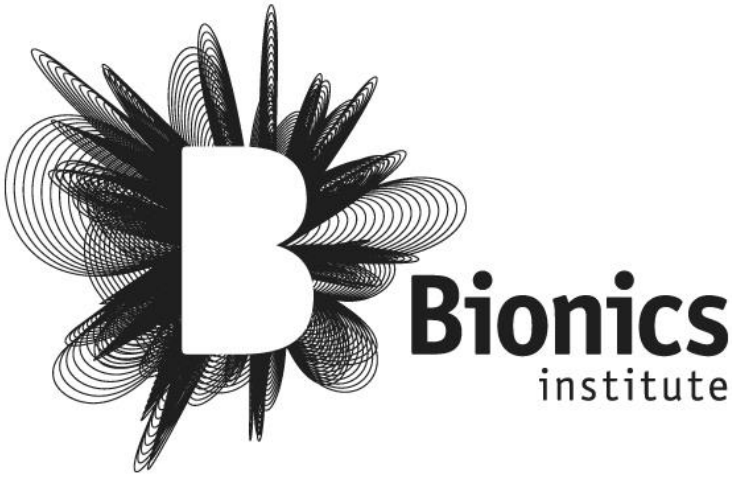

Neural synchrony in ventral cochlear nucleus neuron populations is not mediated by intrinsic processes but is stimulus induced: implications for auditory brainstem implants

Running Head: Neural synchrony in the ventral cochlear nucleus

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Abstract

The aim of this investigation was to elucidate if neural synchrony forms part of the spike time-based theory for coding of sound information in the ventral cochlear nucleus (VCN) of the auditory brainstem. Previous research attempts to quantify the degree of neural synchrony at higher levels of the central auditory system have indicated that synchronized firing of neurons during presentation of an acoustic stimulus could play an important role in coding complex sound features. However, it is unknown whether this synchrony could in fact arise from the VCN as it is the first station in the central auditory pathway. Cross-correlation analysis was conducted on 499 pairs of multiunit clusters recorded in the urethane-anesthetized rat VCN in response to pure tones and combinations of two tones to determine the presence of neural synchrony. The shift predictor correlogram was used as a measure for determining the synchrony owing to the effects of the stimulus. Without subtraction of the shift predictor, over 65% of the pairs of multiunit clusters exhibited significant correlation in neural firing when the frequencies of the tones presented matched their characteristic frequencies (CFs). In addition, this stimulus-evoked neural synchrony was dependent on the physical distance between electrode sites and the CF difference between multiunit clusters as the number of correlated pairs dropped significantly for electrode sites greater than 800 μm apart, and for multiunit cluster pairs with a CF difference greater than 0.5 octaves. However, subtraction of the shift predictor correlograms from the raw correlograms resulted in no remaining correlation between all VCN pairs. These results suggest that while neural synchrony may be a feature of sound coding in the VCN, it is stimulus induced and not due to intrinsic neural interactions within the nucleus. This data provides important implications for stimulation strategies for the auditory brainstem implant, which is used to provide functional hearing to the profoundly deaf through electrical stimulation of the VCN.

1. Introduction

Sound coding by the brain is the process of information transfer through multiple levels of the central auditory system through neural firing of action potentials. It has been suggested that in the central auditory system, information is relayed either exclusively through or by a combination of four main neural firing codes; place, rate, timing (temporal) and ensemble (Cariani 1999). The place or the spatial code is the ability of higher order central processing neurons to encode frequency or pitch based on the location of the neural activity. Together with the rate code, which is dependent on the firing rates of individual neurons, this provides an overall rate-place representation of auditory information where firing rates of neurons at specific locations can reveal the frequency or pitch of the sound being presented (Eggermont 2001).

Temporal coding is a code that involves precise firing of neurons in time to certain events. This type of code has been shown to exist in the phase-locked firing patterns of auditory nerve fibers (ANFs) to frequencies below 5 kHz (Rose et al 1967) and is found to be preserved in the firing of bushy neurons of the ventral cochlear nucleus (VCN), the first processing site of the central auditory system (Britt 1976). Recently, a new dynamic temporal code for processing of high frequency sounds based on timing delays has been proposed in the VCN, particularly involving the D-stellate and T-stellate neurons (Paolini et al 2004, 2005).

Another type of code closely related to this spike-time based code is the simultaneous firing of action potentials by several neurons in a localized region, leading to a synchronized population response. Evidence of neural synchrony has been discussed over the past four decades (Gerstein and Perkel 1969,1972, Perkel et al 1967a, b) and several studies in the auditory system (Davis and Voigt 1997, de Boer et al 1969, Eggermont 1994, 2000, 2001, Epping and Eggermont 1987, Frostig et al 1983, Gochin et al 1989, Johnson 1980, Oertel et al 2000, Voigt and Young 1980, 1988, 1990) have shown the presence of neural synchrony, and have suggested that stimulus-dependent neural synchrony may assist in extracting different

features of sound. While such mechanisms have mainly been proposed in higher midbrain and cortical areas, very little is known about the existence and functional implications of neural coding via synchrony in lower levels of the central auditory system. Some studies (Davis and Voigt 1997, Gochin et al 1989, Voigt and Young 1980, 1988, 1990) have shown evidence of stimulus-dependent neural synchrony between different neuron types of the DCN. In addition, it has been shown that ANFs can themselves synchronize to acoustic stimulation (de Boer et al 1969) and can pass on this synchronous information to neurons of the VCN (Oertel et al 2000, Young and Sachs 2008). However, it is unknown whether neurons of the VCN among themselves show any form of synchrony.

One of the most common experimental techniques to determine the presence of neural synchrony is cross-correlation between spike trains (Davis and Voigt 1997, de Boer et al 1969, Eggermont 1994, 2000, 2001, Epping and Eggermont 1987, Frostig et al 1983, Gochin et al 1989, Johnson 1980, Oertel et al 2000, Voigt and Young 1980, 1988, 1990, Young and Sachs 2008). Cross-correlation can be performed under spontaneous conditions without any stimulus present or, can be determined in the presence of a suitable stimulus in neurons with low spontaneous rates. However, under stimulus conditions, the total neural synchrony found is usually made up of two components, one due to the stimulus effects and the other as a result of neural connectivity (Eggermont 1994). In such cases, an appropriate correction factor known as the shift predictor is applied to determine the component of synchrony solely due to the stimulus (Perkel et al 1967b). The remaining neural synchrony found after subtraction of the shift predictor from the total synchrony represents the synchrony as a result of neural connectivity and is usually comparable to the synchrony found under spontaneous conditions (Perkel et al 1967b, Eggermont 1994).

It has been suggested that neural synchrony between two spike trains is a result of one of two physiological processes; interaction and common input (Perkel et al 1967b). Interaction between two neurons might be a result of direct synaptic connections or indirect connections through interneurons while common input would most likely indicate that the two neurons or

neuron populations receive input from a common source either directly or indirectly (Perkel et al 1967b). The VCN of the rat is known to contain three main classes of neurons known as the bushy, stellate and the octopus neurons (Cant and Benson 2003). Each neuron belonging to either of these classes is known to receive excitatory input from the auditory nerve (AN), however, the degree of AN input may differ for each neuron class (Harrison and Irving 1965, 1966). This excitatory input from the AN, either through spontaneous background activity or stimulus-driven activity may serve as a common input for causing synchronous firing in VCN neurons. In addition, numerous previous studies have shown that VCN neurons can form complex circuits through interconnections, for example the broadly tuned D-stellate neurons are known to inhibit the narrowly tuned T-stellate neurons (Cant and Gaston 1982, Ferragamo et al 1998, Needham and Paolini 2003, Schofield and Cant 1996). Furthermore, it has been hypothesized that T-stellate neurons send excitatory projections to other local T-stellate neurons within the VCN (Ferragamo et al 1998), thus creating the possibility for synchrony via intrinsic neural interactions. Given the evidence for a spike-time based frequency extraction mechanism within the VCN (Paolini et al 2004, 2005), we investigated the possibility that synchrony of neural firing across populations of VCN neurons, if existent, could perhaps further aid in the extraction of frequency features. Furthermore, we investigated the presence of synchrony directly related to the stimulus (henceforth termed as neural synchrony in this study) and the synchrony related to intrinsic neural interactions (henceforth termed as neural correlation in this study) after applying appropriate correction for the stimulus effects as described by Perkel et al (1967b).

The results of this study may have implications on the optimal ways to electrically stimulate the VCN to reproduce sound information using an auditory brainstem implant (ABI). Present ABIs are designed to stimulate the surface of the cochlear nucleus (CN). Since the late 1980s, this device has been implanted in over 700 patients worldwide; however, clinical success has been limited (Kuchta 2007, McCreery 2008, Otto et al 2002, Otto et al 2008). This has been partly attributed to the fact that unlike the cochlear implant which stimulates the tonotopically

arranged neurons of the cochlea; the ABI only stimulates the surface of the CN (Kuchta 2007, Otto et al 2008). Consequently, the ABI is unable to take advantage of the tonotopic organization of neurons inside the CN from low to high frequencies. A simple way to resolve this is by using an electrode array with penetrating electrode sites to stimulate the neurons inside the VCN as opposed to the CN surface (McCreery 2008, Otto et al 2008). However, in doing so one must bear in mind that neurons of the VCN are arranged in a laminar fashion where there are several neurons within each individual lamina that are sensitive to a single acoustic frequency (Rose 1960, Rose et al 1959) and therefore the penetrating electrodes need to be able to access each isofrequency lamina independently for stimulation.

A recent clinical trial using penetrating electrodes in ABI recipients suggested that simply converting the surface electrode array into a penetrating array that allows access to individual isofrequency laminae was not sufficient to improve overall speech perception (McCreery 2008, Otto et al 2008). This leads to the possibility that the lack of improvement in speech perception could be linked to other factors such as the number of electrode sites stimulated within each VCN isofrequency lamina. If VCN neurons within each VCN isofrequency lamina were connected to one another via excitatory synaptic connections, which should be shown by the presence of neural correlation, then electrical stimulation of a single location of an isofrequency lamina should be sufficient to activate a substantial number of neurons within the lamina and provide the required information transfer to higher order centers for speech perception. However, an absence of neural correlation in the VCN would suggest that it may not be sufficient to stimulate only single electrode sites within VCN isofrequency laminae for appropriate information transfer. In a previous study, we have shown that stimulating single sites within VCN isofrequency laminae in the rat, does not always produce a response in the central nucleus of the inferior colliculus (Shivdasani et al 2008). It may therefore be advantageous to incorporate several electrodes in each isofrequency lamina and use simultaneous stimulation of more than one site in each isofrequency lamina to potentially improve speech perception.

By using multichannel electrophysiological techniques we aimed to record multiunit activity simultaneously across the tonotopic array of the VCN in response to single and two-tone stimuli and used cross-correlation analysis under stimulus conditions, as described by Eggermont (1994) to determine the degree of neural synchrony and neural correlation in the VCN.

2. Materials and Methods

2.1 Surgery

Male Hooded Wistar rats (n = 5) weighing 350-450gms were anesthetized using Urethane in water (20% w/v i.p., Sigma, NSW, Australia), placed in a stereotaxic frame and fitted with in-house manufactured hollow ear bars. Following a craniotomy, the cerebellum was aspirated, to expose the brainstem. Using the lateral recess of the 4^{th} ventricle as a guide, a 32-site electrode array (Neuronexus Technologies, Ann Arbor, MI) was inserted into the left VCN along the dorsoventral axis of the nucleus under visual control. The electrode array consisted of 32 iridium sites (200 μm apart), each 413 μm^2 surface area arranged on 4 silicon shanks (200 μm apart, 8 sites per shank). All surgical procedures and protocols were approved by the La Trobe University Animal Ethics Committee (Protocol # 03/13).

2.2 Experiment Protocols and Data Acquisition

Acoustic stimuli generation and simultaneous data acquisition on 32 electrode sites were performed using Tucker Davis Technologies System III hardware (TDT, Alachua, FL) with a custom-designed software package. First, driven multiunit clusters were located by presenting a broadband (1 kHz – 44 kHz) Gaussian distributed noise stimulus (50 ms bursts, 5 ms rise/fall, 500 ms inter-trial interval) while advancing the electrode array into the brain using a motorized micro-drive (Sutter Instruments, Novato, CA). Once the position of the array was fixed, a response area protocol was run using pure tone stimuli (10-80 dB SPL, 10 dB steps, 1-44 kHz, 1 kHz steps, 10 trials each frequency-intensity combination, 50 ms duration, 5 ms rise/fall, 300 ms inter-trial interval) to obtain an acoustic response area and the CF for each individual electrode site.

In order to study correlation amongst pairs of multiunit clusters with similar CFs, tone bursts at various frequencies from 1 kHz to 44 kHz (1 kHz steps) were presented at 65 dB SPL (200 trials at each frequency, 50 ms duration, 300 ms inter-trial interval). For a second correlation

study involving pairs of multiunit clusters with different CFs, two tones, each of 65 dB SPL intensity, were presented simultaneously as multiple tone pairs. For this protocol, one tone was kept at a constant frequency that corresponded to the CF of the first multiunit cluster forming the pair and the other tone frequency was varied from 1-44 kHz (1 kHz steps) and included the CF of the second multiunit cluster forming the pair (200 trials each pair of tones, 50 ms duration, 300 ms inter-trial interval). Simultaneous data acquisition (bandpass filtered from 300 Hz-5000 Hz) from the 32-site electrode array were performed at a sampling rate of 24.4 kHz using an online threshold based spike discriminator and then re-thresholded offline using a Schmitt trigger procedure as described in Shivdasani et al. (2008).

For this procedure, the incoming signal on each site was monitored every two seconds to calculate an estimate of the noise level. The online spike discriminator was triggered each time the signal crossed the noise estimate by more than 4.2 standard deviations. At each trigger, the spike discriminator recorded 31 samples (1.2 ms) of the signal waveform, corresponding to a single multiunit cluster spike. However, while the spike discriminator was recording a waveform, it could not be triggered by another spike until it had recorded 31 samples of the signal. This could result in the recording system not accurately keeping track of all the spikes, especially those which occurred very near in time to each other. Therefore, a re-thresholding procedure was used offline to accurately determine the occurrence of spikes. This procedure involved finding the mean and standard deviation of each 31-sample multiunit cluster spike recorded by the system, and then checking how many times any particular sample out of the 31 exceeded 1.5 standard deviations above the mean. Each threshold crossing was picked up by a Schmitt trigger function, which kept track of the occurrence of multiple spikes within each multiunit cluster spike. This provided a more accurate estimate for spike occurrence (Shivdasani et al 2008).

2.3 Histological Verification

At the end of each experiment, the animal was deeply anesthetized and perfused transcardially using 0.1 M phosphate buffered saline and 10% neutral buffered formalin (Sigma Aldrich, NSW, Australia). Serial coronal sections of 60 µm were collected using a freezing sledge microtome (Thomas Scientific, Swedesboro, NJ, USA) and stained with Thionine. Positions of the electrodes through the VCN were confirmed using bright field microscopy and VCN shank locations were reconstructed by manual drawings using a combination of histological data and known visual placements of the electrodes (See figure 1). Histological data was obtained in four out of the five experimental animals. Due to multiple VCN penetrations, histological data could not be verified for the remaining animal.

2.4 Data Analyses

2.4.1. Acoustic Response Areas. Spike rate calculations were made for each frequency-intensity combination of sound presented using a time window 0-50 ms from stimulus onset. These were then plotted as a function of frequency and intensity to give a response area for each of the 32 recorded sites. The CF for each VCN site was determined from its response area as the frequency which elicited the maximum spike rate at the lowest intensity (dB SPL).

2.4.2. Correlation Test. In order to test for correlation of neural activity, multiunit clusters were paired according to their CFs. In the case of single tone correlation, only pairs with identical CFs or those with a maximum CF difference of 1 kHz were chosen for analyses while for two tone correlation, one multiunit cluster from each pair had a CF equal to the constant tone presented while the other multiunit cluster had a CF within the range of the second tone presented. For each pair of multiunit clusters analyzed, at each frequency of the presented stimuli, a crosscorrelogram was constructed. Correlation was tested in a 60 ms time window following onset of the stimuli only if the number of spikes in each multiunit cluster forming a pair exceeded 200, in 200 trials of the stimulus. The crosscorrelogram $R_{AB}(\tau)$ is defined here as the binning of distances in time between spikes in one multiunit cluster and

the other where τ denotes the lag time (See Figures 2D & 3D). Thus for example a bin count of 100 in the crosscorrelogram for a 1 ms bin width at zero lag time would indicate that there were 100 spike-pairs in multiunit cluster 'B' and multiunit cluster 'A' that occurred within 1 ms of each other. Using similar techniques as Eggermont (1992, 1994), under the assumption of two independent spike trains and assuming a Poisson distribution of bin counts, the expected values (E) and standard deviations (σ) of each crosscorrelogram were computed and the crosscorrelograms were normalized by converting the raw values to Z-scores (Eqs. 1, 2 & 3).

$$E = N_A N_B \Delta / T \qquad \text{Eq. 1}$$

where N_A & N_B are the number of spikes in the multiunit clusters A & B, Δ is the bin width (1 ms used for our data) and T is the duration of the recording (200 trials of 60 ms each trail).

$$\sigma = \sqrt{E} \qquad \text{Eq. 2}$$

$$Z_{AB}(\tau) = [R_{AB}(\tau) - E] / \sigma \qquad \text{Eq. 3}$$

The peaks in the raw correlograms were deemed significant ($p < 0.0001$) if their Z-scores crossed a level of 4 i.e. 4 standard deviations above the expected value (Eggermont 1992). To avoid cases in which a peak crossed the threshold of 4 standard deviations by chance, only those peaks in which three consecutive bins crossed the threshold were taken into consideration. For each significant peak in the crosscorrelogram, a value of correlation coefficient (ρ) was calculated according to Eq. 4 (Eggermont 1992). In order to then determine the total synchrony profile for the multiunit cluster pair, the peak correlation coefficient was plotted as a function of stimulus frequency (figures 2A & 3A). This type of correlation was termed as neural synchrony as it did not involve the calculation or subtraction of the shift predictor.

$$\rho(\tau) = Z_{AB}(\tau)\Delta / T \qquad \text{Eq. 4}$$

2.4.3. Shift Predictor Calculation. Following analyses of the raw crosscorrelograms, the shift predictor crosscorrelogram was calculated to isolate the effects of acoustic stimulation alone on neural synchrony (Perkel et al 1967b, Eggermont 1994). This was performed by computing the crosscorrelogram using spike times of a given multiunit cluster from one

stimulus trial and spike times of a second multiunit cluster shifted by one period of the stimulus (300 ms in this study). The overall shift predictor correlogram was then computed by averaging all possible shifts. Assuming the additive effects of neural synchrony directly related to the stimulus and any underlying spontaneous neural correlation via intrinsic neural interactions between multiunit clusters, the shift predictor correlogram was subtracted bin by bin from the raw correlogram for each multiunit pair. This "corrected" crosscorrelogram, termed as neural correlation was then re-analyzed for the presence of peaks (Perkel et al 1967b).

3. Results

A total of 499 pairs of multiunit clusters were recorded from the VCN of 5 rats (identified recording locations for 4 rats shown in figure 1), and tested for single tone (n = 70) or two-tone synchrony (n = 429). Using the synchrony profiles of each multiunit cluster pair (figures 2A & 2C) without shift predictor subtraction, the actual frequency at which maximum correlation occurred was noted and compared to the expected frequency of maximum correlation. In the case of single tone correlation pairs, the CF of the multiunit clusters forming each pair was taken as the expected frequency of maximum correlation, while for the two-tone correlation pairs the varied tone frequency that matched the CF of the second multiunit cluster forming each pair was taken as the expected frequency of maximum correlation. Pairs of multiunit clusters with a maximum difference of 0.2 octaves between expected frequency of maximum correlation and actual frequency of maximum correlation were classified as "Correlated near CF" (CNCF) while those pairs with a difference greater than 0.2 octaves were classified as "Correlated away from CF" (CACF). Some pairs exhibited no correlation at all stimulus frequencies; these were classified as uncorrelated (UC).

Figure 2 shows examples of pairs of multiunit clusters with similar CFs and their associated crosscorrelograms and correlation coefficients upon presentation of single tones. For a total of 70 such pairs analyzed, 46 pairs (65.7%) fell into the CNCF group while 6 pairs (8.6%) and 18 pairs (25.7%) were classified into the CACF and UC groups respectively. Mean correlation coefficient for all CNCF pairs tested with single tones was 0.172 ± 0.08 (mean ± standard deviation), comparable to those found by Eggermont (1992, 1994). However, subtraction of the overall shift predictor correlograms from the raw crosscorrelograms resulted in mostly crosscorrelograms where the peaks were no longer significant, indicating that the peaks in the raw correlograms were solely due to stimulus effects (shift predictor crosscorrelograms depicted by dashed lines in dark blue in Fig. 2D).

Stimulus dependent neural synchrony was also seen in pairs of multiunit clusters with different CFs (tested by simultaneous presentation of two tones). For this correlation test

(examples shown in figure 3), 273 of 429 pairs (63.6%) were classified as CNCF while 95 pairs (22.1%) and 61 pairs (14.3%) were classified into the CACF and UC groups respectively. Mean correlation coefficient for all CNCF pairs tested for two-tone correlation was 0.176 ± 0.09 (mean ± standard deviation), comparable to those found by Eggermont (1992, 1994). As with the case using single tones, subtraction of the overall shift predictor correlograms from the raw crosscorrelograms resulted in no significant neural correlation.

In addition, neural synchrony (without shift predictor subtraction) was found to be dependent on the electrode separation between multiunit clusters. Figure 4 summarizes the proportion of CNCF, CACF and UC pairs as a function of physical distance between pairs of multiunit clusters. Higher percentages of CNCF pairs were found when the electrode separation was less than 800 μm as compared to the CACF and UC pairs (figure 4A-C). For distances greater than 800 μm, the number of CNCF pairs decreased (figure 4A). The number of CACF pairs remained relatively unchanged with increase in electrode separation (figure 4B) while the number of UC pairs increased progressively with increase in electrode separation (figure 4C). For the multiunit clusters tested with two-tone stimuli, neural synchrony showed a similar pattern of dependence on the CF difference (calculated in octaves) between the multiunit clusters forming each pair (figure 5). The percentage of CNCF and CACF pairs were found to decrease with an increase in CF difference while the percentage of UC pairs increased significantly when the CF difference was greater than 2 octaves.

4. Discussion

This study aimed to find whether neural synchrony under stimulus conditions was a feature of sound coding in VCN neuron populations and further aimed to elucidate if the synchrony was due to stimulus effects or underlying intrinsic interactions between neuron populations. Our results indicate that the neural synchrony found in all analyzed pairs of multiunit clusters was only due to the effects of simultaneously increasing the rate of neural firing in both multiunit clusters as a result of stimulus presentation. Upon removal of these stimulus effects with subtraction of the shift predictor crosscorrelograms from the raw crosscorrelograms, no underlying neural correlation was found. The absence of any neural correlation after removal of stimulus effects in our results suggest that synchrony in VCN neuron populations is predominantly a result of common excitatory input from the AN, in the presence of a common stimulus and not through interactions or synaptic connections between neuron populations.

However, the existence of neural correlation via synaptic connections cannot be ruled out completely by this study. It has been hypothesized previously that T-stellate neurons may receive excitatory inputs from other T-stellate neurons of the VCN (Ferragamo et al 1998), presumably within the same isofrequency lamina, thus creating the possibility of neural correlation between single T-stellate neurons. Furthermore, isolated VCN neurons or discrete neuron populations that share input from the same ANFs should have some degree of neural correlation, however our results did not show this. This could be partly related to the fact that we made use of multiunit activity to estimate neural correlation. Hence the crosscorrelograms in this study would most likely be calculated from a complex mixture of responses from multiple neuron types. We therefore cannot comment on the neural correlation arising from spontaneous activity between particular neuron types either due to synaptic connections between themselves or because of shared ANF input without the presence of a driving stimulus. Given the nature of multiunit activity in our study and the likelihood of summing of stimulus-evoked spikes, it was not possible to sort the multiunit clusters from our data into

single neuron activity. It remains to be tested then if indeed isolated neurons within each individual VCN isofrequency lamina show neural correlation among themselves through spontaneous activity without the presence of any stimulus, or after stimulus corrections have been applied. The main purpose of this study was to determine if VCN neuron populations would show stimulus-dependent neural synchrony and whether this synchrony was due to intrinsic neural interactions or solely due to stimulus effects. Thus multiunit recordings served this purpose. In addition, although multiunit activity cannot distinguish between neuron types, Bedenbaugh and Gerstein (1997) using cross-correlation techniques in rat somatosensory cortical recordings suggested that cross-correlation with multiunit activity can be a more sensitive detector of relationships between neurons compared to single-unit cross-correlation. As a result we cannot rule out the existence of neural correlations in the VCN that are exclusive of stimulus driving, however our results have shown that in the presence of a stimulus, these correlations are not seen and that the effects of stimulus driving are dominant under the conditions of this study.

The implications of having solely stimulus-induced neural synchrony and no neural correlation via intrinsic interactions between different neuron populations of the VCN could perhaps motivate the need to change the existing design of ABIs that aim to restore hearing in patients with Neurofibromatosis Type 2 (NF2) vestibular schwannomas. The present commercial ABI results for open-set speech perception are far from those of CI users (Kuchta 2007, Otto et al 2008). Even recent results from a clinical trial in ten patients with a penetrating electrode in the cochlear nucleus (Otto et al 2008) and another trial in three patients with a penetrating electrode at the level of the inferior colliculus (IC) (Lim et al 2007) have not shown any better open-set speech perception in NF2 patients than those obtained with the ABI. It has been hypothesized that perhaps ABIs are not well suited to NF2 patients owing to the compression of the VCN (Crea et al 2009) or more specifically due to the destruction of specialized neurons that are crucial to speech perception as these devices when fitted in some non-tumour patients show exceptional results in terms of speech

perception (Colletti and Shannon 2005). It is more likely however, that the stimulation strategy and the electrode design of the ABI need to be altered as present ABIs as well as the auditory midbrain implant at the level of the IC use conventional stimulation strategies more suited for CIs. One of the possible drawbacks of present ABI stimulation strategies is that they do not incorporate multi-site stimulation within VCN isofrequency laminae. The results from this study showing stimulus induced synchrony between similar CF neuron populations suggest that stimulating only single sites within VCN isofrequency laminae would not represent synchronous patterns in VCN neurons as seen with acoustic stimulation and it may not be enough to activate the necessary pathways required for pitch perception. It is possible that stimulation of multiple areas of each VCN isofrequency lamina may be required to reach a certain threshold for adequate information transfer to higher centers for pitch perception, thus mimicking the synchronous common input found with acoustic stimulation. Furthermore, given that we found neural synchrony in similar CF multiunit pairs up to 800 μm apart, our results indicate that as long as multiple regions of each VCN isofrequency lamina are stimulated simultaneously through either several small electrode sites in each lamina or a macro-electrode site along each lamina, it could improve speech perception for a PABI.

In conclusion, these results suggest that frequency or pitch coding in the VCN may predominantly be mediated through location of neural firing, neural firing rates and precise timing of neural firing either exclusively or in a combination. Neural interactions intrinsically within the VCN may perhaps not be a frequency coding mechanism. Neural correlation like that found in the auditory cortex for example, would more likely aid in population coding of species-specific complex sounds such as speech and animal vocalizations (Eggermont 1994). Furthermore, to account for this stimulus induced synchronous nature of VCN neurons, ABI stimulation strategies may need to be altered by including more sites in individual isofrequency laminae and implementing simultaneous stimulation of these multiple sites to ensure adequate information transfer to higher levels for improved speech and pitch perception.

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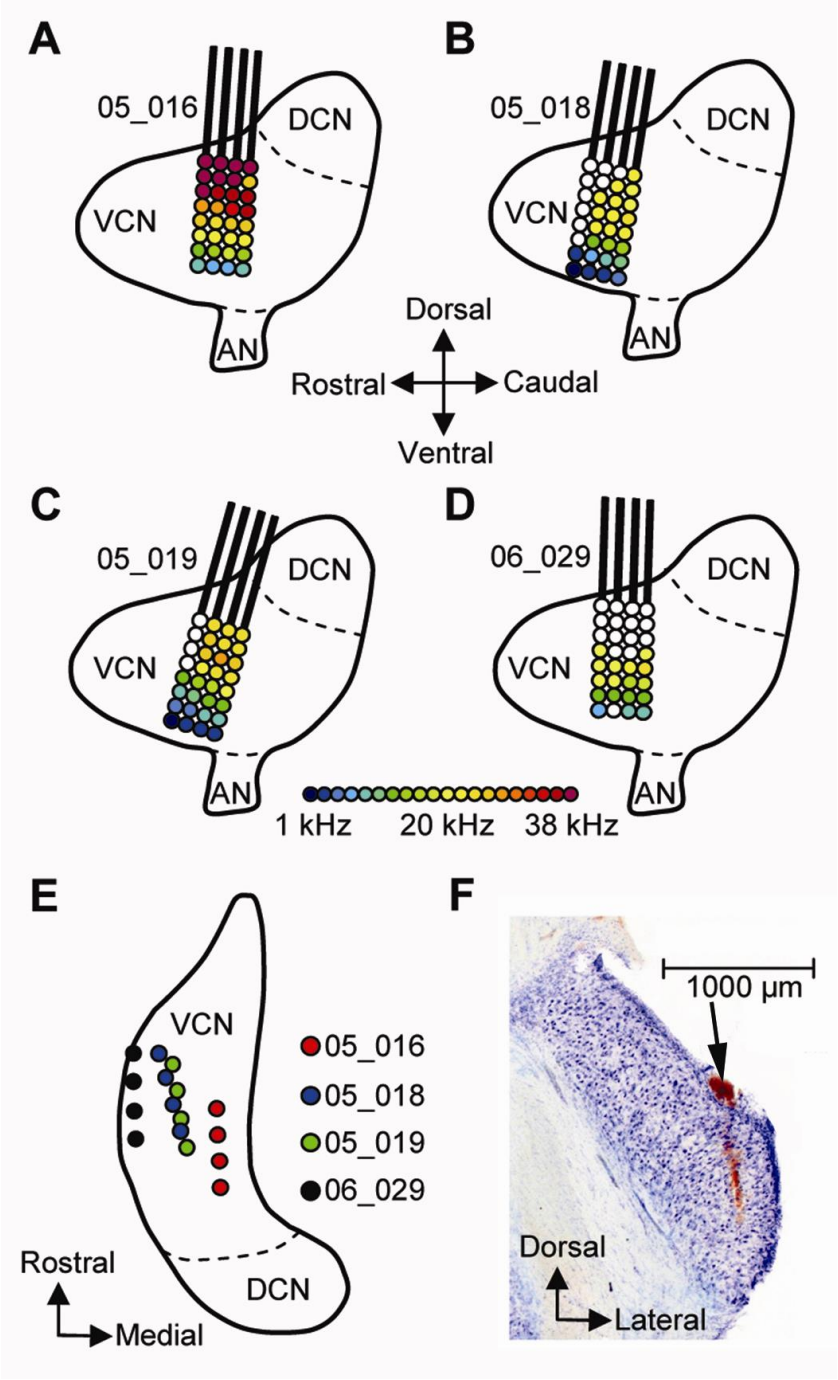

Figure 1. Electrode placements and tonotopic maps. (A-D) Schematic diagrams of the cochlear nucleus (CN, parasagittal view) indicating location of electrode placements in four animals. Dashed lines indicate approximate extents of the DCN, VCN and the AN. The CF of the multiunit cluster recorded at each site is depicted by the color gradient (1 kHz, blue; 38 kHz, red; no multiunit activity is shown as white). (E) Dorsal view showing electrode penetration points for each of the experiments in (A-D). (F) Histology depicting example of an electrode track through a coronal 60 µm section of the CN (arrow).

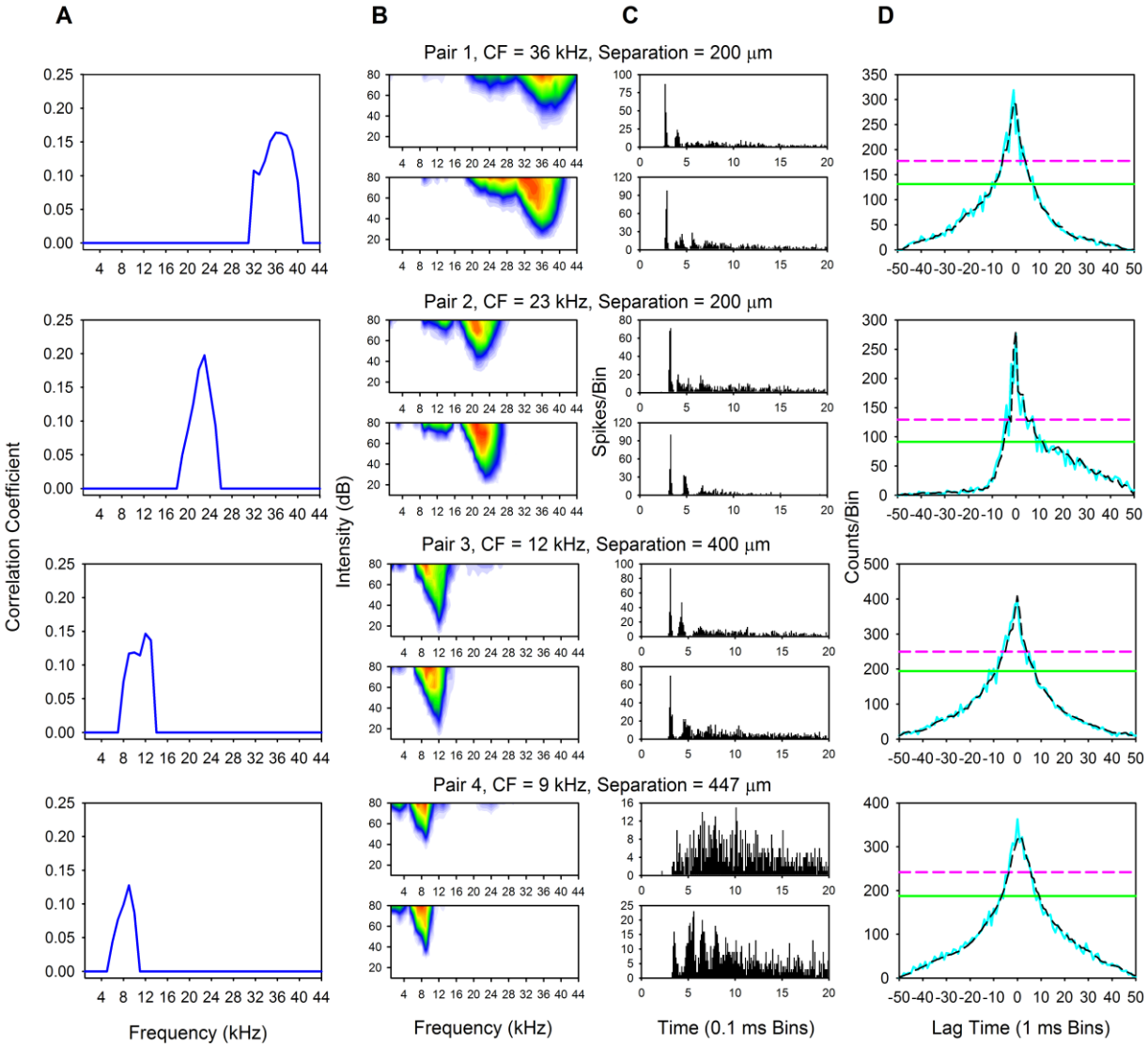

Figure 2. Four examples of pairs of multiunit clusters with similar CFs. (A) Correlation coefficient as a function of stimulus frequency for each of the examples. (B) Response areas for each of the multiunit clusters indicating pairs with similar CFs. (C) Post-Stimulus Time Histograms (PSTHs) for the first 20 ms of the stimulus duration (Bin width = 0.1 ms). Note chopping behavior in some PSTHs indicating some presence of T-stellate neuron activity. (D) Raw crosscorrelograms for the multiunit cluster pairs indicating peaks within a 10 ms lag time (Blue lines, Bin width = 1 ms). Dashed lines in dark blue indicate the shift predictor crosscorrelograms obtained by averaging all possible trial shifts. Green lines indicate the expected value (E) of correlated firing under the assumption of the independence of the two spike trains. Significance levels for the raw crosscorrelograms (E + 4*s.d.) are indicated by pink dashed lines.

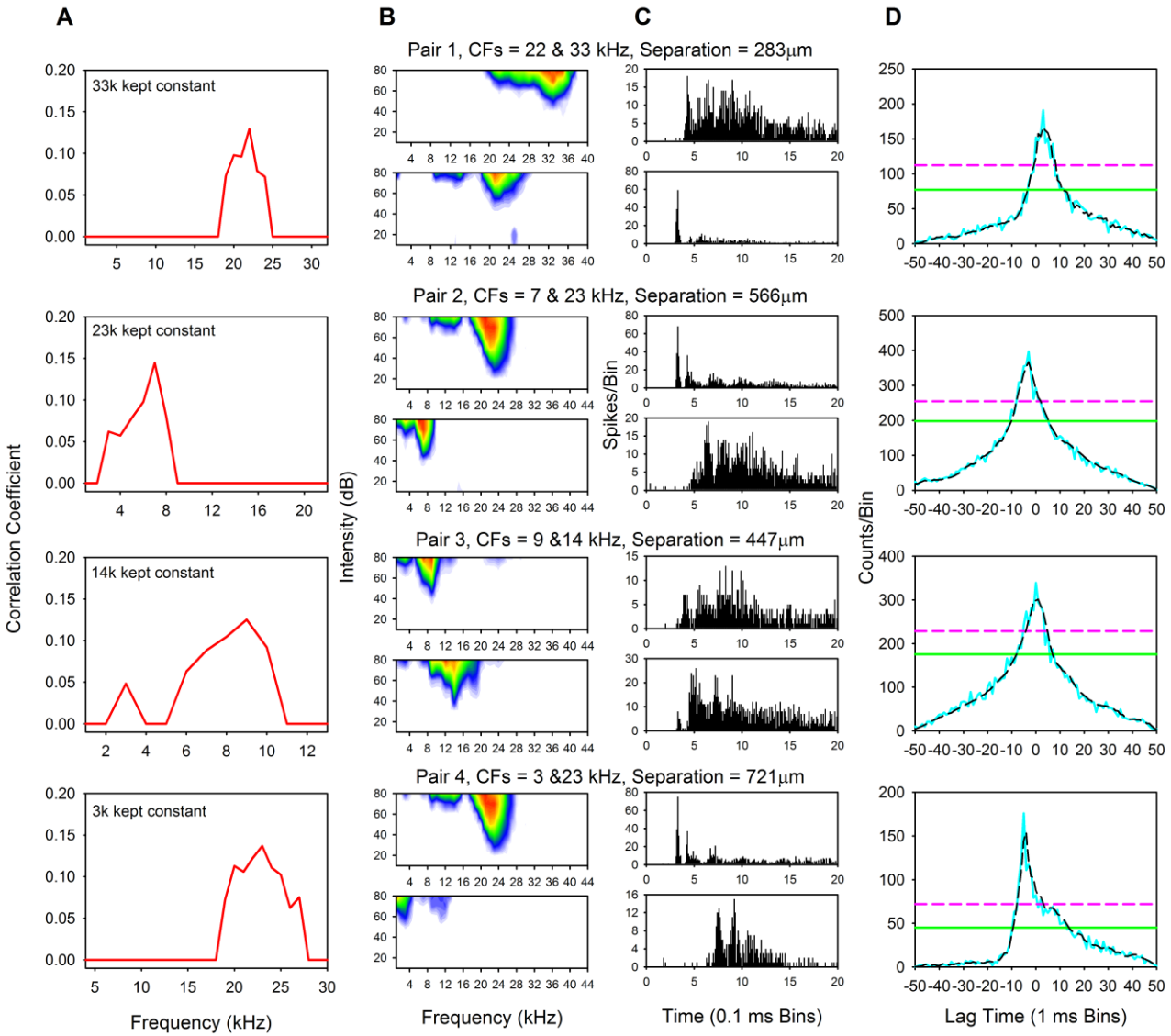

Figure 3. Four examples of pairs of multiunit clusters with different CFs. Panels are outlined as in Figure 2. For the two-tone stimuli pairs, one tone frequency was kept constant and the other tone frequency varied from the frequency range tested (1-44 kHz). Both tones were presented at 65 dB SPL.

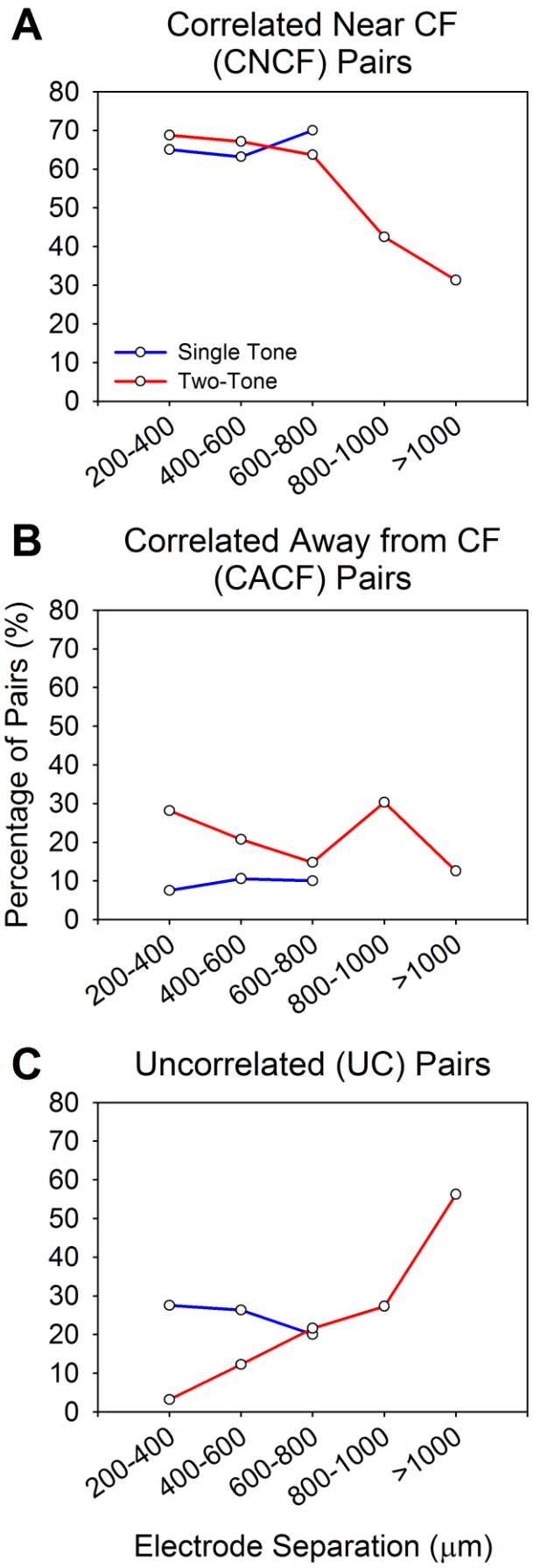

Figure 4. Dependence of neural synchrony on electrode separation. Percentage of (A) Correlated near CF (CNCF) pairs, (B) Correlated away from CF (CACF) pairs and (C) Uncorrelated (UC) pairs according to the electrode separation where the multiunit clusters were recorded (blue lines, single tone; red lines, two-tone).

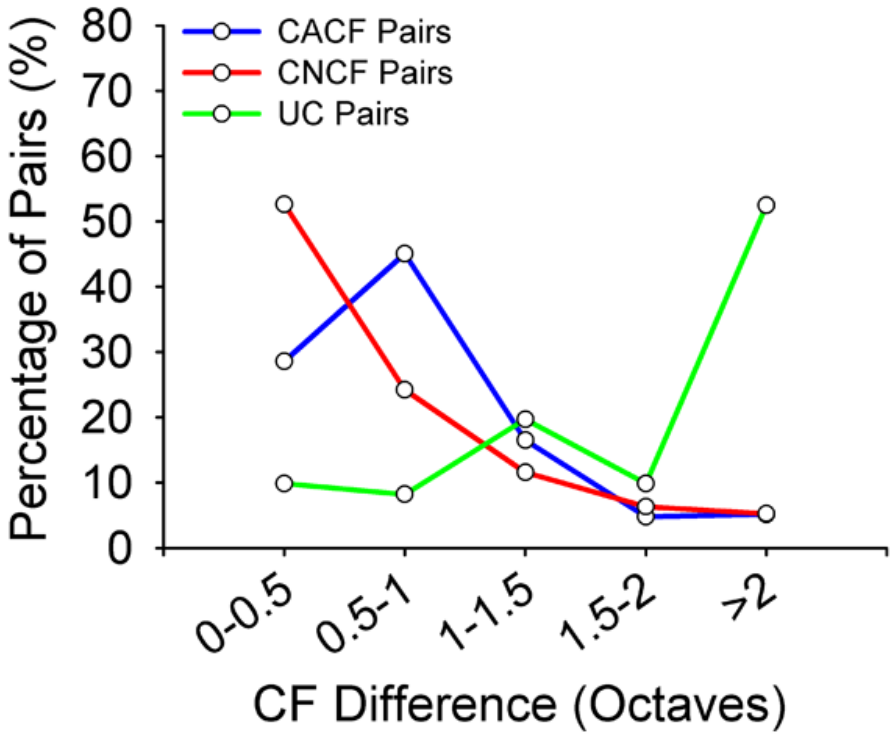

Figure 5. Dependence of two-tone neural synchrony on CF difference. Percentage of Correlated CNCF, CACF and UC pairs tested with two-tone stimuli according to the CF difference (octaves) between the multiunit clusters forming the pairs.

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