Tinnitus-related pathophysiology in the cochlear nucleus of the cat

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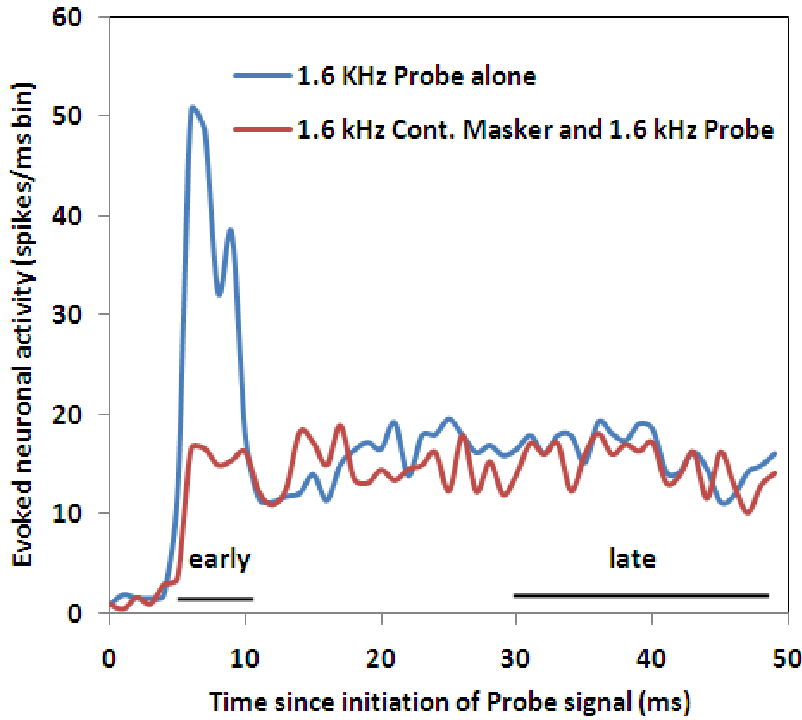
Animal implants.

Two cats were chronically implanted in the ventral cochlear nucleus (CN) with a silicon-based array, consisting of 4 shanks, with 2 shanks in the rostral-caudal row and 2 rows arranged medially and laterally. Each shank has 4 sites, spaced dorso-ventrally. Overall, there are 16 recording sites in the implanted array. The impedance spectroscopy records, obtained at 2-4 months post-implantation show little change in the total impedance (~300 kOm at 1 kHz), indicating the long-term stability of the electrode-tissue interface. We were able to record spontaneous single-unit activity and acoustically-evoked multi-unit neuronal activity from most of the electrode sites in the array. These animal will be subjected to the narrow-band acoustic trauma, followed by physiological evaluation of tinnitus, using our novel approach described below.

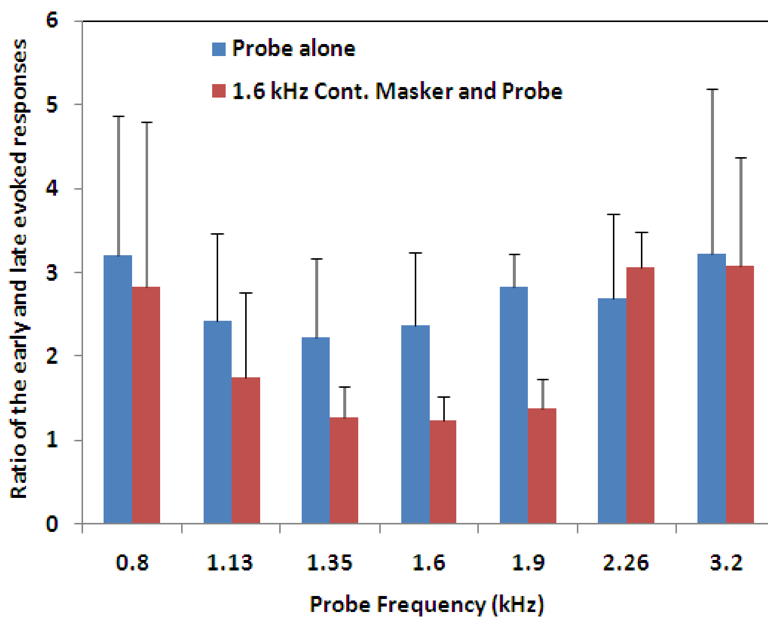
Physiological evaluation of tinnitus.

The method relies on physiological phenomenon of gap detection, called *forward masking*, which requires absolutely no training or aversive stimuli and can be conducted even under light general anesthesia. The method consists of applying an acoustical stimulus, the probe signal. When another sound, such as the tinnitus phantom tone, is simultaneously present in the CN neuronal circuitry, it produces the forward masking effect on a subsequent probe signal. This approach can be easily evaluated in normal animals by substituting a tinnitus percept with a continuously applied sound (the masker).

We have evaluated the sensitivity of the forward masking method in one normal-hearing implanted cat, anesthetized with Propofol. Using the recording site #4, we detected broadly tuned activity, ranging ± one octave from 1.6 kHz. We then simulated the tinnitus by producing a continuous tone at 1.6 kHz with an amplitude of 70 dB SPL at the cat's pinna and evaluated our ability to detect this pseudo-tinnitus by varying the frequency of the probe signal, also delivered at 70 dB SPL. The responses to probe alone versus probe with masker applied at the same frequency were recorded (Figure 1.A). These responses were subdivided into the early (6-11 ms) and late (30-49 ms) phases, relative to the onset of the probe, and confirmed that forward masking was evident only during the early phase. The 6-ms delay until the early response is due to auditory signal propagation from the speaker to the CN. Then, forward masking was evaluated for a range of frequency shifts between the Probe and Masker and found it to be effective for one quarter-octave shifts, but not for one octave shift (Figure 1.B).



A



B

Figure 1. Evoked multi-unit neuronal activity recorded using chronically-implanted silicon-based array in the feline CN on channel 4 following acoustic stimulation with either Probe alone or 1.6 kHz continuous Masker combined with Probe. (A) Example of the evoked activity following stimulation with a Probe frequency of 1.6 Hz, identical to the frequency of the Masker. Evoked neuronal activity was measured as the post-stimulus time histogram of the total number of spikes per 1-ms bin during 30 consecutive presentation of the Probe stimulus. (B) Analysis of a series of acoustic tests, with the Probe frequency ranging ± one octave from the Masker frequency. Error bars indicate standard deviations of the responses to each of the 3 repetitions of the test.

Analysis of synchronous neuronal activity in CN

An important measure of tinnitus is the appearance of broadly-synchronized activity in the CN. We have developed a procedure for conducting such an analysis. The analysis begins by generation of the cross-correlation histograms (CCHs) for each pair of active neuronal units and computation of their *maximum peak values* and the *time lags* relative to the stimulus. In evaluating the large number of CCHs, it becomes very important to discard weak and presumably less important correlations in order to better visualize the strong ones, the task that is commonly done using clustering algorithms. The functional significance of clustering lies in a high degree of temporal correlation of spiking activity within each cluster. Among the many methods for characterizing the interactions in large populations of neurons, we have examined the five most commonly-used visualization and clustering methods: 1) correlation linkage mapping; 2) hierarchical clustering, 3) gravity method, 4) meta-k-means, and 5) multiscale data mining approach. The key features of each method, such as its ability to reduce the N-dimensional complexity and its flexibility (i.e. adaptability and self-adjustment for individual data sets, thus reducing bias in the results) are presented below (Table 1).

Method	Reduction of N-D complexity	Flexibility	Limitations
Correlation linkage mapping	None	None	Preserves complexity
Hierarchical clustering	2-D	Minimal	A priori optimal cluster number
Gravity analysis	2-D or 3-D	Some (time lag correction)	A priori optimal cluster number
Meta-k-means	2-D	Adaptive	Multiple local minima
Multiscale data mining	2-D	Highly adaptive	

Table 1. Comparison of visualization and clustering methods used in analysis of correlations for large populations of neurons.

Among the evaluated methods previously applied for partitioning of the neurons into clusters, the multiscale data mining approach appears to provide the best performance for large numbers of neurons and superior adaptability to time variance in the neuronal discharge probability. However, none of the methods can effectively separate the stimulus-induced correlations from the trial-to-trial variability arising from animal's movement, respiration, or heartbeat. To overcome these limitations, the recording will be repeated in multiple trials and under light Propofol anesthesia.

PEP19 immunohistochemistry in the CN

In order to localize the electrode site locations relative to the populations in the CN, we have used a neuronal marker PEP19. This marker is very useful for observing the general anatomy of the CN at low magnification (Figure 2) as well as for identification of the morphological features of individual cells at high magnification (Figure 3). In the anteroventral CN PEP19 selectively stains neuronal populations of spherical and globular bushy but not granule cells; in the posteroventral CN it labels multipolar and octopus but not granule cells; and in the dorsal CN it strongly labels cartwheel and fusiform cells. Therefore, a combined use of this immunomarker

with the neural recording data can associate neuronal activity with specific populations in the ventral and dorsal CN. The importance of morphological identification of the cell types is especially important for the ventral CN, because the multipolar cells (the primary cell type) are intermixed with spherical and octopus cells, and these three cell types have broadly varying functions, based on their physiological properties.

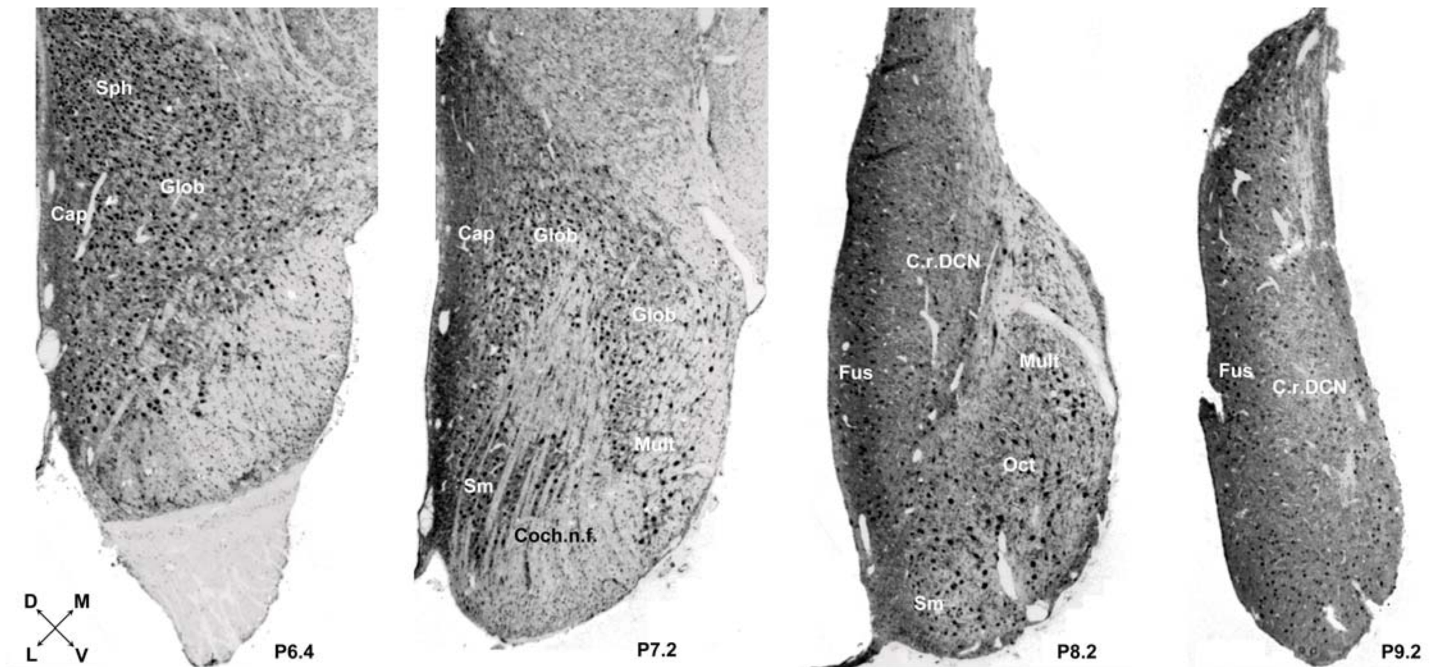


Figure 2. Microphotographs of serial transverse sections through the feline CN immunostained with PEP19, arranged from rostral (P6.4) to caudal (P9.2). Abbreviations: Coch.n.f. – cochlear nerve fibers, C.r. –central region, D – dorsal, Fus – fusiform cell layer, Glob – globular cells, L – lateral, M – medial, Mult – multipolar cells, Oct – octopus cells, P – posterior, Sm – small cells, Sph – spherical cells, V – ventral.

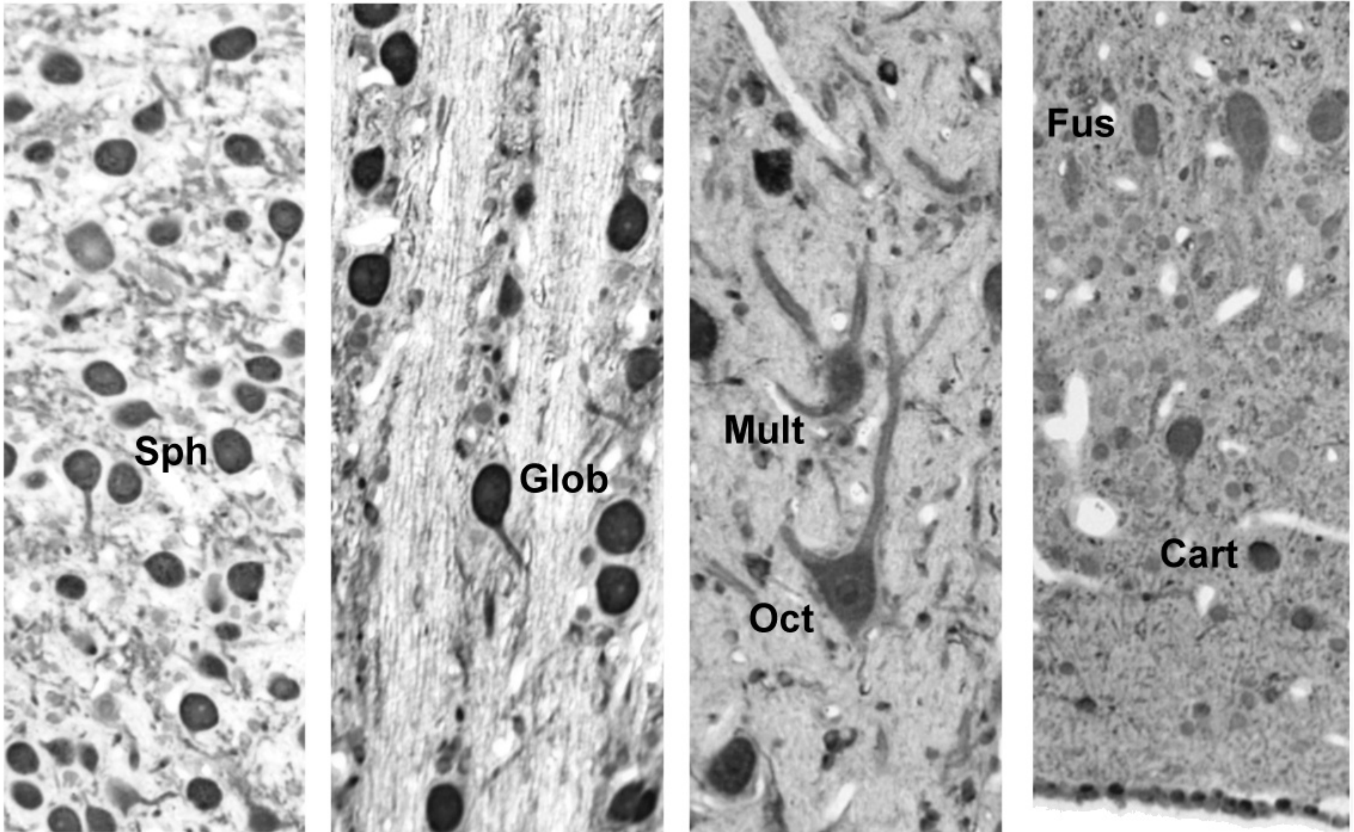


Figure 3. Morphologies of major cell types in dorsal in ventral CN immunostained with PEP19. Abbreviations: Cart – cartwheel cells, Fus – fusiform cell layer, Glob – globular cells, Mult – multipolar cells, Oct – octopus cells, Sph – spherical cells.

Summary.

In the course of this year-long project, we have made advances toward all objectives of the project. Specifically, we have implanted two animals with the silicon-based microelectrode array in the ventral CN and were able to record from many electrode sites in the array. We have confirmed the suitability of our forward gap masking method for detecting simulated tinnitus condition. We have also examined the existing methods for detecting synchrony in large neuronal populations and selected the the multiscale data mining analysis, which allows determination of both spatial and temporal features of the synchronous neuronal firing and provides superior adaptability to time variance in the neuronal discharge probability. Finally, we have used the CN tissue from previously implanted animal to evaluate the PEP19 immunohistochemistry. We found it to be useful in locating major subdivisions of the ventral and dorsal CN and in morphological identification of the major cell types in the CN.