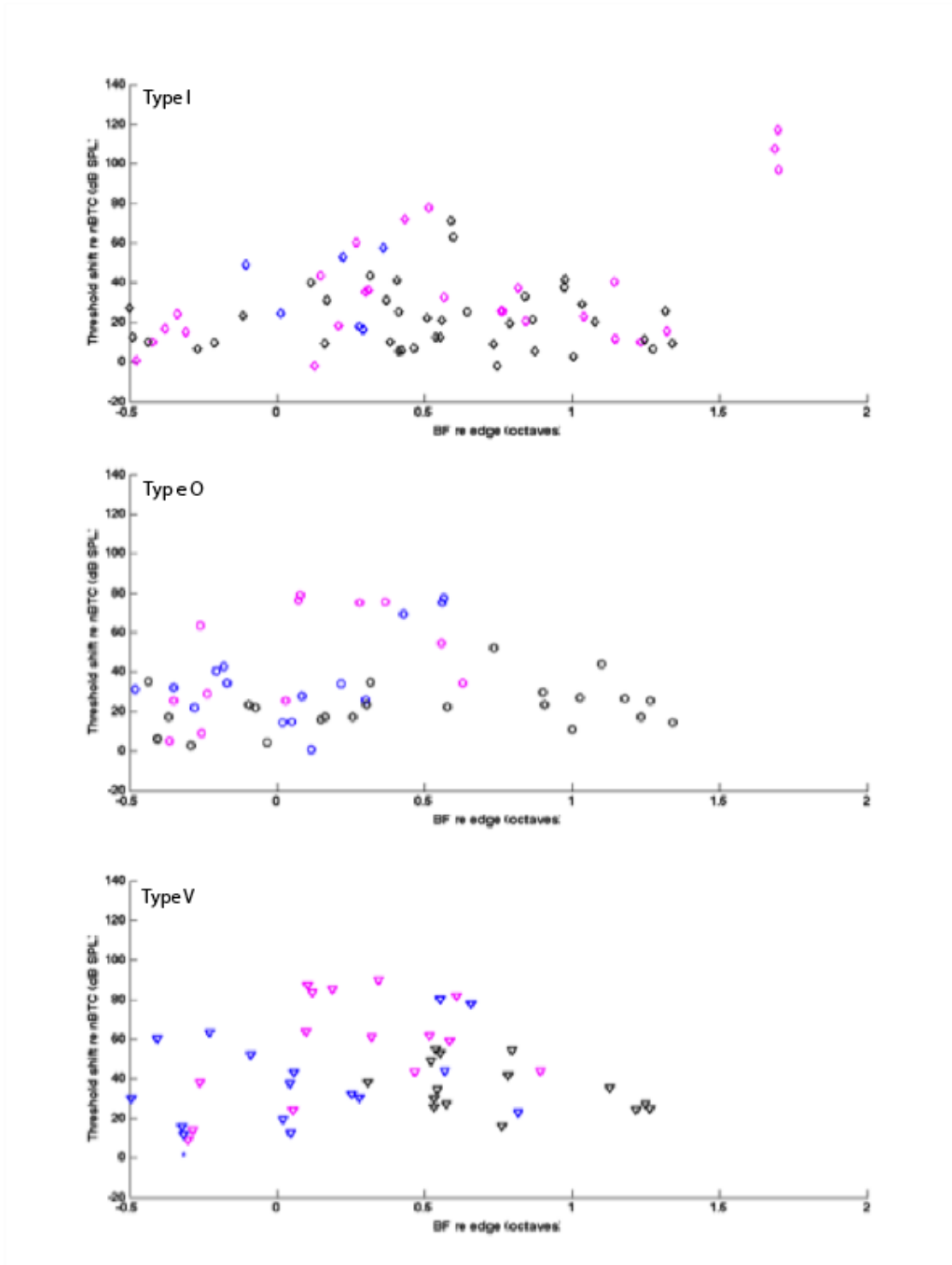
Figure 3.11- Thresholds by unit type.

As in previous figures, black denotes unexposed units, magenta denotes tinnitus negative units and blue denotes tinnitus positive units. A, B and C are Type I, O and V units respectively. The only apparent increase in threshold occurs in type O units.

CHAPTER 3- PHYSIOLOGICAL CHANGES

Threshold shift by Unit Type

An analysis of the degree of threshold shift (re nBTC) by type is shown in Table

3.3. This shows the average threshold by unit type for all units in the edge and above-

edge region. Significance testing was done using ranksums across the rows of the table.
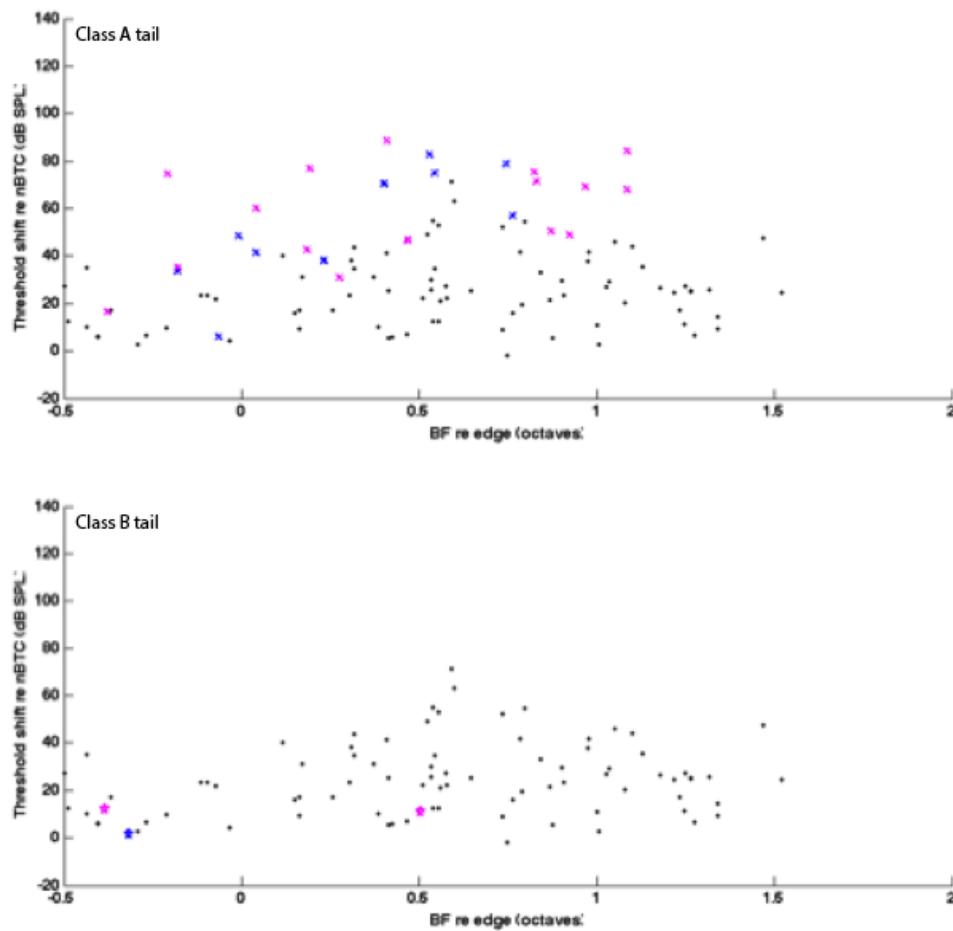
Figure 3.12- Thresholds for tail units.

A and B show thresholds for class A tail and class C tail units respectively. The black dots are the thresholds for all recorded unexposed units. While class A tail units have markedly higher thresholds than the unexposed units, class C tail units have more normal looking threshold values

The only unit type that showed a significant threshold shift from unexposed thresholds was the type O units of tinnitus negative animals. However, it is notable that the thresholds of class A tail units are significantly increased relative to all of the unexposed units, while class C tail units have normal thresholds. Figure 3.11 shows the thresholds of each "normal" unit type for each group of animals. The elevation of type O unit thresholds can be clearly seen in this figure. Figure 11 shows the thresholds of all the normal units types in black, the tail unit types are plotted against these units. This figure shows a distinct increase in threshold in class A tail units, while the small number of class C tail units have normal threshold levels.

In summary, there is a loss of type I units in tinnitus positive animals. This suggests a loss of inhibition and of narrowly tuned neurons.   In both exposed groups, there is a decrease in type I units and an increase in type V units in the edge regions. Only tinnitus positive animals show a decrease in type I units in the above edge region. The magnitude of threshold shift in both groups of exposed animals is about the same in both edge and above-edge regions. While the excitatory tail units (class A) have thresholds significantly higher than unexposed thresholds, inhibitory tail units have thresholds with the normal range of unexposed unit thresholds.

Chapter 4

Spontaneous Activity

The aim of this chapter is to look at the profile of hyperactivity in rat CIC after sound exposure. The animals in this study have been classified as tinnitus positive or tinnitus negative based on the results from a Gap Inhibition based behavioral screening. The units are lined up such that the best frequencies are reported relative to the frequency of the edge of the threshold shift produced by sound exposure. Based on the data shown in the previous chapter (Tables 3.1 and 3.2), neurons generally have higher thresholds in the edge and above-edge frequency regions, especially the abnormal tail neurons. Since the degree of damage to the auditory system is relative to the edge frequency, we investigated possible differences in spontaneous rate in the above-edge region (more than a half octave above the edge) and in the edge region (a half octave below to a half octave above the edge).

Results

Spontaneous Activity

The spontaneous rates (SR) of the three groups of animals were compared relative to the edge frequency. Figure 4.1 shows the spontaneous-rate data plotted with respect to the best frequency relative to the edge frequency (BF re edge). The spontaneous rates were widely distributed in the population and there is no clear separation between groups

in this scatter plot. Since prior studies have looked at differences in the average SR between exposed and normal groups, we looked at the averages of our spontaneous rates (Table 4.1) and saw that there was no significant difference in average SR (averaged over the edge and above-edge regions) between the three animal groups (ANOVA, p=0.14). Tests for the equivalence of distributions also failed to describe a difference between the three animal groups (KS test, p=0.86 unexposed/tinnneg, p= 0.06 unexposed/tinnpos).
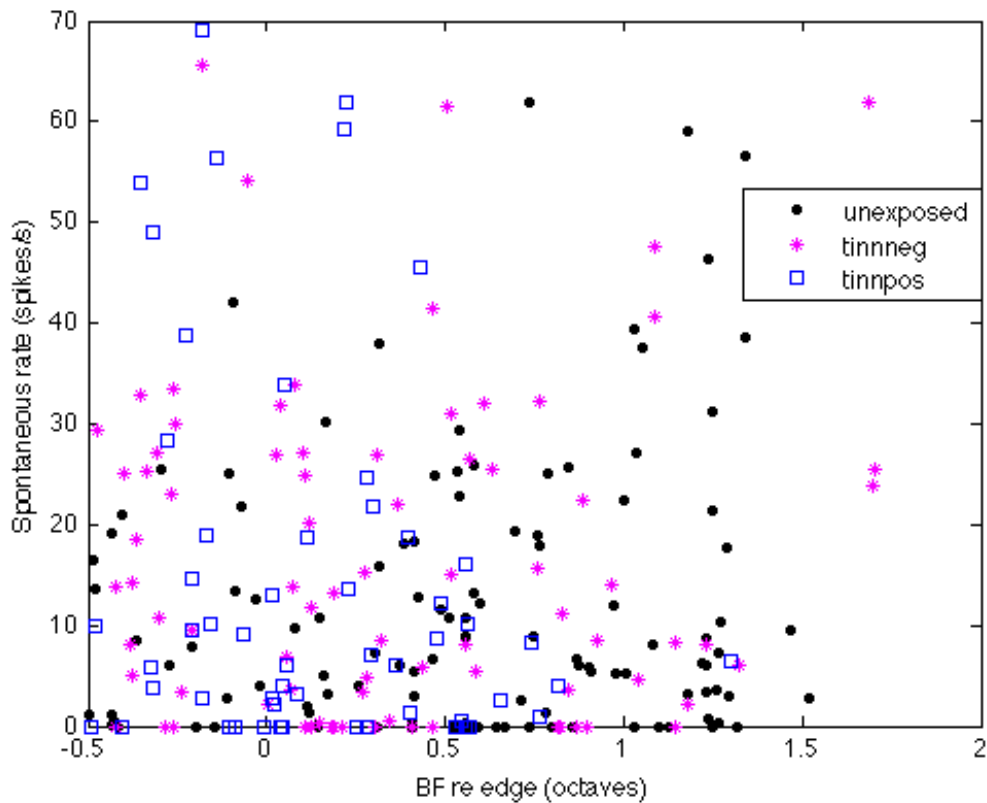
Figure 4.1- Spontaneous rates.

Spontaneous rates of single units collected from unexposed animals (black dots), animals that tested positive for Tinnitus (tinnpos- blue squares) and animals that did not test positive for Tinnitus (tinnneg- magenta stars). The abscissa is the BF with respect to the edge, calculated by taking the logarithm of the unit BF divided by the animal's edge frequency as determined from the ABR. For unexposed animals, the median of the exposed edge frequencies is used (18.931kHz). Note that the spontaneous rates of unexposed single units are as variable as the exposed animal spontaneous rates.

BF re edge>=-0.5 octaves	unexposed	Tinn -ve	Tinn +ve
All units	11.79	16.24	14.43
Type V	3.60	11.18	4.20
Type I	9.44	14.25	18.79
Type O	19.68	21.10	27.08
Tail units	-	18.05	7.77
Unclassified	20.08	20.11	17.48

Table 4.1- Average spontaneous rates by type.

Average spontaneous rates of single units in the edge (BFs between a half octave below and a half octave above the edge frequency) and the above-edge (BFs over a half octave above the edge frequency) regions in unexposed, Tinn –ve and Tinn +ve animals.   There is no significant change in the spontaneous rates across the three animal groups.
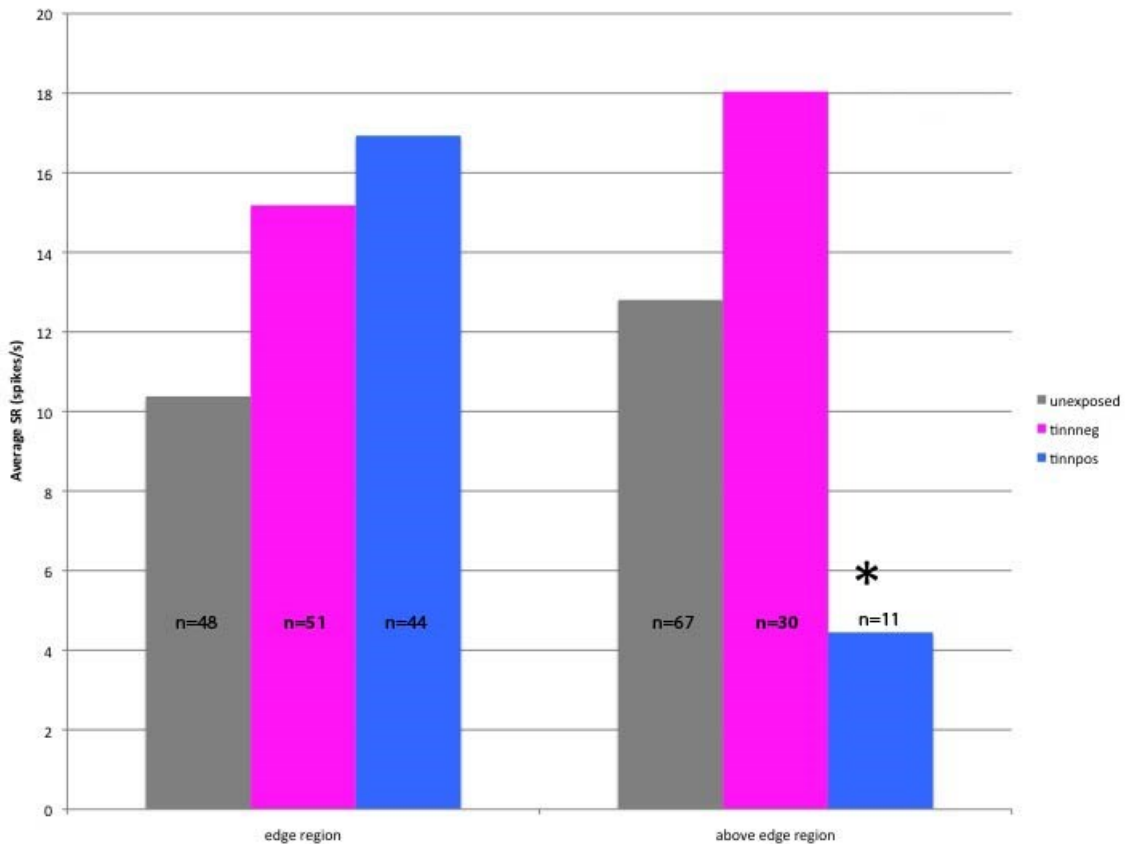
Figure 4.2- Average spontaneous rates for the three animal groups in the edge region and in the above-edge region.

Only the lower SR in the tinnitus-positive animals in the above-edge region is significantly different. This suggests differences in the spontaneous rates in the two edge regions as defined. Single units in the above-edge region have greater threshold shifts and are expected to show signs of damage post-exposure.

However, differences in spontaneous rate were found when the edge and above-edge groups were analyzed separately (figure 4.2). In the edge region, average spontaneous rates are not significantly different (ANOVA, p=0.11), while in the above-edge region, the difference in SR between groups is significant (ANOVA, p<0.05).

Defining Hyperactivity

Average spontaneous rates do not give a complete picture of changes in the spontaneous rate since the distributions are skewed to the right (see figure 4.3). Thus a better way to define hyperactivity is by looking at the cumulative density functions (CDF) of the spontaneous rates (figure 4.4). The CDFs suggest two criteria for hyperactivity: SR>= 1 spike/s and SR>= 19 spikes/s.   One spike/s correlates well with the divergence of the tinnitus negative CDF from the unexposed CDF curve, and 19 spikes/s, correlates well the divergence point of the tinnitus positive CDF from the unexposed CDF curve. These criteria also follow the classification of low (<1 spike/s), medium (1 to 19 spikes/s) and high (>19 spikes/s) spontaneous rate units in the auditory nerve (Liberman, 1978).
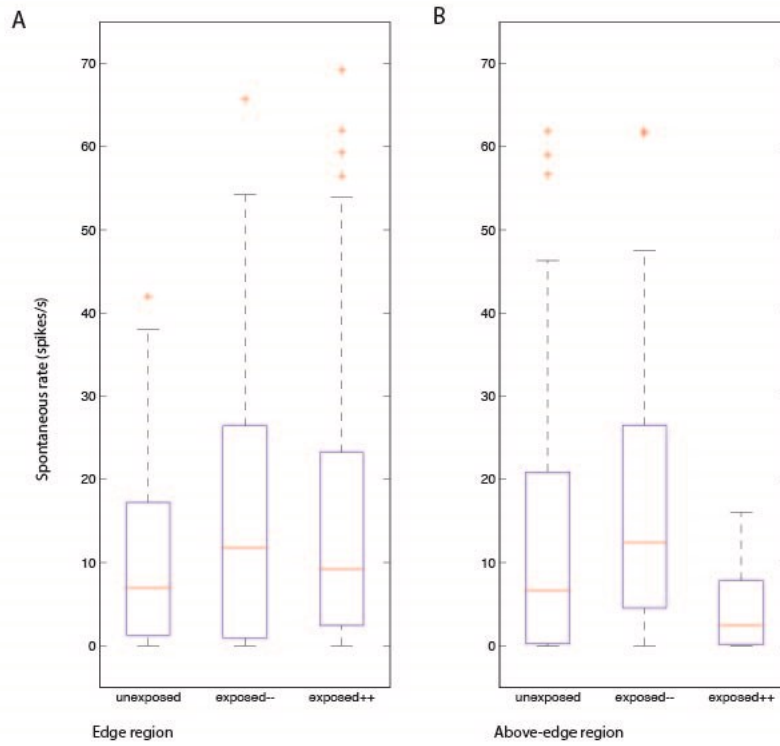
Figure 4.3- Box plots showing the median and quartile distributions of spontaneous rates.
These box plot show the SRs in the three groups of animals in the edge region (A) and in the above-edge region (B). This shows that the average is not a good representation of the data in each group, since the data have a significant tail.
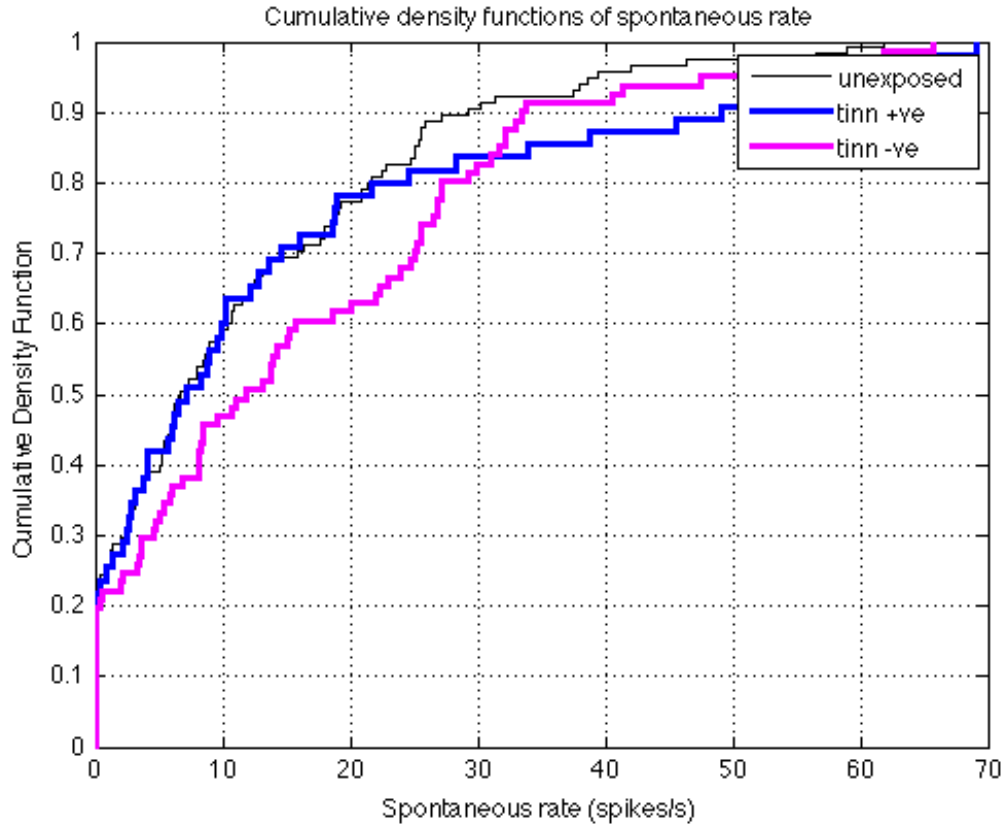
Figure 4.4- The cumulative density functions for the three groups of animals in the edge and above-edge region.

This depicts the probability of a given spontaneous rate or less. Note the immediate shift to the right of the tinnitus-negative curve from the unexposed curve, suggesting hyperactivity in the tinnitus-negative units. The tinnitus-positive curve on the other hand follows the unexposed curves until higher SR values. This suggests looking at the behavior of the spontaneous rates at different rate levels might give insight to the behavior differences between the two exposed groups. Unit totals by animal type: unexposed: N=115; tinnitus negative: N=81; tinnitus positive: N=55.

Figure 4.5 shows the SR data broken into these three spontaneous rate groups.

Note that this suggests a different profile of spontaneous activity in tinnitus positive

versus tinnitus negative animals, and that the profile of spontaneous activity in tinnitus

positive animals is similar to the profile seen in unexposed animals.
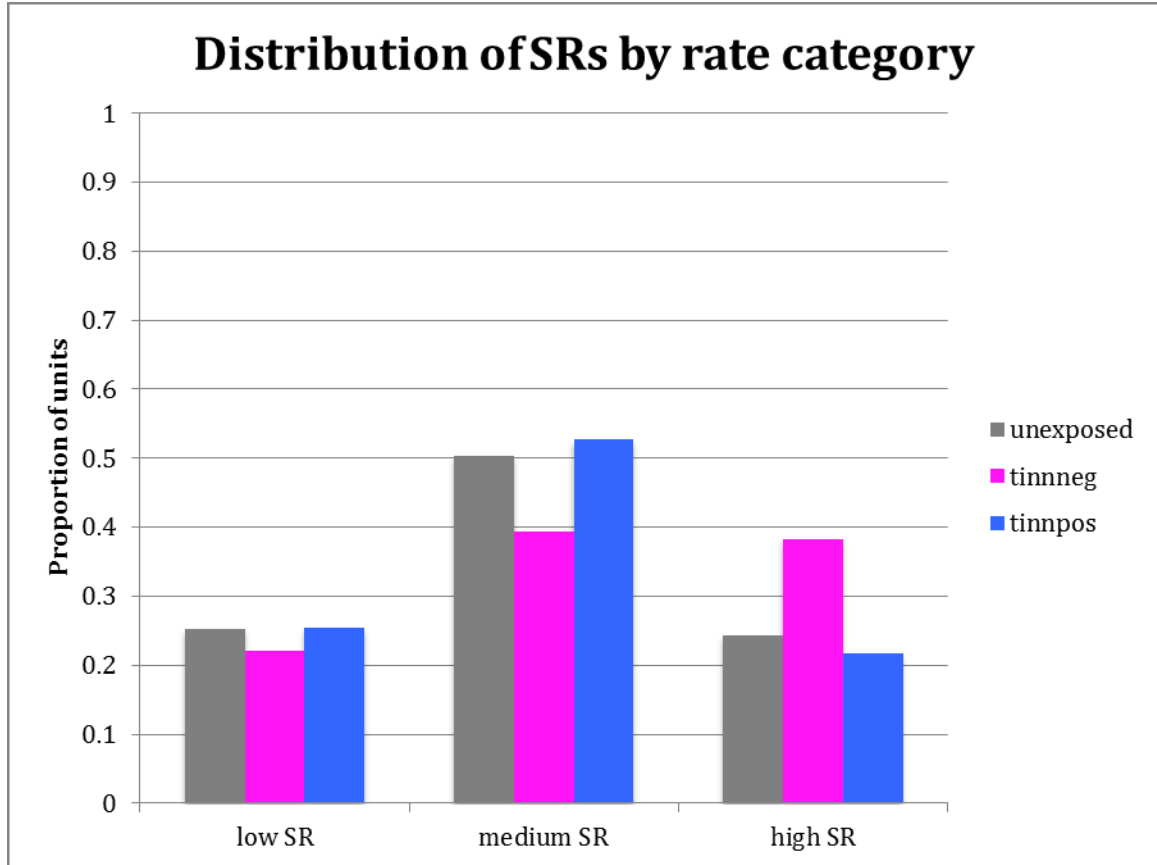
Figure 4.5- Proportions of units in SR class.

Note the differences in the distribution profile across the animal groups- the distribution for unexposed and tinnitus-positive animal groups are uni-modal, while the tinnitus-negative group looks more uniform. Unit totals by animal type- unexposed: n=115; tinnitus negative: n=81; tinnitus positive: n=55

To further explore changes in hyperactivity across the animal groups, we investigated the changes in high spontaneous rate units in the regions about the edge and above the edge. Under the first criterion for hyperactivity (SR>1 spike/s), there is no significant change in the proportion of units in the edge region or in the above-edge region (figure 4.6). Under the second criterion for hyperactivity (SR>19 spikes/s), there are high spontaneous rate units in the tinnitus negative animals in both frequency regions- figure 4.7. However, the profile of the tinnitus positive group is quite different- there is a

significant decrease in the proportion of hyperactive units in the above-edge region in the tinnitus positive group ($p<0.01$), while the edge region is not significantly different from the unexposed group.
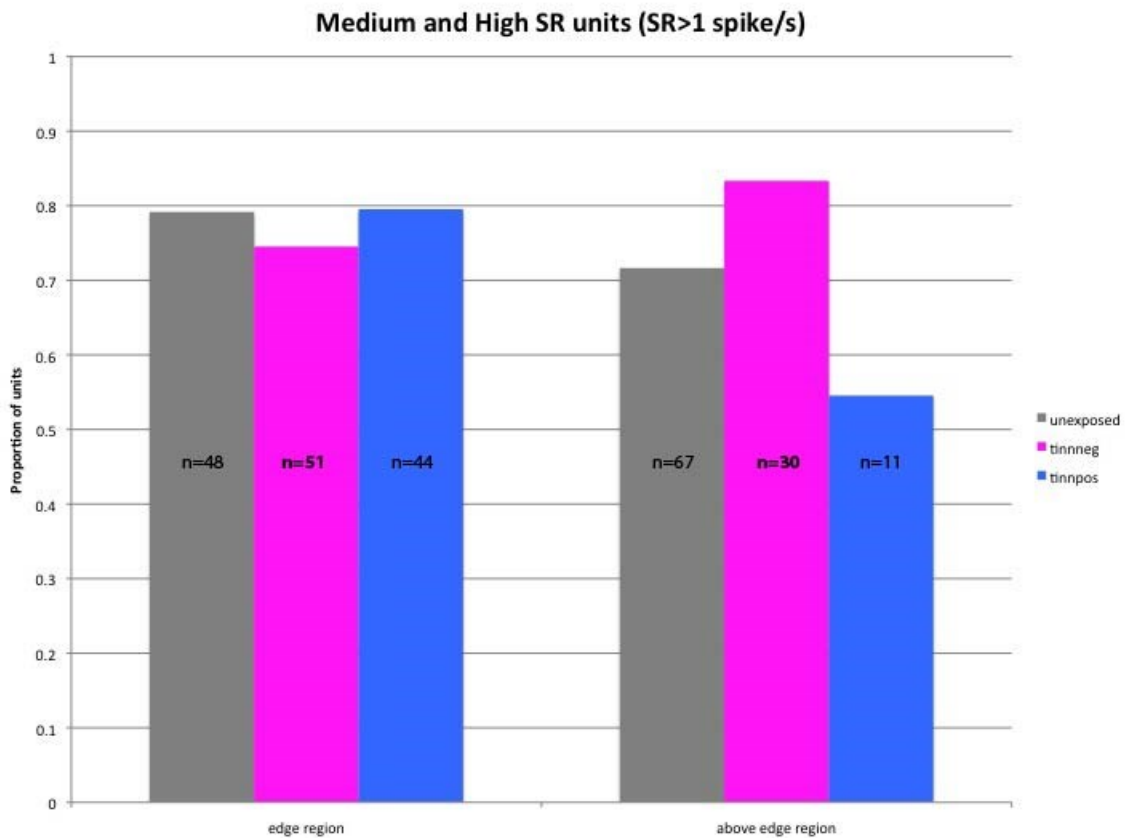
Figure 4.6- Hyperactivity (criterion 1: SR>1 spike/s).

This figure shows the proportion of units in animal group satisfying the hyperactivity criterion in the edge region (A) and in the above-edge region (B). There is no significant change across the animal groups: edge region (Chi-squared, $p=0.8$), above-edge region (Chi-squared, $p=0.16$).
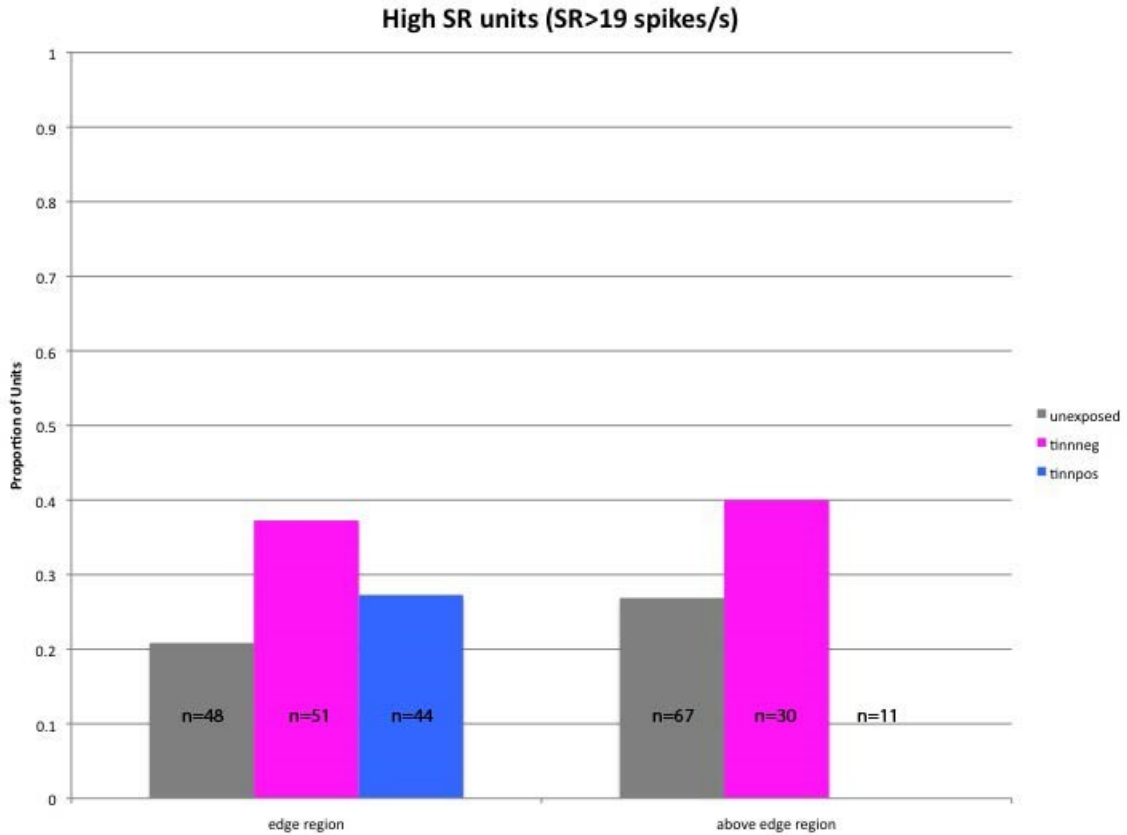
Figure 4.7- Hyperactivity (criterion 2: SR>19 spikes/s).

This figure shows the proportion of units in animal group satisfying the hyperactivity criterion in the edge region (A) and in the above-edge region (B). There is no significant change across the animal groups in the edge region (Chi-squared, p=0.21), but there is a significant change in the above-edge region (Chi-squared, p<0.05).

Therefore, noise exposure causes an increase in the proportion of high SR units. In tinnitus negative animals, this increase is broadband- it is prevalent both in the edge region and the above-edge region. In tinnitus positive animals, there is an increase in high SR units the edge region, and a significant decrease in high SR units in the above-edge region.   Perhaps this narrowband, or edge effect, hyperactivity can explain a tinnitus percept.

Identification of Hyperactive Unit Types

To further explore hyperactivity (defined as an increase in the proportion of high spontaneous rate neurons) in the CIC, we looked at the spontaneous rates of the units based on their physiological type. The physiological type classification is detailed in Chapter 2, but includes Type V, I, O and unclassified units in all animal groups, as well as tail units in the exposed animal groups. Table 4.2 shows the number of units in each type class by frequency location (re edge).

	unexposed		tinnitus negative		tinnitus positive	
	Edge	Above	Edge	Above	Edge	Above
type V	5	21	11	4	12	4
type I	23	25	12	14	6	0
type O	19	16	10	2	13	2
unclassified	1	5	7	2	6	1
class A tail	-	-	10	7	6	4
class B tail	-	-	-	-	-	-
class C tail	-	-	1	1	1	0

Table 4.2- Unit counts by type in the edge region and in the above-edge region

Type V units usually have lower spontaneous rates in unexposed animals, compared to inhibitory type units (figure 8A). By setting the criteria for hyperactivity as before, we see an increase in high SR units in both the edge and above-edge regions in both tinnitus positive and tinnitus negative animals at the lower criterion. At the second criterion level, while there is an increase in the proportion of high SR units in the tinnitus negative animals, there are no hyperactive excitatory units in the above-edge region in the tinnitus positive animals.
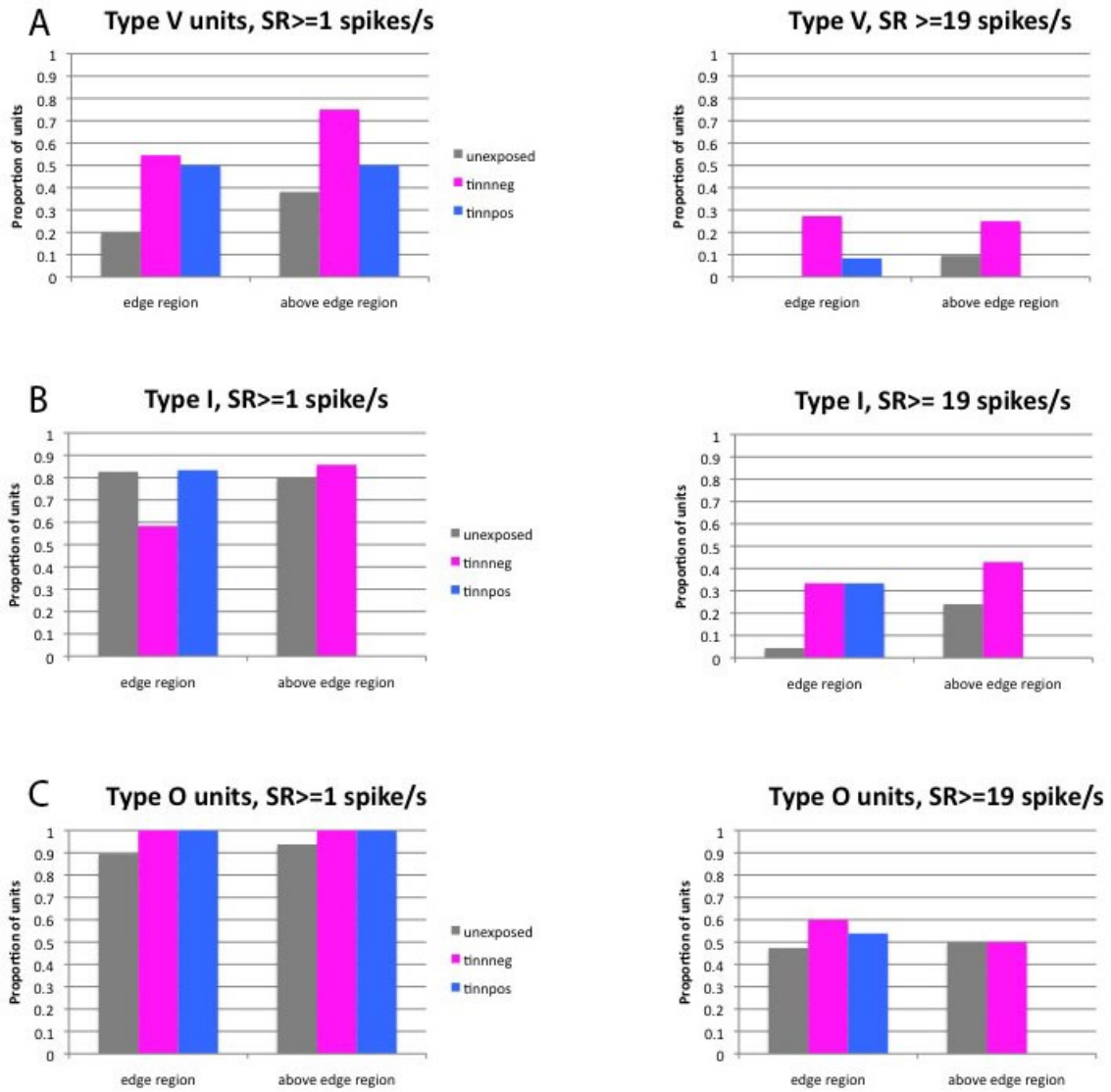
Figure 4.8- Hyperactivity by unit type (unexposed unit types).

This figure shows the levels of hyperactivity in the edge and above-edge regions in the three animal groups according to the 1 and 19 spikes/s hyperactivity criteria. The bars represent the portion of the units (per animal group) in the frequency region that satisfies the hyperactivity criterion. For example, 20% of the type V units in the edge region of normal animals have SRs greater than 1 spike/s, 80% of the unexposed Type V units in this frequency region have SR<1 spike/s. The type V and Type I unit proportions reflect the overall proportions seen in figures 4.6 and 4.7. Note that there is a significant increase in the proportion of hyperactive Type I units in the edge region in both exposed animal groups. The Type O units show no significant change in the proportion of hyperactive units.

Type I units show similar trends in hyperactivity as type V units (see figure 4.8B). Type I units have higher spontaneous rates than type V units in unexposed animals. But, like type V units, there is an increase in hyperactivity in the edge region of both tinnitus positive and tinnitus negative animals, and hyperactivity in the above-edge region in tinnitus negative animals. This is a significant increase (p<0.05) in proportion in the exposed animals. There is insufficient data to assess the effect on tinnitus positive animals in the above-edge region.

Type O units show no change in hyperactive units across the animal groups at both SR criterion levels (figure 4.8C). It is clear from this figure that the type O units are the high SR units in unexposed animals. After sound exposure, the other unit types, which have low spontaneous rates in unexposed animals, become hyperactive.
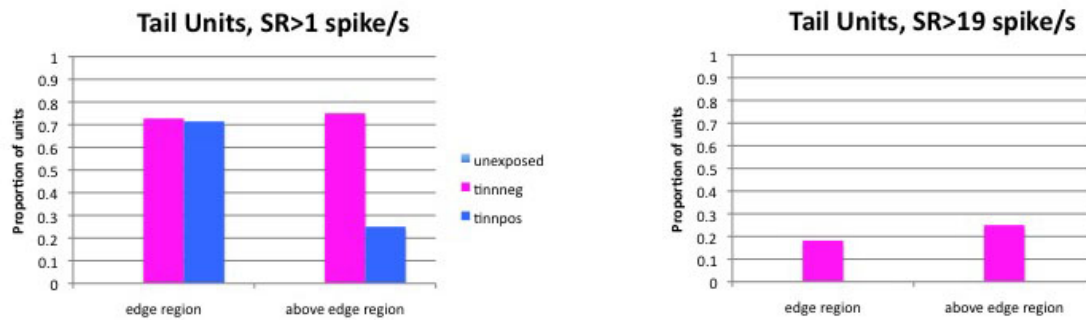

Figure 4.9- Tail units in the exposed animal groups are hyperactive.

These units reflect the overall hyperactive behavior observed- i.e., broadband hyperactivity in the tinnitus negative animals and a narrowband hyperactivity in the edge region, flanked by hypoactivity in the above-edge region.

The tail units (figure 4.9) in tinnitus negative animals are more hyperactive than those in tinnitus positive animals. There are no tail units in the tinnitus positive animals that fit the higher SR criteria. The tail units in the tinnitus positive animals are hyperactive in both frequency regions.   Interestingly, while tail units seem to suggest some physiological change within the nucleus since their responses are abnormally broadband excitatory or inhibitory, they do not have abnormally high spontaneous rates. Combining the class A tail units (excitatory tails) with type V units and the class C tail units (inhibitory tail units) with the type O units does not change the distributions of these unit types.

Thus hyperactivity in the CIC seems to be mediated by a change in the spontaneous rates of Type V, Type I and tail units, and not by Type O units.   Type O units have direct projections from the DCN, so this correlates with Brozoski's (2012) finding that the DCN was not necessary for the expression of tinnitus. The rise in hyperactivity in type V points to disinhibition as a possible consequence of noise exposure. The edge effect appears in these units in tinnitus animals, possibly suggesting that frequency specific changes in these units may be the root of tinnitus perception.

Ipsilateral Spontaneous Activity

Thus far we have described hyperactivity in the CIC contralateral to the sound exposed ear. Some data was collected from the CIC ipsilateral to the exposed ear. Here we will summarize this data. Acoustic stimulation in these recordings, as with all the

other data previously reported, is contralateral to the CIC being recorded from. There were 59 units in total recorded from the protected side in 7 exposed animals. Six of these animals were tinnitus negative (N=46 units recorded), one was tinnitus positive (N=13 units recorded). The edge frequency for each animal was set as the edge frequency from the exposed ear. Of the 46 units in the tinnitus negative animals, nine were in the edge region, 24 in the above-edge region. Of the 13 units recorded in tinnitus positive animals, 11 were in the edge region and one was in the above-edge region. The physiological thresholds of the units are significantly higher in these units compared to unexposed-ear physiological thresholds ($p \ll 0.01$, Ranksum). The median single unit threshold for the protected ear units was 40.33, compared to 17.65 in the unexposed units. However, average spontaneous rates are not significantly different from average unexposed spontaneous rates ($p=0.32$, Ranksum).

Figures 4.10 and 4.11 show the proportion of hyperactive units in the CIC iplilateral to the exposed ear at the two criterion levels for hyperactivity described earlier in the chapter. There is no significant change in the proportion of hyperactive units in tinnitus-negative animals compared to unexposed animals. Because only one neuron was recorded above the edge in the exposed and tinnitus-positive animals, there is no comparison bar for this case.

Figure 4.10- Hyperactivity, criterion 1, protected ear data.

Hyperactivity criterion 1 (SR>1 spike/s) for unexposed units and protected ear units in tinnitus negative and tinnitus positive animals. There is no significant change in the proportion of hyperactive units.
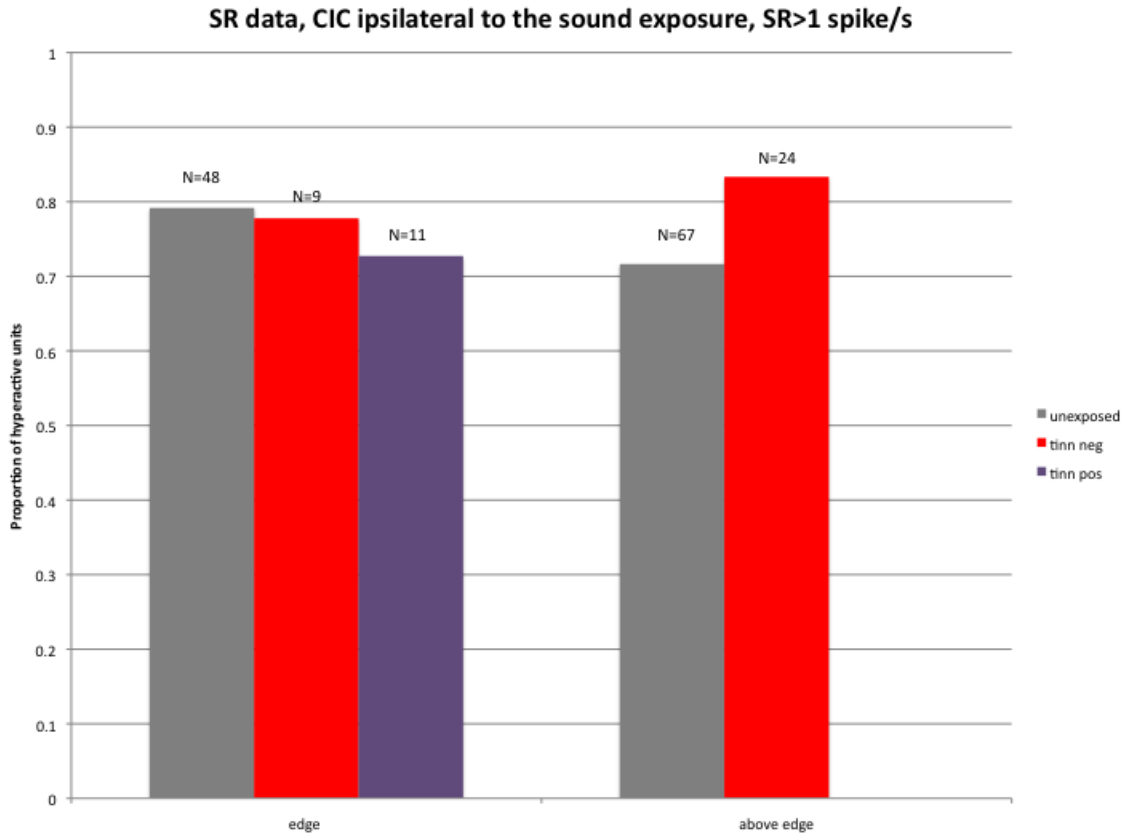
Figure 4.11- Hyperactivity, criterion 2, protected ear data.

Hyperactivity criterion 2 (>=19 spikes/s) for unexposed units and protected ear units in tinnitus negative and tinnitus positive animals. There is no significant change in the proportion of hyperactive units.

Chapter 5

Variability analysis

This chapter describes the regularity of neuronal spiking in single units in normal versus sound exposed rats, suggesting the possibility of changes in spike train dynamics as a possible neural code for tinnitus. This continues some of the work presented in Ben Letham's Masters Thesis (2007), which hypothesized an anomaly in the spontaneous activity of tinnitus positive animals as an attribute of tinnitus, rather than a change in the spontaneous rate itself. Here we use standard statistical methods for looking at regularity in spike timing and spike count (Gabbiani and Koch, 1996). We demonstrate that neurons in the inferior colliculus display the same long-range dependence behavior seen in the auditory nerve, and characterize changes in regularity across the three groups of animals.

Results

For the spike timing analysis, we use the coefficient of variation of the interspike intervals of the spontaneous recordings. For the spike rate analysis we use the Fano Factor to assess changes in spike rate statistics. These analyses are performed on units with spontaneous rates over one spike per second, since lower rate neurons yield unreliable statistics within the time limitation of the spontaneous recordings (a 10-minute interval). For a further analysis of regularity, the hyperactivity parameters established in

the previous chapter are used to look at the regularity of hyperactive units in three animal groups.

Spike Timing

While regularity of spontaneous rates of CIC neurons has not been reported previously, Rees et. al. (1997) report regularity in guinea pig CIC neurons in response to stimulation to tones 15-25 dB above the units threshold. The classification scheme used in that paper established highly regular neurons as ones with a CV less than 0.35, and regular units as neurons with a CV less than 0.5. Table 5.1 summarizes the values of CV measured in unexposed, tinnitus negative and tinnitus positive animals following these parameter guidelines. While there is an increase in the number of regular units in tinnitus positive animals compared to unexposed or tinnitus negative animals, this change is not significant. Note that there are no units with highly regular spontaneous rates in any of the animal groups studied here.

	CV<=0.35	0.35<CV<=0.5	0.5<CV<=1	CV>1	total # of units
unexposed	-	1 (1.2%)	64 (75.3%)	20 (23.5%)	85
tinnneg	-	1 (1.7%)	40 (66.7%)	19 (31.6%)	60
tinnpos	-	3 (7.5%)	23 (57.5%)	14 (35.0%)	40

Table 5.1- This chart emulates the driven rate CIC CV values presented in Rees et al. (1997).

In response to low sound level stimulation (15-25 dB above threshold), driven rates achieve CV values below 0.35. Neither unexposed nor exposed units have comparable spike timing regularity as those seen in driven responses. The distribution of CV values is not significantly different across the animal groups (chi-squared, p=0.12).

Figure 5.1 shows the standard deviation vs. the mean interspike intervals as measured from the spontaneous recordings of single units in unexposed animals. CV values of 1 and 0.5 are plotted with the data to illustrate bounds on the CV values. This

figure is plotted on a log-log scale in order to inspect all of the data points clearly on a single diagram. Note that the CV value increases with mean ISI.
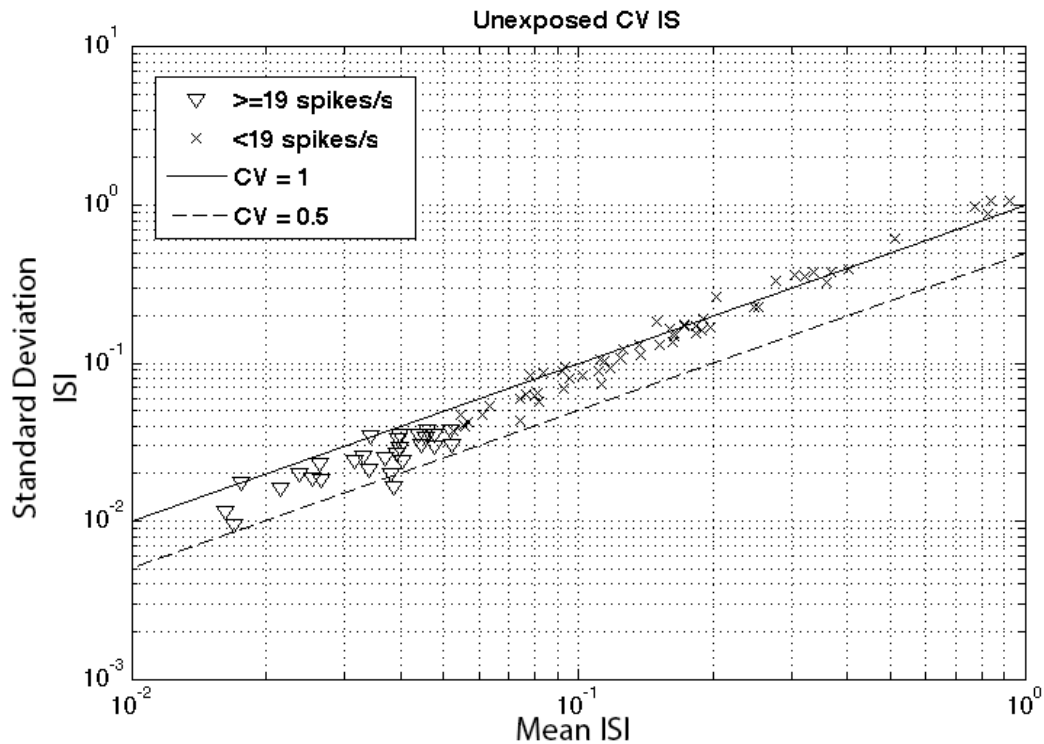
Figure 5.1- Standard deviation vs. Mean interspike interval values for all unexposed data points.

This graph is plotted on log-log axes so that the behavior of all of the data can be visualized. The medium SR data lies on the upper right, the high SR data is on the lower left. It is evident from this plot that there are two types of CV behavior in the dataset- medium SR units (x) tend to have CVs close to 1 (solid line), while high SR units (triangle) have lower CV values between 0.5 (dashed line) and 1.

Figures 5.2A through D show a similar presentation of the tinnitus negative and tinnitus positive data, the gray data points in the background are the unexposed data from figure 5.1. From these figures it is clear that there are two trends in the CV data- at low spontaneous rates (5.1; 5.2A and C), the CV tends to hover about 1, while at higher

spontaneous rates (5.1; 5.2B and D), the CV varies between 0.5 and 1. The CVs from

normal and from exposed animals seem to follow the same pattern.
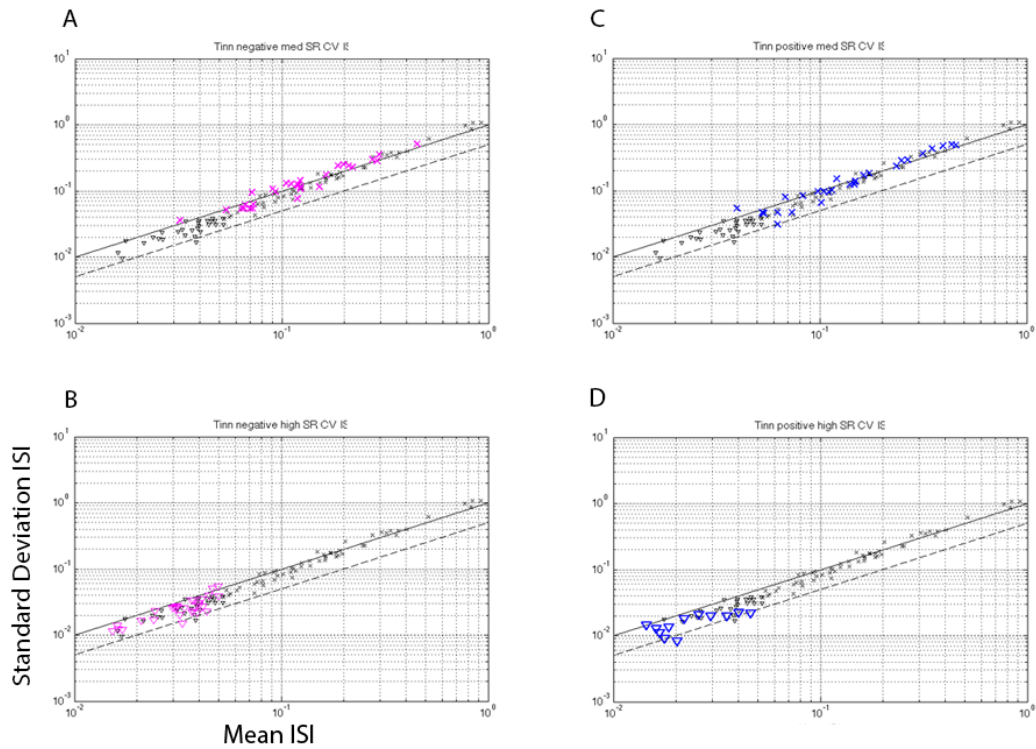
Figure 5.2- Standard deviation vs. mean interspike interval.

Standard deviation vs. mean ISI plots for tinnitus negative (A and B, magenta) and tinnitus positive (C and D, blue) units; colored symbols are data from sound exposed animals, shown superimposed on the data from Fig. 1 for unexposed animals. Again, the medium SR units (A and C) have values close to 1, while the medium SR units (B and D) have lower CV values. The CV values of the tinnitus positive animals (D) tend closer to the CV = 0.5 line that in the unexposed or tinnitus negative units.

Figure 5.3 shows the data at two SR levels, in keeping with the medium (<19 spikes/s) and high SR (>=19 spikes/s) distinctions defined in the previous chapter. Figure 5.3A and B are unexposed-animal data for medium and high SR units respectively. An estimate of the regularity of the group of data points is made using the slope of the line fitting the data points. Fitting the unexposed data points with a linear regression, we find that the slope for these points is 1.21 ($r^2 = 0.992$) for medium SR units and 0.65 ($r^2 = 0.836$) for high SR units. Similarly, for tinnitus negative medium SR units, the slope is 1.16 ($r^2 = 0.969$) and 0.87 ($r^2 = 0.811$) for high SR units (figures 5.4A and B). Finally, for tinnitus positive medium SR units, the slope is 1.19 ($r^2 = 0.991$) and 0.39 ($r^2 = 0.799$) for high SR units (figures 5.5A and B). The goodness of fit is comparable across the animal groups within SR group.

A bootstrap analysis is used to test for significant differences in the slopes of high SR units between the three groups of animals. The samples within each animal group are bootstrapped and the linear regression is calculated 500 times. The resulting histograms are shown in figure 5.6A-C. The values are significantly different ($p<<0.01$, ANOVA).

While the medium SR units are Poisson-like (randomly firing) spike trains, the high SR units in the tinnitus positive animals fire more regularly timed spikes than unexposed units, and tinnitus negative high SR units have more irregular spike timing.
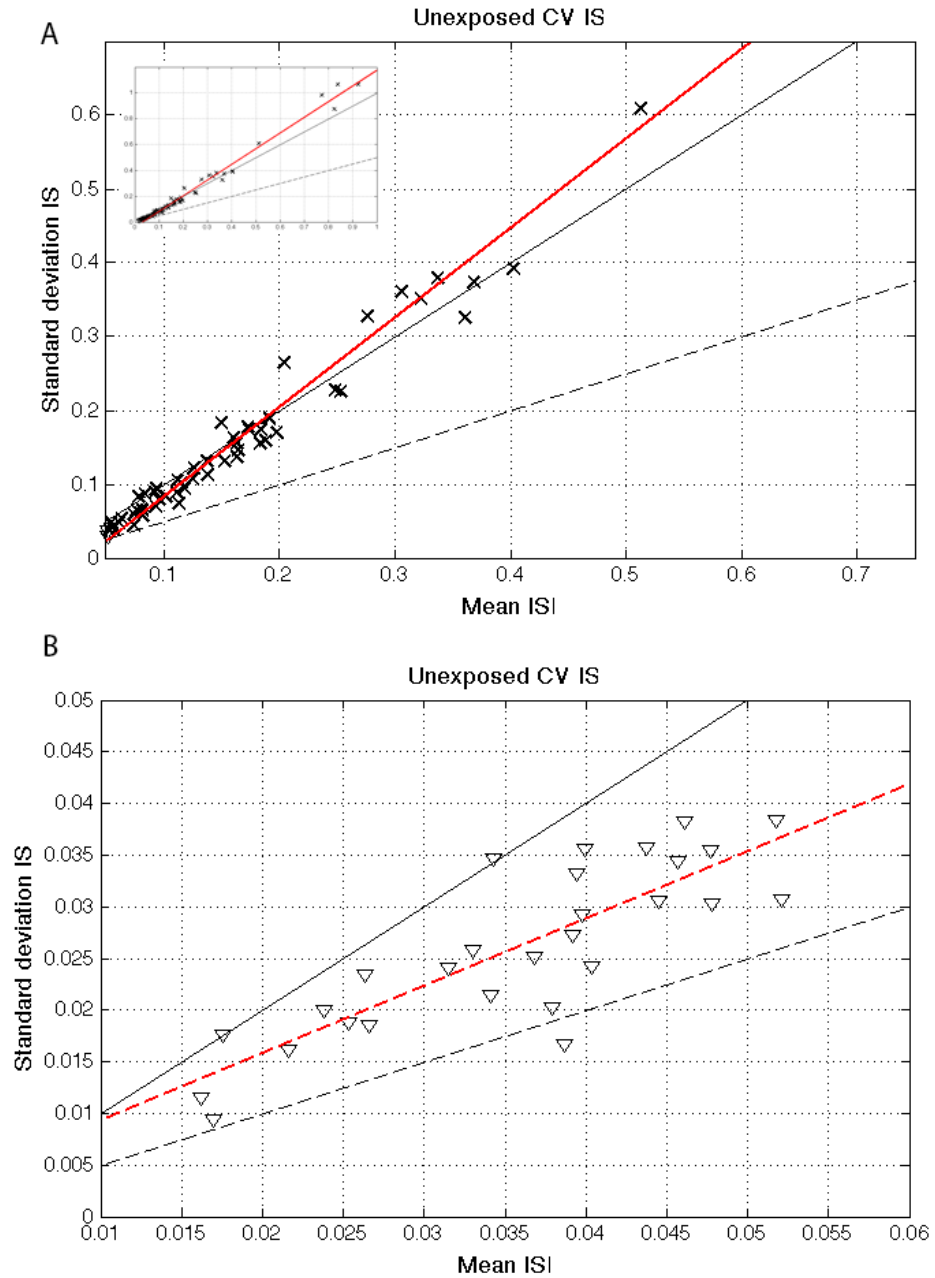
Figure 5.3- Linear regression estimates of regularity.

A and B show the same data represented in figure 5.1, here on a linear plot and zoomed in to see the behavior or each group of points. (A) shows a zoomed in picture of the medium SR units, the full range of medium ISI values is shown in the inset. The linear regression in A yields a slope of 1.21 (r = 0.996). Fitting the medium SR units (B) yields a CV of 0.65 (r = 0.91).
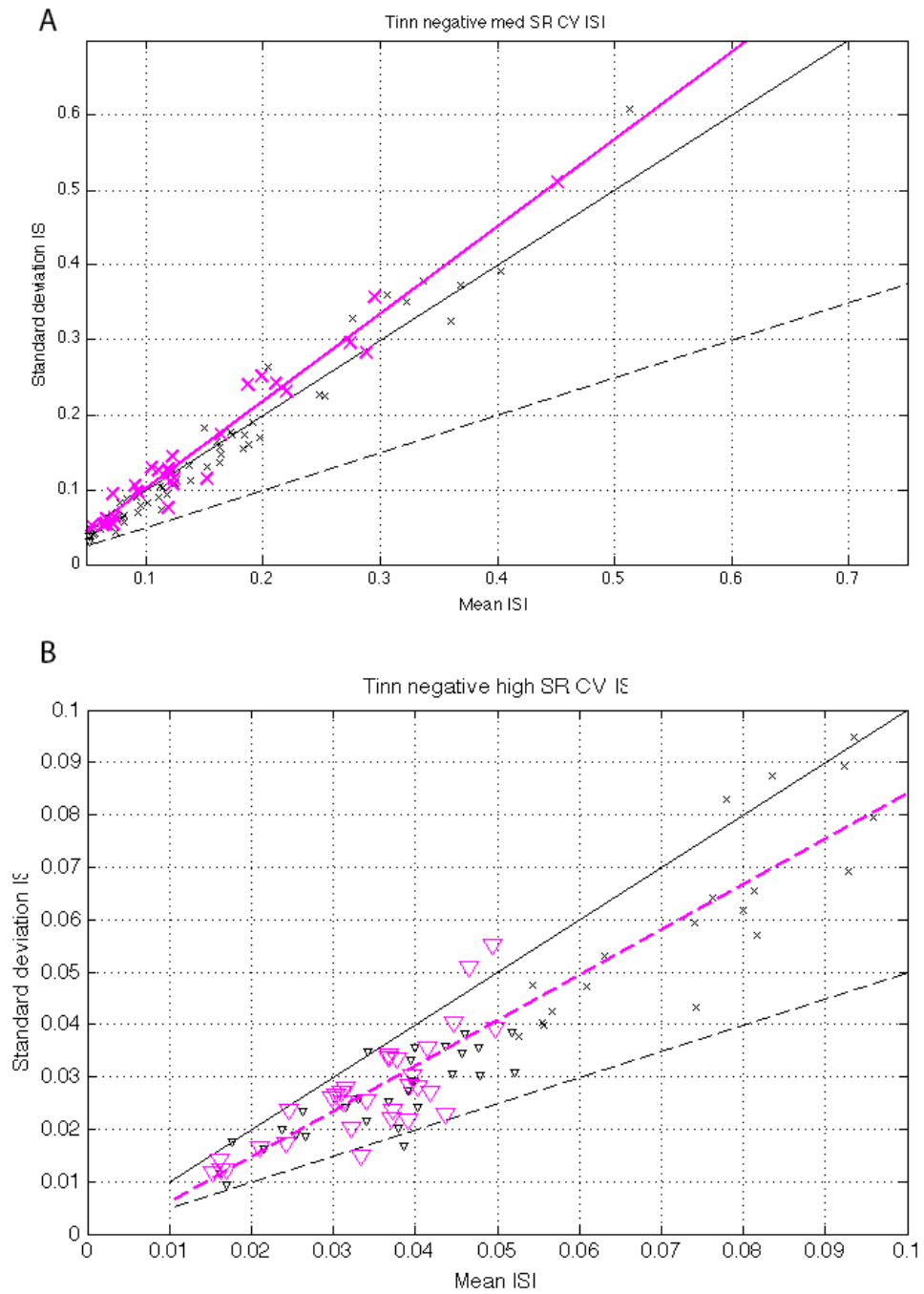
Fig

ure 5.4- Linear regression estimates of regularity, tinnitus negative units.

A and B show the same data represented in figures 5.2A and B, here on a linear plot and zoomed in to see the behavior or each group of points. A shows a zoomed in picture of the medium SR units. The linear regression in A yields a slope of 1.16 (r = 0.98). Fitting the medium SR units (B) yields a CV of 0.87 (r = 0.90).
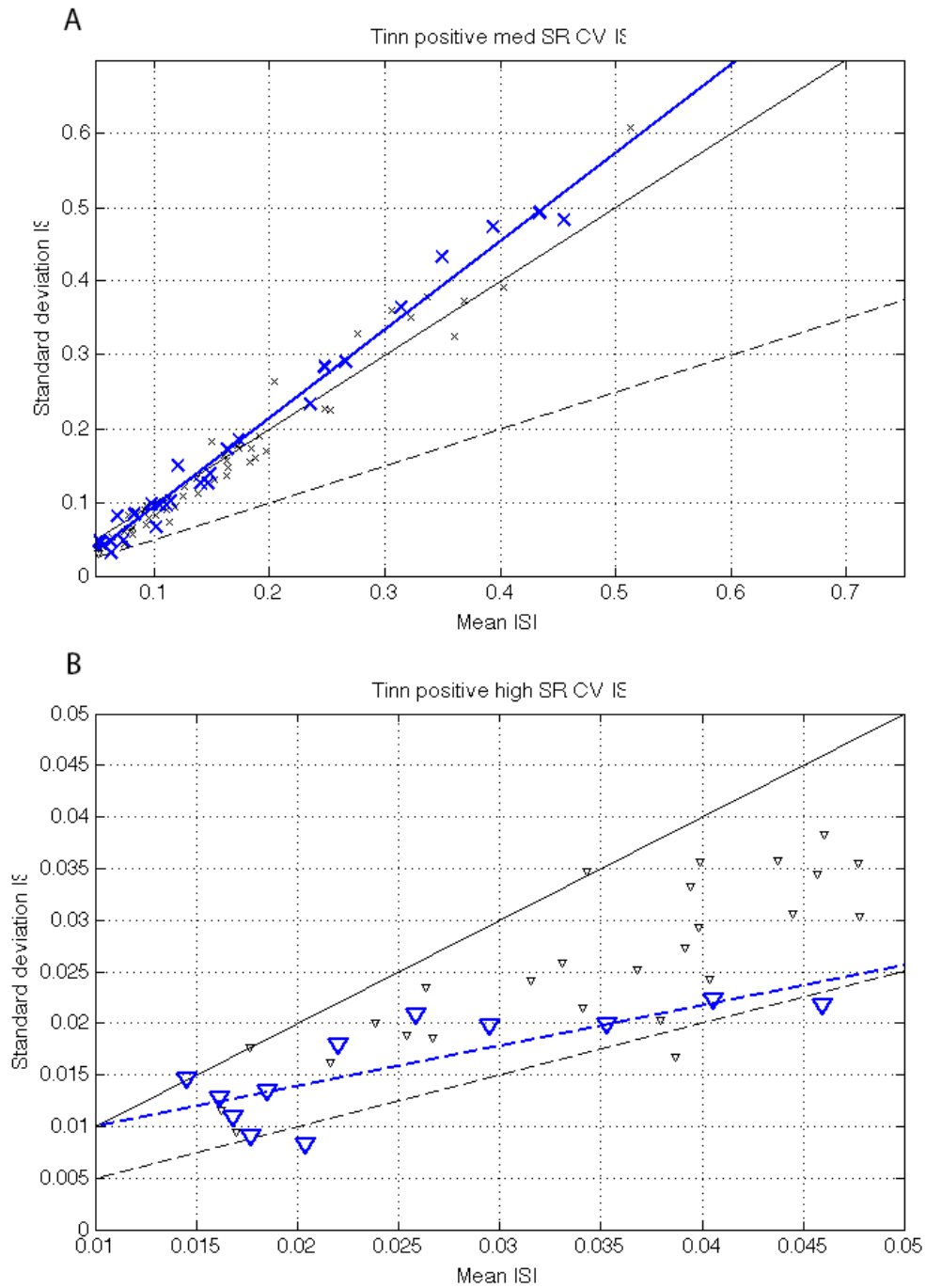
Figure 5.5- Linear regression estimates of regularity, tinnitus positive units.

A and B show the same data represented in figures 5.2C and D, here on a linear plot and zoomed in to see the behavior or each group of points. A shows a zoomed in picture of the medium SR units. The linear regression in A yields a slope of 1.19 (r = 0.99). Fitting the medium SR units (B) yields a CV of 0.39 (r = 0.89).
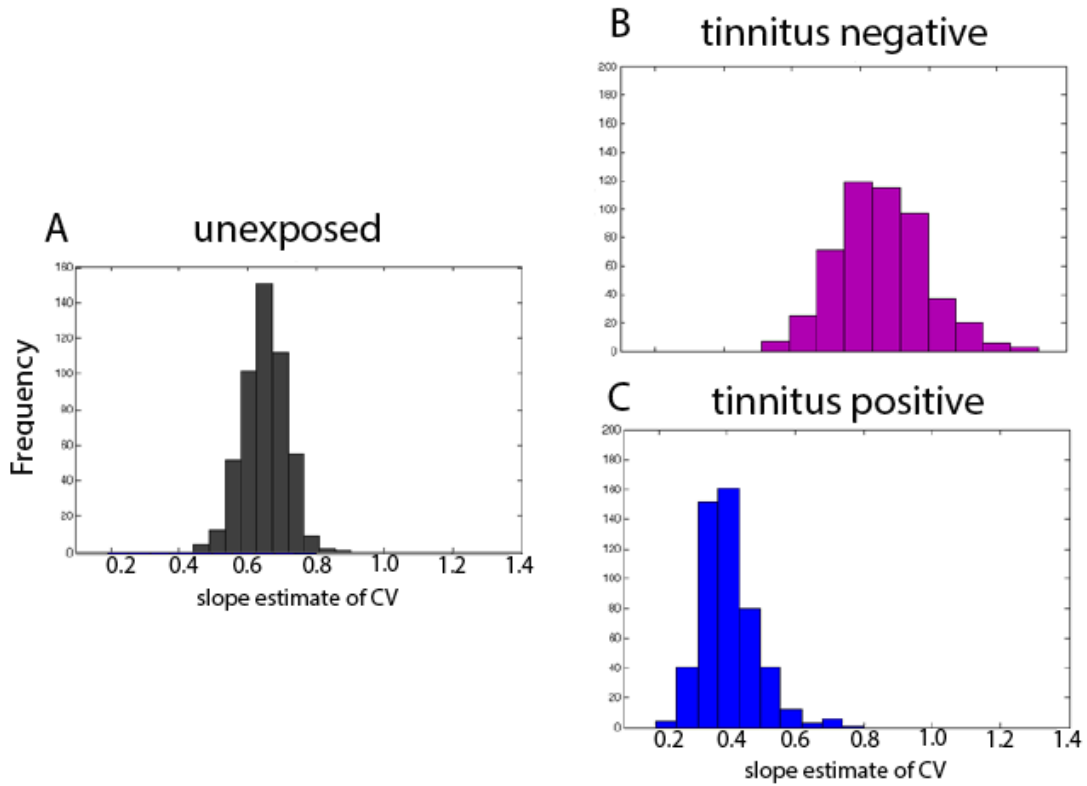
Figure 5.6- Statistical analysis of linear regression regularity analysis.

Bootstrap statistics showing the estimates of the linear fit of regularity in high SR units, with 500 repetitions of the bootstrapping technique to look at statistical significance of the fits. The tinnitus negative values are significantly lower than the unexposed values, the tinnitus negative values are statistically higher than the unexposed values.

Spike Rate

Teich and Khanna (1985) present a spike count analysis of auditory-nerve fibers in cats, comparing the spike count distributions to some of the classical models for neuron spiking. Teich et al. (1990) describe the Fano factor function for the spontaneous spike train of an auditory nerve fiber. The characteristics of this function are as follows:

1. the curve starts with Poisson process like behavior for small counting windows (at window lengths less than the refractory period),

2. it dips down, becoming more regular, and

3. turns up sharply, exhibiting power-law type behavior.

This power-law type behavior is a fractal effect in the spike train that is time dependent, unlike the behavior seen in a Poisson process in which the Fano factor would be constant at 1, with no time dependence. The fractal effect, called long-range dependence, is the maintenance of the variation of the firing rate as the time bin increases (figure 5.7). If the data is shuffled (by randomly selecting the ISIs without replacement), removing the time dependency from the analysis, this temporal structure is lost while the average firing rate is maintained(figure 5.8). The Fano factor relates alterations in the firing pattern, not assessed alone by firing rate. We get two pieces of information from Fano factor functions- an assessment of time-scaled rate regularity and an estimate of long-range dependence.

While some of the Fano factor functions of unexposed animals are similar to the ones seen in auditory nerve (Teich, 1990), figure 5.9, others have a more accentuated deviation from the unity line, as seen in lateral superior olive neurons (Teich 1990),

figure 5.9. Some CIC neurons do not exhibit this long-range dependence behavior and

have a fairly flat Fano factor function (figure 5.10).
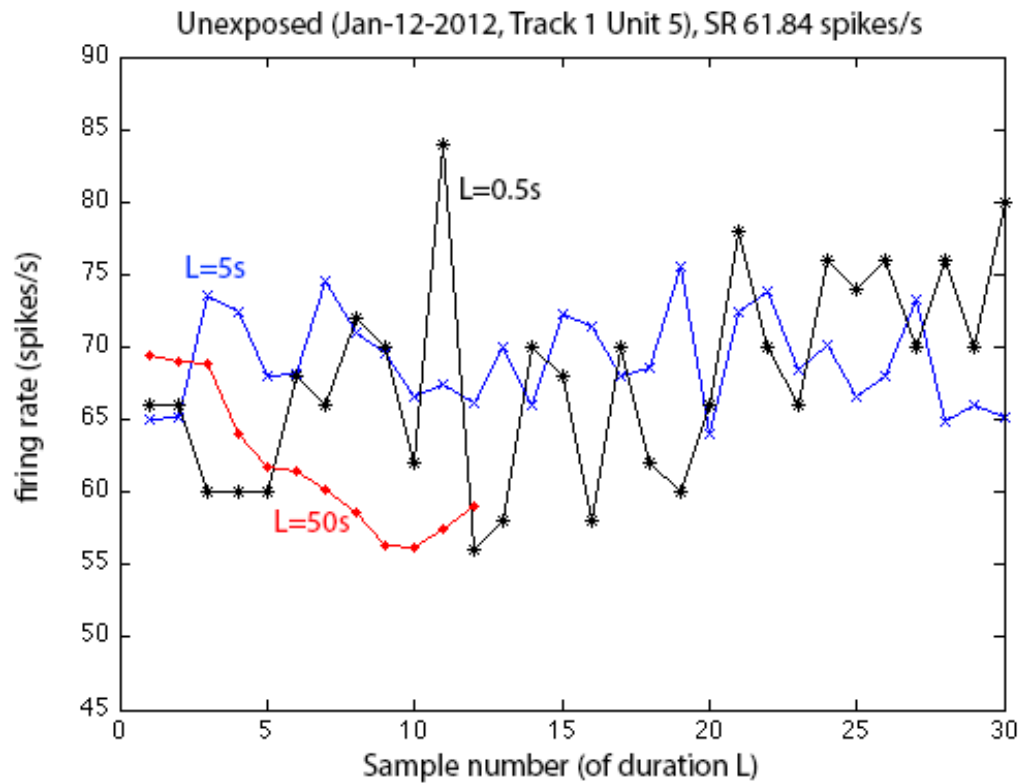
Figure 5.7- Long-range dependence in CIC spontaneous activity.

This figures shows the spontaneous rate on a variety of window sizes-- L=0.5, 5 and 50 seconds-- the spikes are binned by windows of size L and the counts are averaged by the bin width to yield the firing rate in that bin. Note that the rate continues to wander even at the large bin width (L=50 seconds). This is because of the fractal nature of the spike train and its time dependence on long time intervals. A Poisson process binned under these same conditions loses its fluctuations at large bin width, but preserves the spike rate. The unit used in this analysis is from an unexposed animal. SR = 61.84 spikes/s.
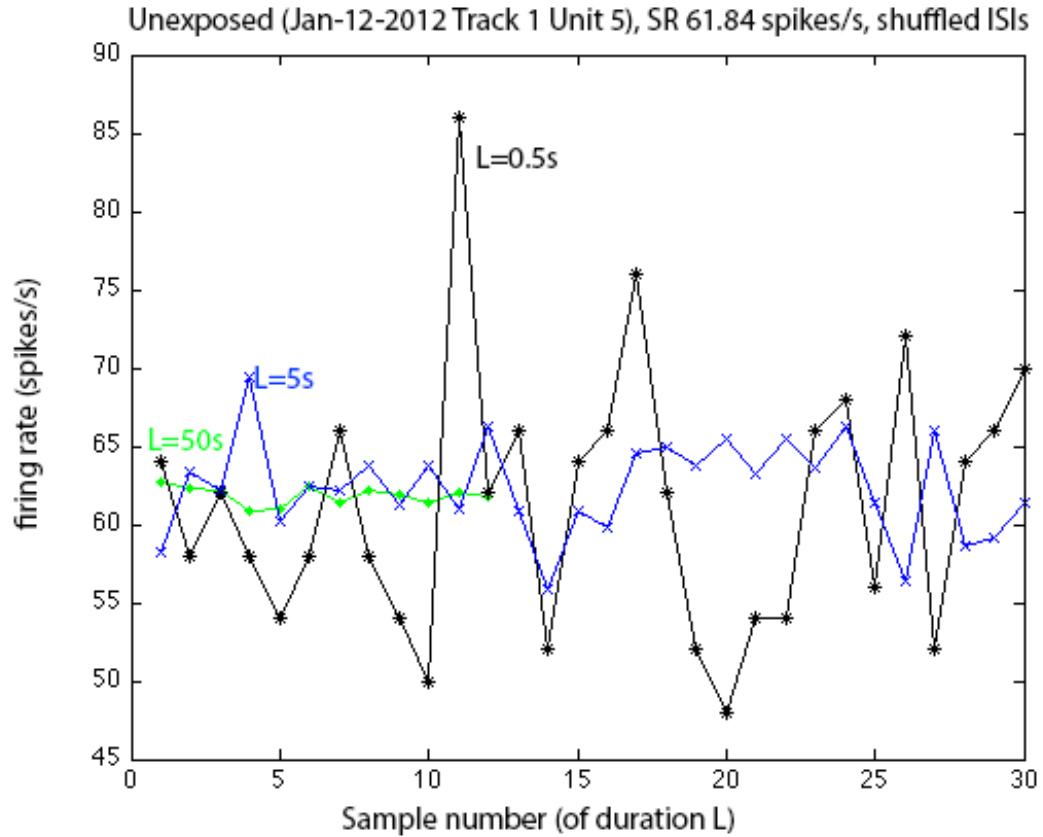
Figure 5.8- Shuffling the interspike intervals destroys the long-range dependence in the spike train.

This figure shows the same unit as in the prior figure, but here the ISIs have been randomly shuffled. While the variability persists at low counting windows, at higher counting windows the variability in the spike rate disappears. This is because shuffling the interspike intervals removes the temporal dependence in the spike train.
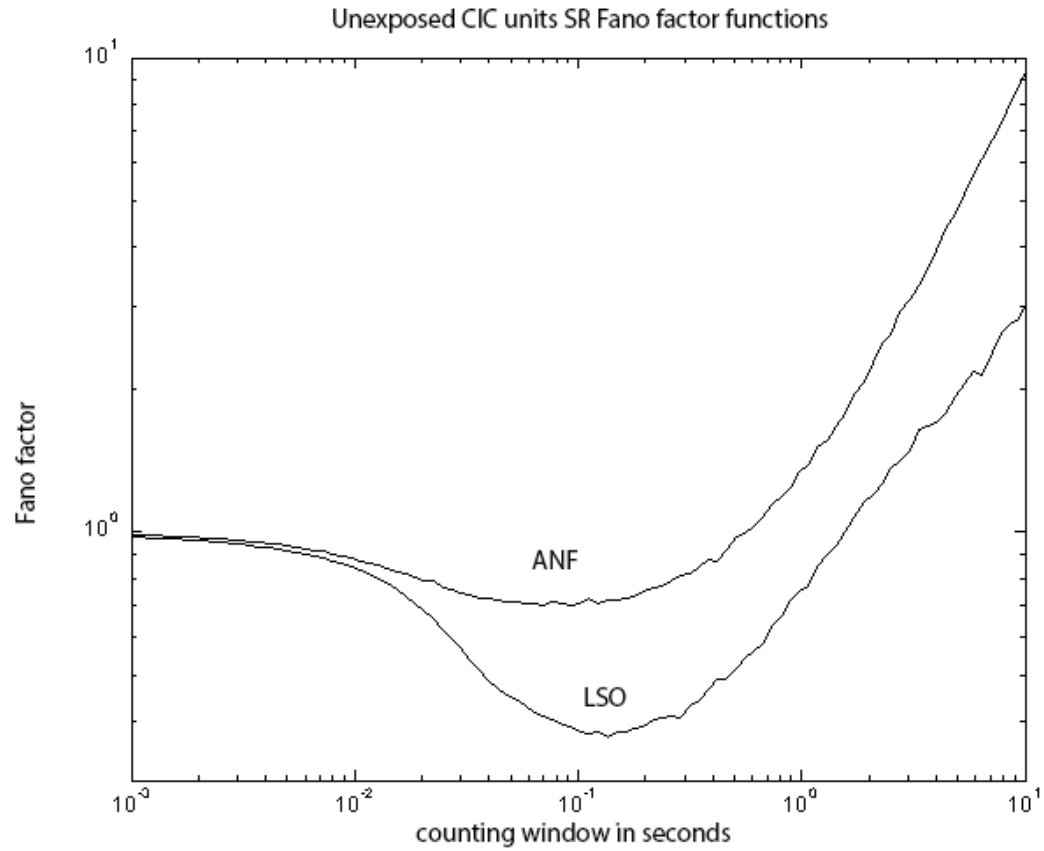
Figure 5.9- Fano factor functions from CIC units.

These Fano factor functions resemble the Fano factor functions in ANF and LSO neurons reported by Teich (1985,1991).
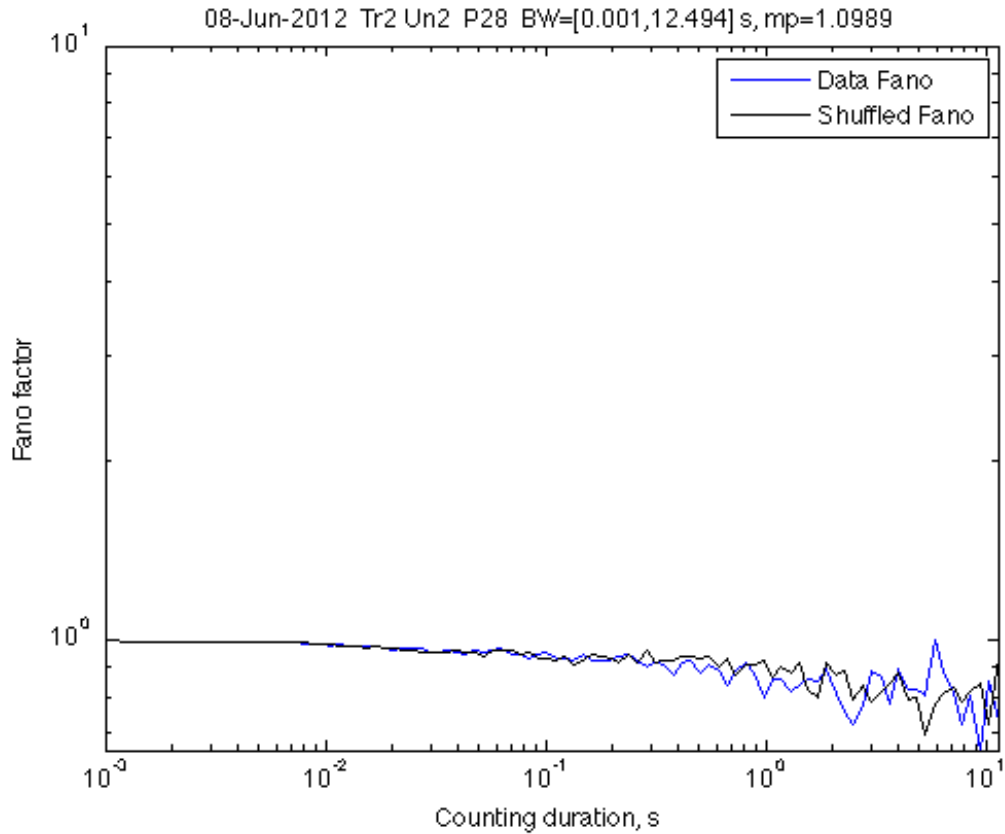
Figure 5.10- Fano factor function of a CIC neuron with spontaneous activity that exhibits no long-range dependence.

This unit, from an unexposed animal, did not show the long-range dependence behavior described by Teich- i.e., the curve does not turn up at high counting windows, instead the Fano factor tends to remain Poisson-like at high counting windows.

This characteristic Fano function behavior, showing long-range dependence as described by Teich, occurs in 80% of unexposed units (N=85). Seventy-two percent of tinnitus negative units (N=61) and 75% of tinnitus positive units (N=40) exhibit this behavior. These counts are not significantly different.
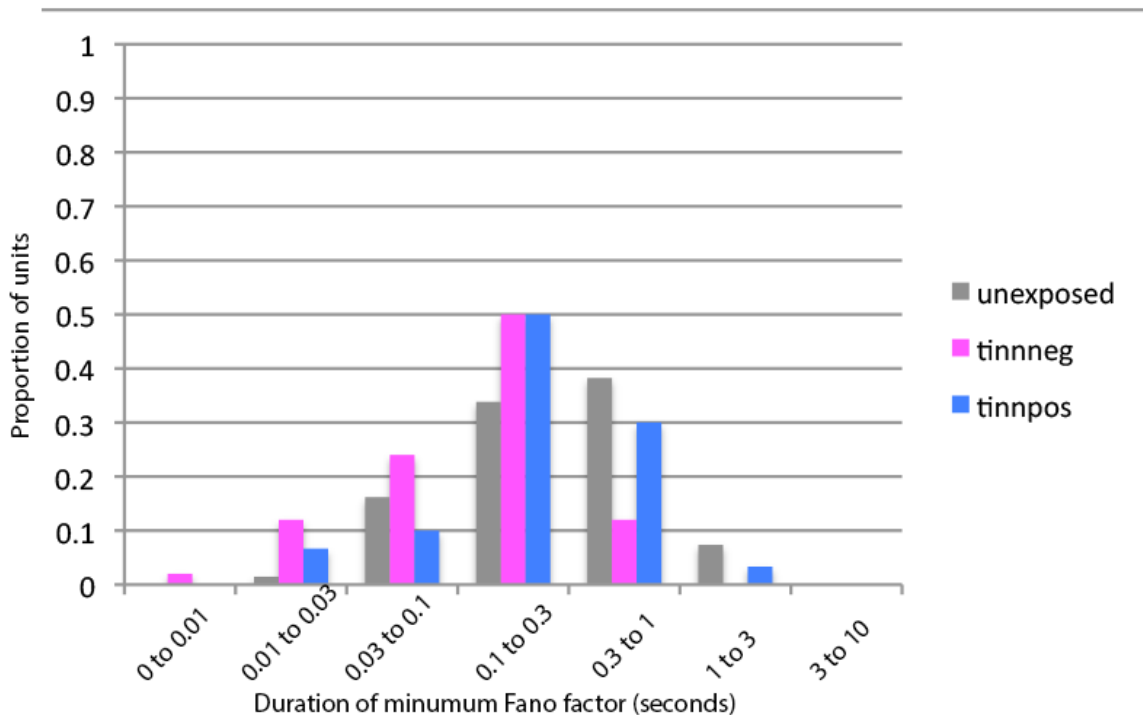
Figure 5.11- The duration of the minimum Fano factor value.

The duration (L value) corresponding to the minimum value of the Fano factor function in unexposed, tinnitus negative and tinnitus positive units. The binwidths on this histogram are logarithmically spaced since the Fano factor function is plotted on log-log axes. From this, the minimum Fano factor values tend to occur between 100ms and 1 second, i.e.- the largest deviation from the Poisson process value of 1 occurs at about 100ms. For unexposed units N=68, for tinnitus negative units N= 50 and for tinnitus positive units N=30.

Teich et al. (1990) describes the minimum Fano factor values of the spike count between 0.6 and 0.9 for spontaneous rates of single auditory nerve fibers. The minimum Fano factor values (figure 5.11) in our unexposed units ranged from 0.17 to 0.91. The minimum Fano factor values in our tinnitus negative units ranged from 0.18 to 0.99 and from 0.19 to 0.99 in tinnitus positive animals. These values were not significantly different ($p=0.06$, ANOVA).

For the first part of the analysis we will look at the Fano factor at 100ms time intervals, as in Teich and Khanna (1985), because it is a time point where the Fano factor function tends to deviate from Poisson-like behavior (figure 5.11 and 5.12). This is typically how the Fano factor is used in variability studies (Eden and Kramer, 2010). Figure 5.13 shows the Fano factor (100 ms) of all units with spontaneous rates over one spike/s for unexposed animals by BF. Figure 5.14 shows that there is a rate relationship in the Fano factor in unexposed animals, the measure is more variable at lower spontaneous rates. In keeping with the conventions of the prior chapters, we will look separately at the behavior of medium SR units (units with rates from 1 to 19 spike/s) and high SR unit (units with rates over 19 spikes/s). Figure 5.15 shows the median Fano factor for medium and high SR units by animal group. The medium SR units are significantly less regular (higher Fano) in both exposed groups in the edge and above-edge regions. However, in the high SR units, the units in tinnitus positive animals are significantly more regular (smaller Fano) than the other unit groups. Most of this difference is attributable to the edge region (all the high SR units in the tinnitus positive group are in the edge region), as shown in Fig. 5.16.
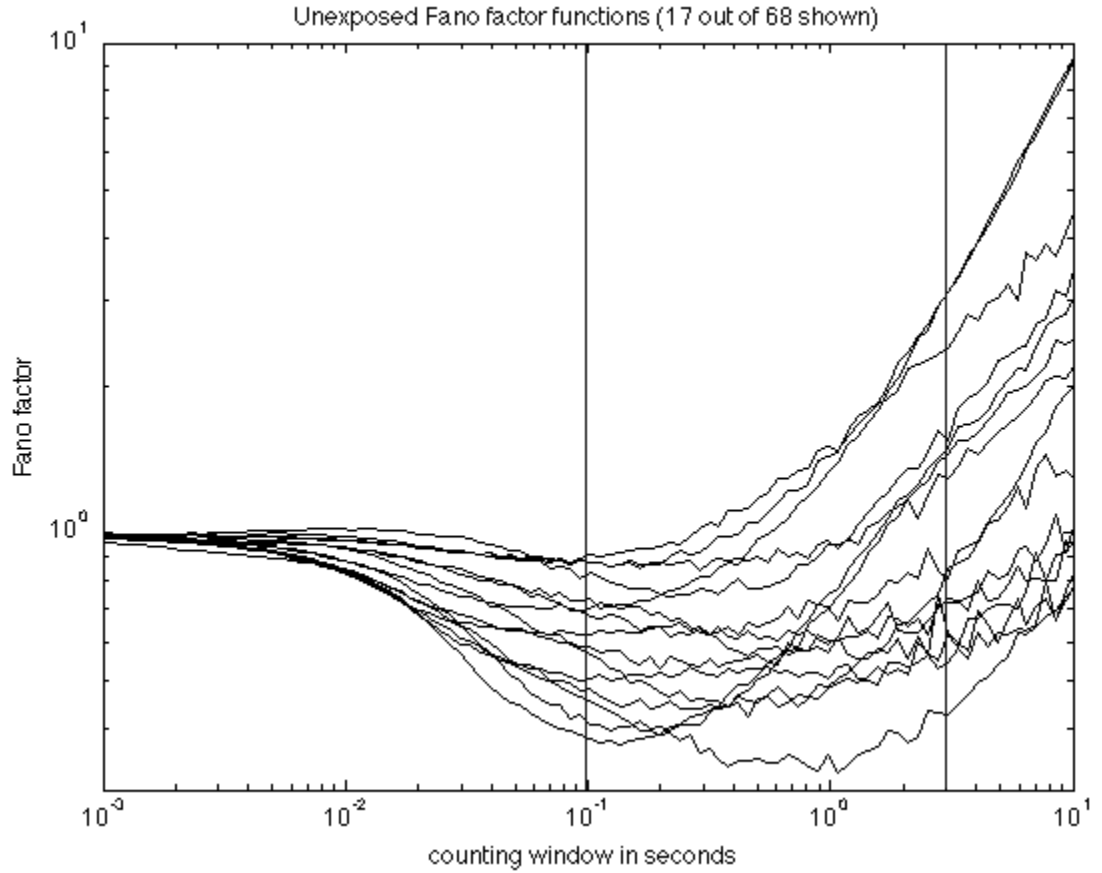
Figure 5.12- Fano factor functions for 17 of the 68 unexposed units that display fractal behavior.

This is a sample of the Fano factor functions used in the following Fano factor analysis. The counting window lengths used in the analysis are marked. The vertical lines mark L = 0.1 seconds and L = 3 seconds. The right endpoint of the graph is L = 10seconds.
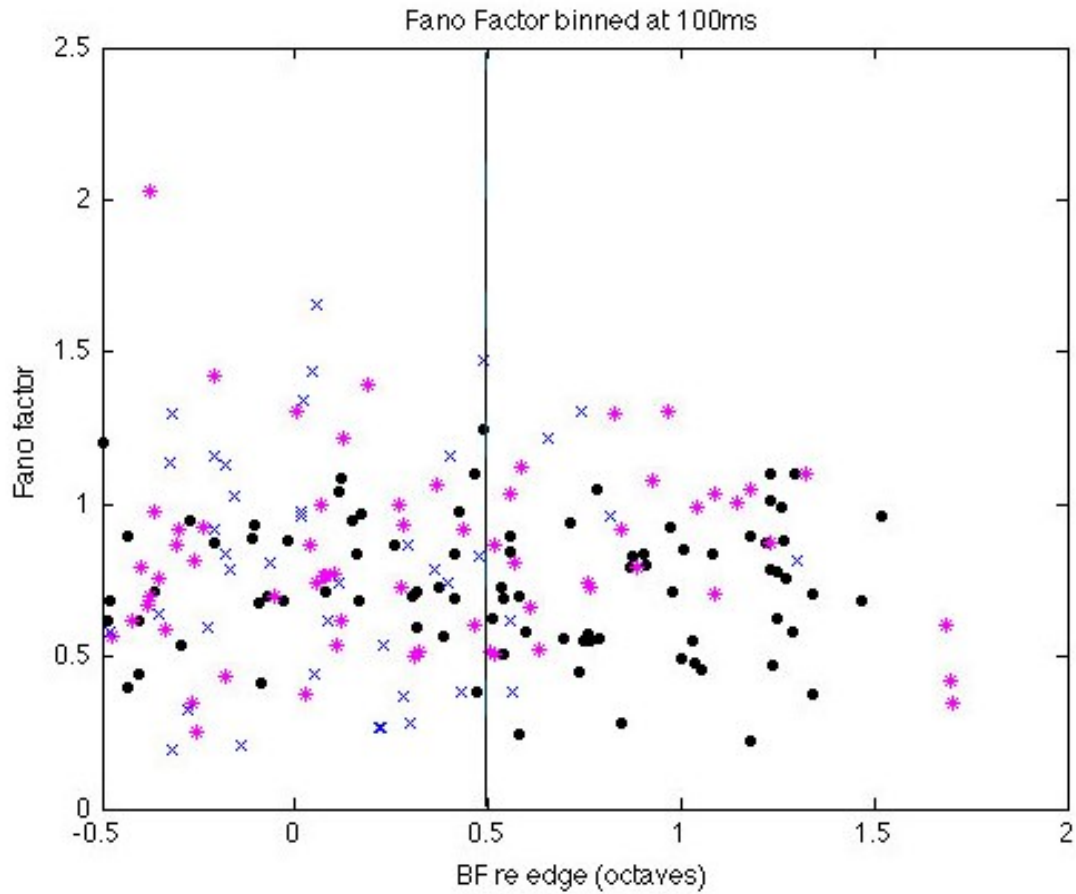
Figure 5.13- Fano factor (100ms) vs. BF.

Fano factor (100ms) values vs. BF re edge for unexposed (black dots, N=85), tinn neg (magenta stars, N=60), tinn pos (blue x's, N=40) units. The tinnitus positive units in the edge region (-0.5 to 0.5 octaves from the edge frequency) tend to lower Fano factor (more regular spike rate) values.
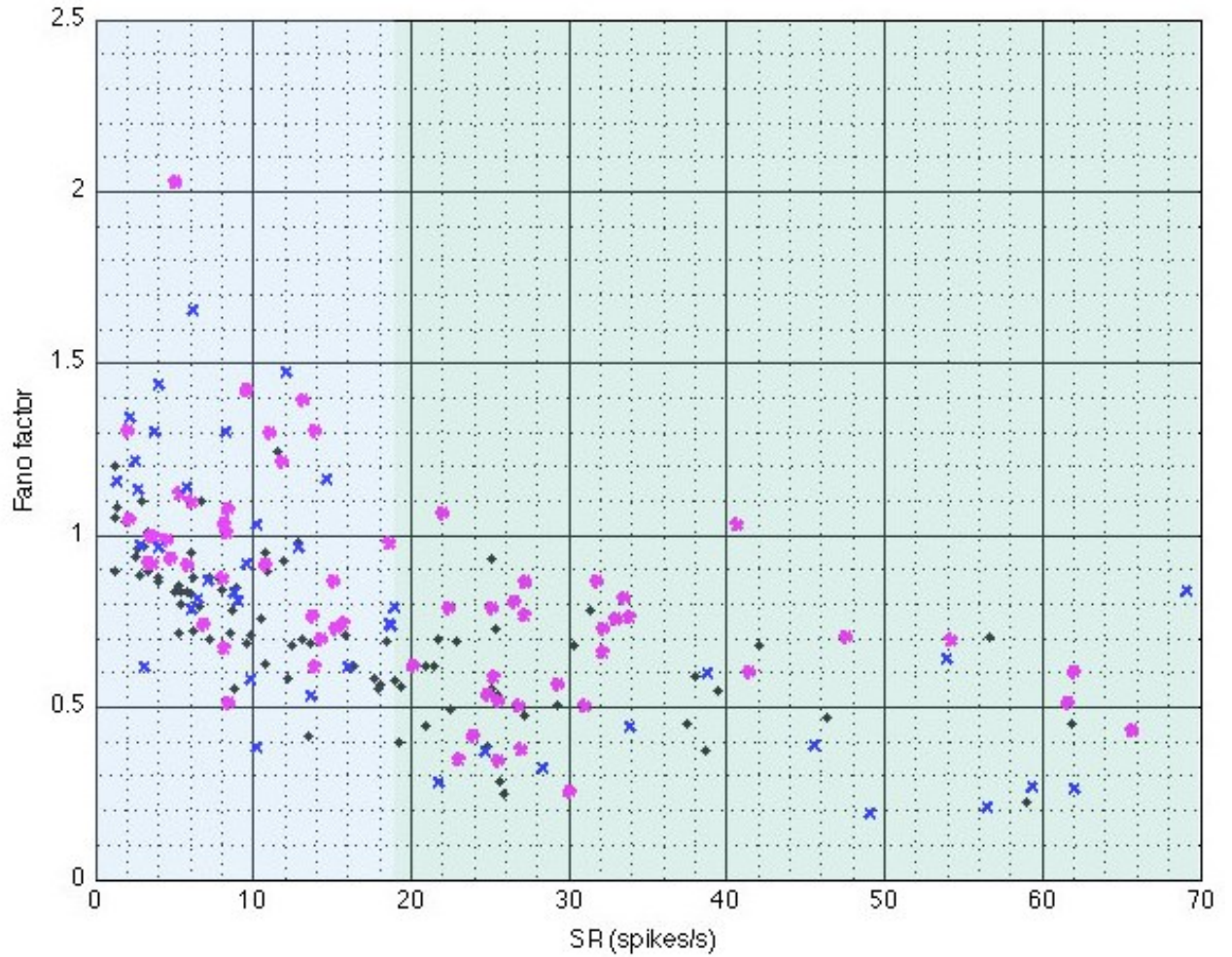
Figure 5.14- Fano factor (100ms) vs SR.

Fano factor vs. Spontaneous rate in unexposed, tinnitus negative and tinnitus positive units. The area shaded in green are the high SR units, the blue area indicates the medium SR units. There is more variation in the Fano factor in the medium SR units and there are notably lower Fano factor values in the high SR units of tinnitus positive animals.
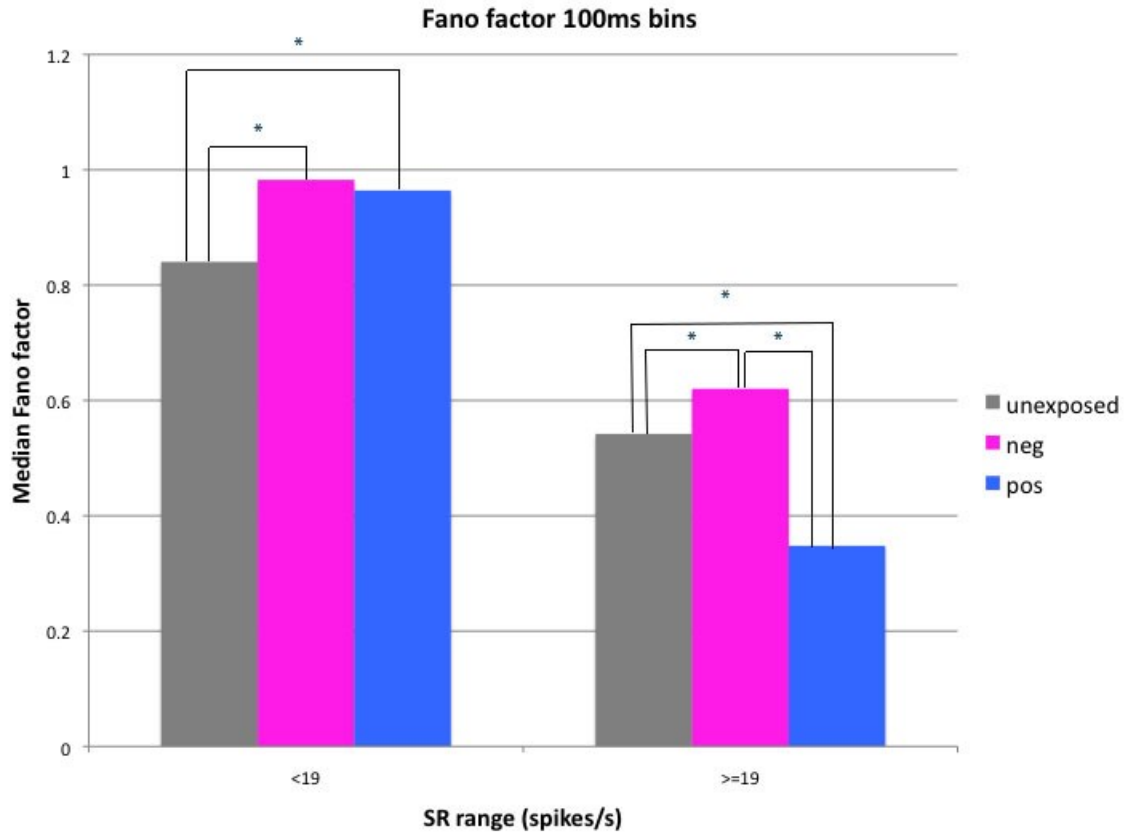
Figure 5.15- Median Fano factor values across animal groups.

Within the SR groupings the values are significantly different (1- way ANOVA, p<0.05), the pair-wise tests for significance (ranksum) are indicated by stars (*, p<0.05). Both exposed groups have significantly higher Fano factor values for medium SR units. The high SR units have significantly higher Fano factor values in tinnitus negative animals and significantly lower values (more regular spike rates) in tinnitus positive units. This analysis is done across all BFs>=-0.5 octaves from the edge frequency.
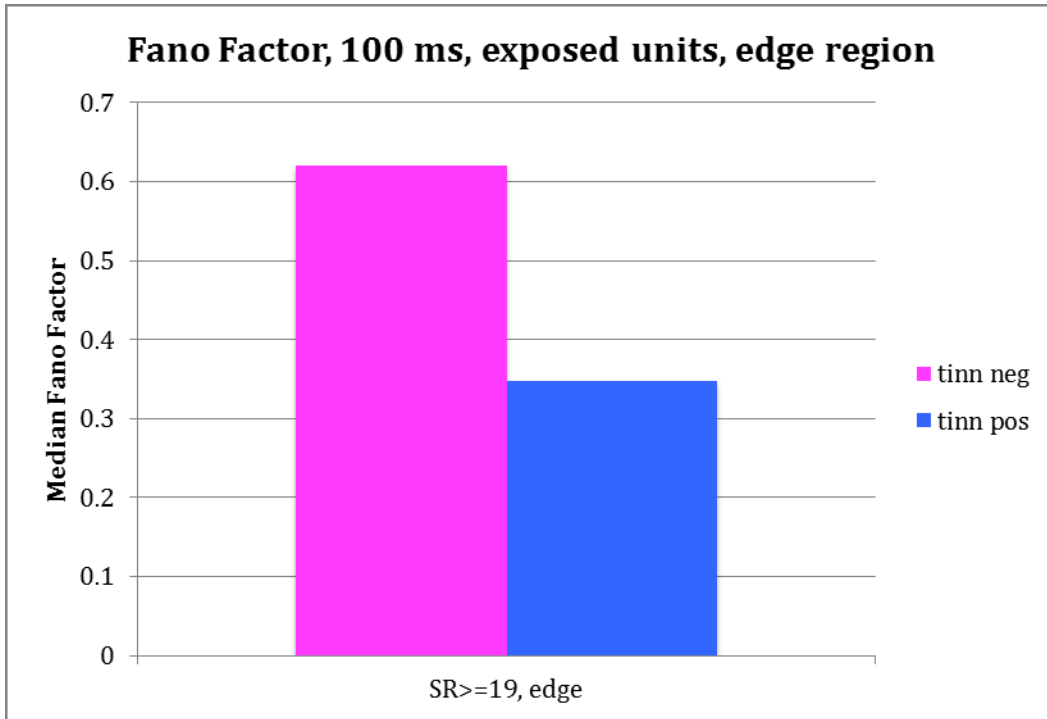
Figure 5.16- Median Fano factor values for exposed high SR units in the edge region.

The Fano factor values of high SR units in the edge region for tinnitus positive animals is significantly lower than that of tinnitus negative high SR units in the same frequency region (one-way ANOVA, p<0.01).
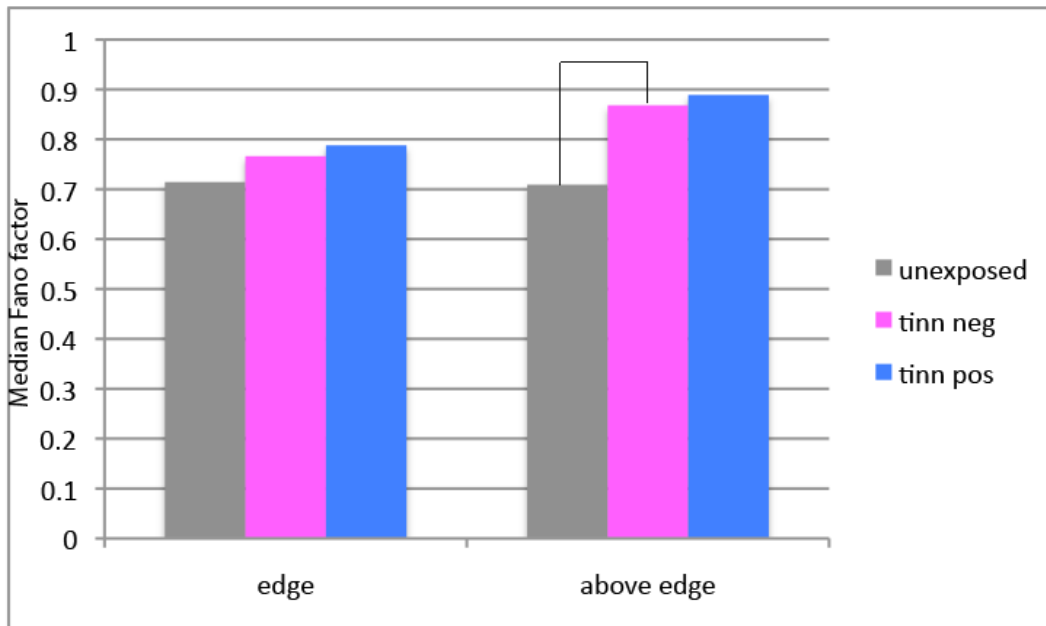
Figure 5.17- Median Fano factor (100ms) in the edge (octave band, centered about the edge frequency) and above-edge (>0.5 octaves from edge frequency) regions.

The values in the edge frequency area are not significantly different (p=0.81, 1-way ANOVA). In the above-edge region, the tinnitus negative values are significantly higher than the unexposed values (p<0.05, ranksum).

Again, to follow the analysis of the previous chapter, we look at the Fano factor in the edge region and the above-edge region (see figure 5.17). The only significant difference is an increase in the Fano factor of the tinnitus negative units in the edge region ($p < 0.05$, 1-way ANOVA; $p < 0.05$ Ranksum between the unexposed and tinnitus negative units).

Thus, in tinnitus positive animals, there is an increase in the proportion of high SR units in the region about the edge of the exposure, and these units have more regular firing rates than units of similar SR in unexposed or tinnitus negative animals.

The second part of this analysis looks at the Fano factor at 3s and 10s. Like in the previous analysis, the graph of the Fano factor values vs. BF (figures 5.18) and Fano factor vs. SR (figure 5.19) are shown. The Fano factor at 3s is significantly higher in tinnitus negative units ($p << 0.01$, 1-way ANOVA, figure 5.20A). The tinnitus positive units are not significantly different from the unexposed units. Breaking the data into edge and above-edge BF regions, the tinnitus negative units in the above-edge region have Fano factor values (3s) statistically higher ($p < 0.05$) than unexposed units (figure 5.20B). This difference is observed in the medium SR units (figure 5.20C). There are no significant differences in the 10s Fano factor values (data not shown).

Changes in the 3s Fano factor may be explained by the shift of minimum Fano factor values to lower counting windows (figure 5.11) in tinnitus negative units. If the minimum Fano factor is at a higher counting window (figure 5.12) the curve may still be in a recovery period at 3s. Because of this, and the notion that long-range behavior is reflected in the slope of the Fano factor curve at large counting windows, the slope between the 3s and 10s Fano factor values was computed. Calculations of the slope

between 3s and 10s Fano factor values yielded no significant change across the three groups (data not shown).
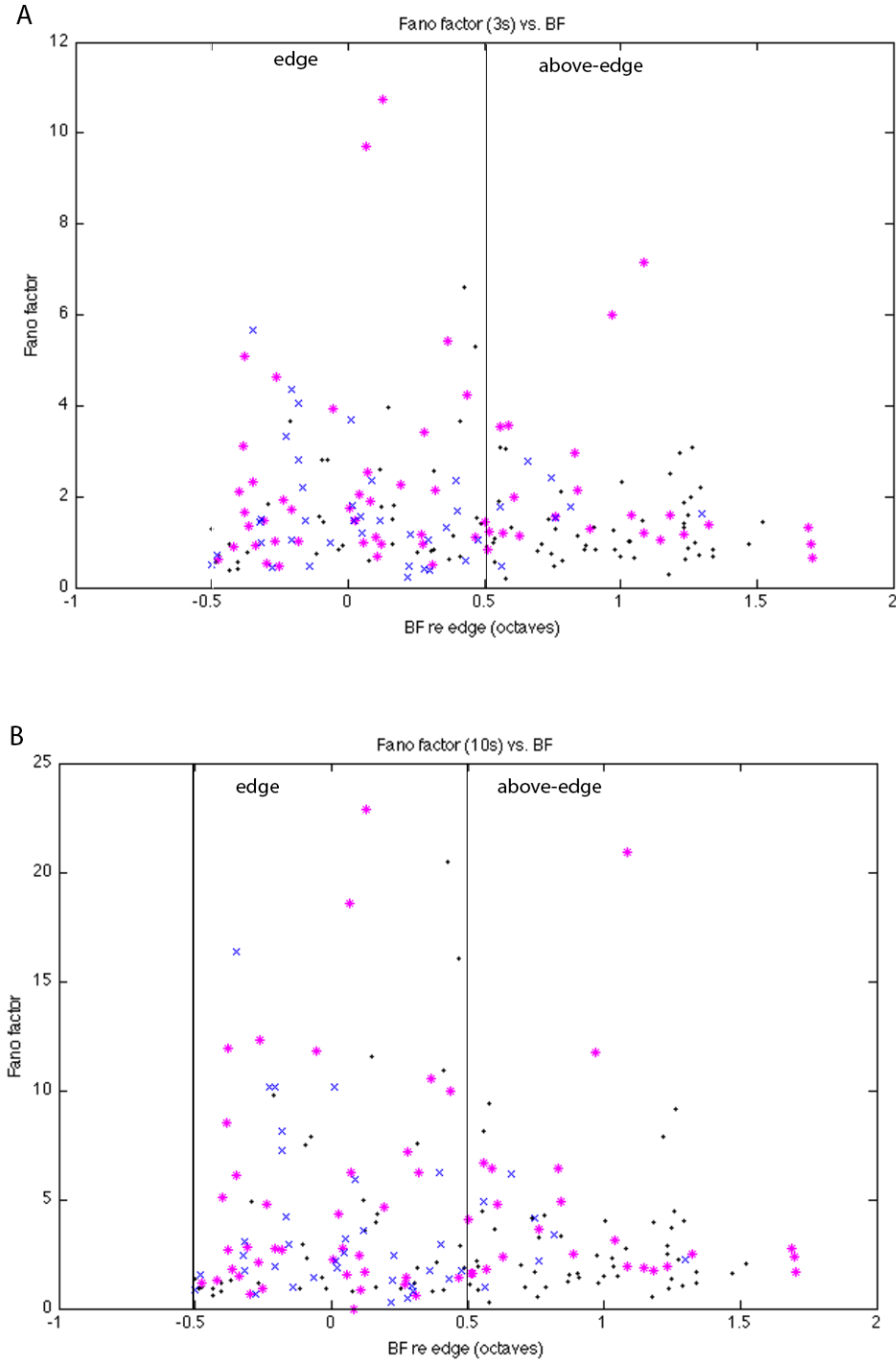
Figure 5.18- Fano factor values versus BF re edge at (A) 3 seconds, and (B) 10 seconds.

This figure shows the Fano factor values at higher counting windows in the edge and above-edge regions.

A

B
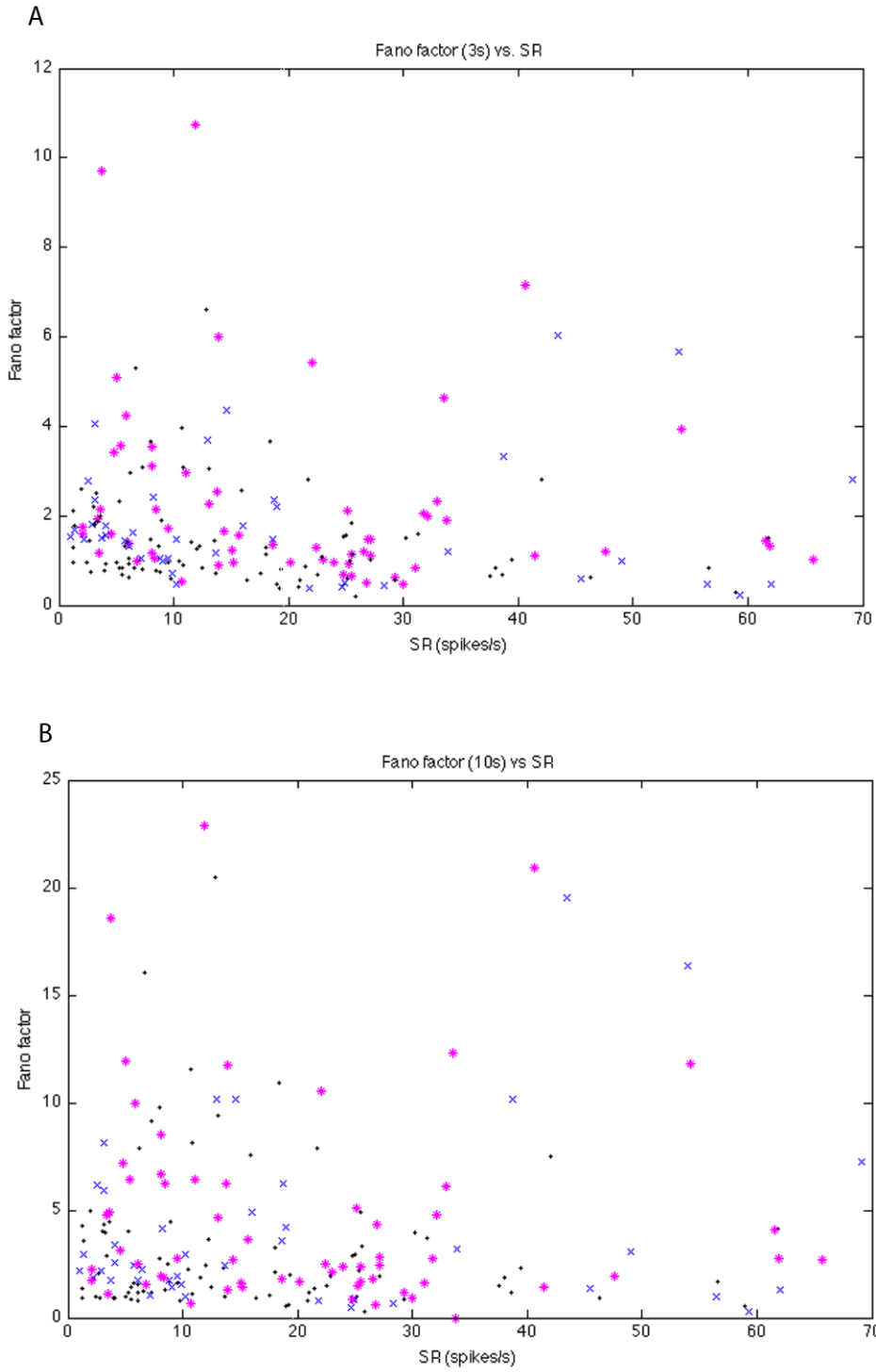
Figure 5.19- Fano factor values versus the spontaneous firing rate at (A) 3 seconds, and (B) 10 seconds.
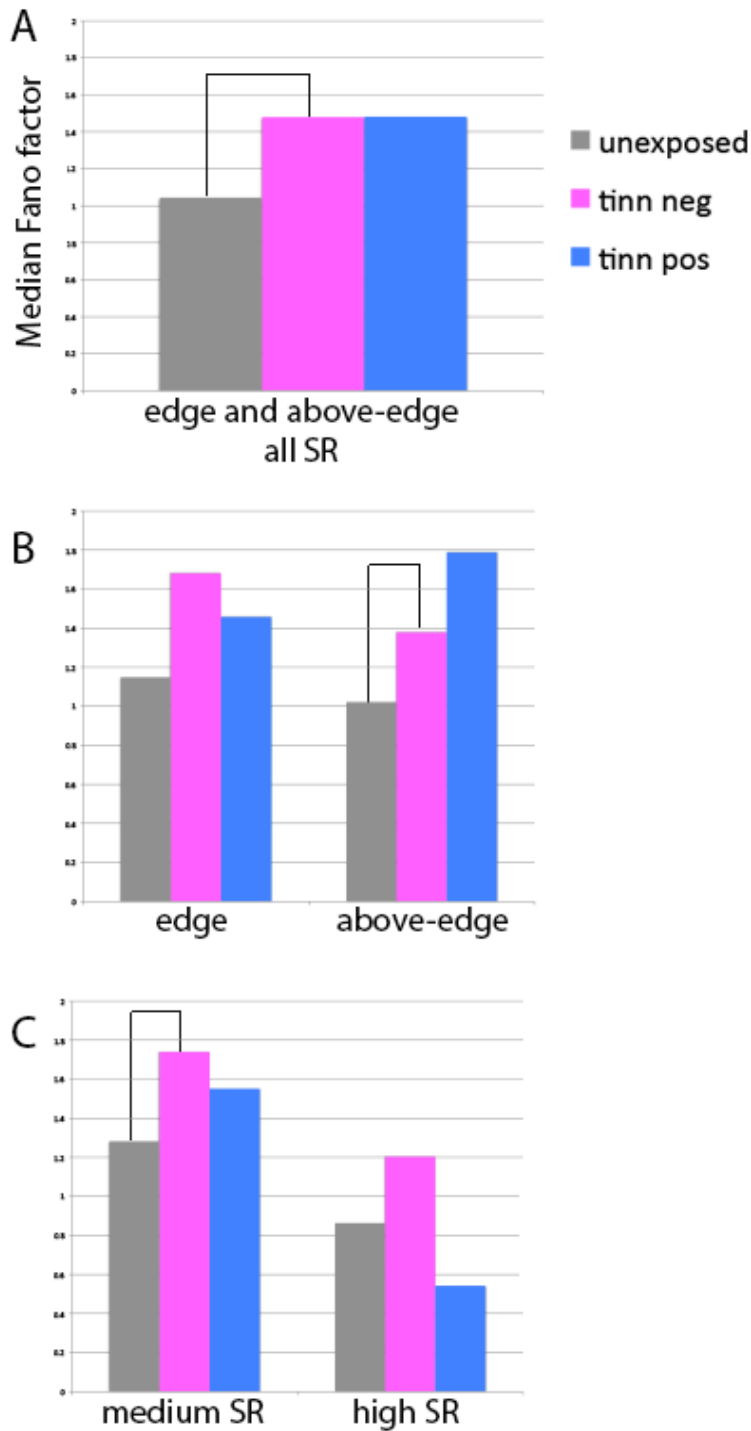
Figure 5.20- Statistical plots for 3s Fano factor analysis.

(A) Shows the median Fano factor values (L=3s) for all data in the edge and above-edge regions. (B) shows the medians for the data dividing in to edge region and above-edge region. The edge region is not significantly different (p=0.1544, 1-way ANOVA), the above-edge region has a significant increase in tinnitus negative units (p<0.05). (C) shows the medians for the data divided by spontaneous firing rate. The high SR units show no significant change in Fano factor (p=0.1179), but the median SR units show a significant increase in tinnitus negative units over the unexposed Fano factor values.

In summary, while there is increased regularity in high SR tinnitus positive units at 100ms, at higher counting windows, 3s and 10s, the tinnitus positive units do not show a significant difference.   This suggests that there is a possible change in rate coding in tinnitus positive units at 100ms. At higher time windows, the tinnitus positive neurons show no significantly different behavior than their unexposed matches. Tinnitus negative neurons show very different spike rate dynamics from their unexposed matches. Tinnitus negative units show irregularity of spike timing at 100ms and 3s, compared to unexposed units.

Chapter 6

Discussion and Future Directions

Discussion

To summarize the physiological findings of this thesis, we see a loss of inhibition in both tinnitus-positive and -negative exposed animals in single units of the CIC. This loss of inhibition correlated well with a loss of rate control, apparent by the increase of hyperactivity. The hyperactivity profile is different in the two groups of exposed animals (tinnitus negative and tinnitus positive). There is broadband hyperactivity in tinnitus negative animals, accompanied by firing irregularities. In tinnitus positive animals, there is loss of inhibition, with an increase in type V units and a decrease in type I units, but the loss of type I units is pronounced in the above edge region. Tinnitus positive units also have a completely different and unique profile of hyperactivity. Firstly, there is hyperactivity in the region about the edge frequency (octave band about the edge, centered at the edge frequency), but this is flanked by low spontaneous activity, perhaps even hypoactivity, in the above-edge region. This is congruent with the edge effect described by Komiya and Eggermont (2000) in auditory cortex. Furthermore, the hyperactive units in the edge region fire more regularly, producing more regular interspike intervals. Spike rate regularity analysis suggests that hyperactive tinnitus positive units have more regular rates on 100ms (and 300ms, data not shown) intervals. These results suggest two possible neural correlates for tinnitus: 1. an edge effect, i.e.-- a region of hyperactivity, flanked by hypoactivity, and/or 2. encoding by regularly firing hyperactive neurons in the edge region.

Hyperactivity after sound exposure in the CIC is specific to unit type. We show that type O units, which are thought to receive excitatory inputs primarily from type IV units in the DCN, show no change in hyperactivity after sound exposure. Yang Li (MSE thesis, 2011) showed no significant increase in the spontaneous rates of type IV units in the rat DCN with a similar sound exposure, which correlates well with our findings in type O units of CIC. Type O units have a high proportion of hyperactive units in unexposed animals as well, average unexposed SR = 18.7320; type I SR = 8.89; type V SR = 2.56 (significantly different, $p \ll 0.01$, 1-way ANOVA). Type I and V units however, become hyperactive after sound exposure.

The identification of hyperactive classes of units in CIC is unique to this study. Other studies of hyperactivity in tinnitus take a global multiunit approach (e.g. Bauer et al., 2008; Kaltenbach et al., 2004). Post-exposure multiunit recordings describe hyperactivity in the nucleus based on average spontaneous rates. Multiunit recordings are biased to record from spontaneously active units, and are a misrepresentation of the proportion of low or zero spontaneous rate units at any particular recording site. There are also anesthesia related differences between the data presented here and previously published data. Barbiturate anesthetized animals have considerably lower spontaneous rates than unanesthetized animals (Schumacher et al., 2011). If barbiturates were to cause differential silencing of certain unit types, say type O units, a multiunit spontaneous rate recording in an animal under barbiturate anesthesia misrepresents the spontaneous rate in the nucleus. The anesthetic routine used in this study, Ketamine/Acepromazine, has less effect on spontaneous activity. Evidence for this

conclusion is provided by the time course of spontaneous rate following drug administration; no effect on the spontaneous rate is seen in these data (not shown).

Hernandez et al. (2005) describe the inferior colliculus in rat as a primarily excitatory nucleus, with very little spontaneous activity. This varies from the findings in this study because of frequency range differences. Indeed, we find more type V excitatory units at lower frequencies (below 10kHz), but inhibition plays a strong role in higher frequencies in the rat CIC. Because of the locus of our sound exposure, the focus of our study is in the higher frequency regions where robust spontaneous activity and a balance of excitation and inhibition are prevalent in the unexposed nucleus. It is the change in these high frequency dynamics after exposure, with and without tinnitus perception, which are described here. The changes in physiological response types shown here suggest changes in the circuitry after sound exposure, and furthermore suggest a different effect in the tinnitus positive CIC. Unfortunately, physiological types have not been correlated to anatomical neuronal type and functional dynamics of the CIC. Furthermore, the physiological classification becomes blurred after sound exposure- i.e., no further conclusions can be made about the redistribution of units other than the change in distribution itself without further study.

Future Directions

Several studies have been conducted on the changes that occur in the auditory system after sound exposure that suggest changes in the system from the periphery to the auditory cortex (reviewed by Eggermont, 2013). Unfortunately, many of these studies that are aimed at studying tinnitus fail to utilize an assay for tinnitus to correlate tinnitus

perception with their physiological findings. Thus the effects of the method of inducing hearing loss are intermingled with the mechanisms of tinnitus perception. While this study does not definitively give causal evidence for the physiological changes in tinnitus positive animals, it does suggest neural changes that should be further investigated as correlates of tinnitus. The dilemma is finding a behavioral test that accurately and reliably determines whether or not an animal has a tinnitus percept. The GIASR paradigm developed by Turner et al. (2006), and used here in a modified form, serves as a reliable assay for tinnitus with a few caveats. The first is the ability of the animal to startle and show reasonably reliable startle suppression is compulsory for this paradigm. About half the animals failed this precondition. The second is that we assume that animals that do not have a robust startle response with inhibition of the startle modulated by the introduction of a gap before the startle stimulus do not have some auditory abnormality that should exclude them from the study. ABRs were not taken in unexposed animals, whether or not they were behaviorally prescreened. The third is that we assume that a unilateral exposure, necessary for the use of this behavioral paradigm, does not present a unique case of hearing loss and tinnitus percept generation. The fourth is that the test has limitations of the type of tinnitus that is detectable- it's frequency, quality, bandwidth, etc. In theory, an exhaustive list of trials could be conducted to determine tinnitus quality, but in practice this is not practical, given realistic time limitations for testing the animals. The fifth is that one must assume that with repeated testing, the animal does not learn the task and is not exhibiting a habituated response. The conclusions of this paper make the assumption that the false negative rate is low and the false positive rate is miniscule. Implementation of a more reliable assay, that not only

detects a wide variety of tinnitus percepts (in correlation with a wide variation of hearing loss resulting from a fixed parameter sound exposure), but has a low false negative rate, and a high success rate of tinnitus induction, perhaps used in tandem with the behavioral methods described here, is necessary.

While the physiological preparation used here gives a conceptual idea of changes that occur in the CIC after sound exposure and with tinnitus, it still fails to identify mechanisms on the single neuron level that might be associated with tinnitus. Partly, this is because CIC circuitry is still an enigma. A physiological assay that can better correlate the anatomical structure of the CIC and its physiological responses would be ideal for being able to interpret the changes that occur in the nucleus post-exposure. Simply implementing a binaural acoustic stimulation scheme, as used in Davis et al. (1999), will give a clearer interpretation of the physiological type changes post-exposure. Answering questions such as:

1. Do changes in the nucleus post-exposure, broadening of tuning and a loss of inhibitory input for example, make type I units appear to be type V units?

2. What are the changes that occur in the nucleus that can explain the origins of tail units?

Iontopheresis studies may also help demystify these questions (as in Spirou et al. (1999)).

A recording scheme that implements recording multiple single unit recordings, possible by using tetrode technology or electrode arrays, would give more concrete answers about changes in neural synchrony. Studies in auditory cortex (Eggermont and Roberts, 2004) suggest that increased neural synchrony is a neural correlate of tinnitus.

CHAPTER 6- DISCUSSION AND FUTURE DIRECTIONS

Recording simultaneously from multiple neurons is the only way to really assess such changes in the CIC.

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