Harvard-MIT Division of Health Sciences and Technology
HST.723: Neural Coding and Perception of Sound
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Cochlear Nucleus Unit Classification from Spike Trains

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READ THE ENTIRE INSTRUCTIONS BEFORE PROCEEDING

In this laboratory, you will classify single units recorded from the cochlear nucleus on the basis of their PST and interspike interval histogram shapes. The data were recorded from an anesthetized preparation in an experiment performed by Dr. Peter Cariani. You will be provided with a tape recording of spikes and a computer program that enables you to construct the histograms. You will work in groups for the lab, but please write separate write-ups of your results. For your lab write-up, please **limit the text to 4 pages** (excluding figures).

Lab write-ups are due Friday April 4.

I. Equipment Set-up

The equipment for the lab is in Chamber 4 of the Eaton-Peabody Laboratory. A Wiring Diagram for Chamber 4 Hardware is shown below. Verify that the connections shown in solid black lines are made. Other wiring is part of the Chamber IV standard set-up and should not be changed.

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due to copyright
considerations.

Oscilloscopes:

Tektronix TDS 2014: Triggers when stimulus gate indicates presentation of a tone burst.

Tektronix 2213A: Triggers when a spike is sent to Event Timer. When a waveform causes a trigger to be sent to the Event Timer, it appears with a bright "hat" on top of the waveform. You control this triggering by adjusting the Triggering Gain Control on the Audio Monitor. 1 V/div (Channel 1) and 2 msec/div (time base). Triggering: Ext.

Audio Monitor:

The Triggering Gain Control is adjusted until the Tektronix 2213A oscilloscope triggers, indicating that a spike has been sent to the Event Timer. Adjust this Gain so that you are triggering on every spike but not so high that you are triggering on the noise (Note: tape must be playing to adjust this level). This adjustment will have to be made for each unit, and sometimes for each data run on a unit. High- and Low-pass Filters may help eliminate some of the noise. Try different filtering and see how it affects the triggering and resulting histograms.

Audio Volume:

Adjusts gain of audio amplifier for listening to spikes via the speaker.

Spike Trigger and TTL Pulse Monitor:

Accepts an input signal (spikes or Stimulus Gate) and outputs a pulse when the input exceeds a certain voltage. You should not have to adjust their triggering level. Instead, control the triggering by adjusting the Triggering Gain Control of the Audio Monitor.

Event Timer:

Accepts stimulus onset triggering pulses on Channel 0 to start the PST histogram, and spike triggering pulses on Channel 1 to count the responses.

II. Tape

Insert into the Magnavox VCR the tape "Master #2 CN Recordings 8/93". Rewind the tape. Reset the tape counter by pressing the Counter button, then the Reset button on the VCR. See chart on UNIT DATA page to find the tape counter locations for your units. Remember that the tape counter locations are only approximate and may be off by as much as 0010 or 0020, especially if you do a lot of starting and stopping. To be sure that you have the right unit, make sure that there are the right number of "runs" (see below), the correct amount of spontaneous activity, and if possible (i.e., for low-frequency runs), that the tone bursts are at the correct frequency.

The tape has three simultaneously recorded channels:

Channel	Information
1	Spikes from single-unit micro-electrode
2	Voltage delivered to earphone to produce the tone burst *
3	Stimulus Gate (square wave going positive at the start of the tone burst)

*Due to the limited bandwidth of the tape recorder, this voltage is too small to be seen for high frequencies.

The data for each unit are on tape between certain tape counter readings (see UNIT DATA page). The data are arranged as follows: For each unit, there is a series of just over 100 or 200 tone bursts presented at the Start Level. Then there is a 3 sec. pause. Then there is another series at the Start Level plus the Incr. Level. There is another 3 sec. pause. This sequence continues until the Stop Level is reached. In between units, there is a "blip" on the recording before the next unit's recording starts. Tone bursts are on for 50 msec, and then off for 50 msec. All tone bursts are presented at the unit's CF.

III. Software

To bring up the histogram program:

On the Dell computer, double click on (to open) the following:

1. Experiment VIs
2. MCB VIs
3. HST 722 Lab
4. INT & PST Histogr…

LabVIEW (an icon-based programming language) will start, and the program (VI or virtual instrument) "INT & PST Histogram 3 (LV3)" will start and display its Front Panel. Numbers in boxes (digital controls) can be changed by "highlighting" them by running the mouse over them and then typing new numbers. Use the following parameters:

Num Sweeps: Enter either 100 or 200, depending on how many Repetitions per Level are on the UNIT DATA page for your unit.

INTERVAL: Num Bins: 200, Bin Width 100 msec

PST: Num Bins: 1000, Bin Width: 100 msec

The x-axis (time axis) is Bin Width * Num Bins so it will be 100 msec for PST and 20 msec for INT.

The best time to start the program is during the 3-sec. pause between tone-burst runs. Start the program by pushing the run arrow (second box from left in upper left menu). A stop-sign will form that can be pressed to stop the program. The program will execute until it has received the Num Sweeps specified or until you press the stop sign (note: the data will not be saved if you press the stop sign). The Overflow bins count the number of spikes that were not within the time windows for each histogram. After the program has stopped you can enter Notes (like the unit number) in the Notes window.

To Print (on the Chamber II printer): File-> Print Window.

IV. Correct Triggering

When a waveform causes a trigger to be sent to the event timer, it appears with a bright "hat" on top of the waveform on the Tektronix 2213A oscilloscope. When the triggering is set correctly, each spike should have the same waveform. "False" triggers, often from the triggering set too low in amplitude, are evident when some triggered waveforms look different than others. Also watch out in some recordings for a "gross" response, an evoked potential that is picked up by the electrode. Contamination with a gross response will make correct triggering difficult. If the contamination has lower frequencies than the spikes, the recording can be improved by high-pass filtering (adjust filters on Audio Monitor). If you are unsure how to obtain correct triggering, consult the instructor.

V. Construction of histograms and write-up

Play the tape for your unit to be sure that you have the right unit and the correct triggering level. Even for one unit, you may have to adjust the triggering level from run to run.

For each of your units, investigate the following:

1. *Spike quality:* Play the tape through to get an idea of the unit's spikes. Make a judgment of the "quality" of the spikes of your unit ("a", "b", or "c" on RATING page). This judgment is a measure of your confidence in the triggering. During construction of your histograms, make note of whether you have any false triggers, or whether any of your spikes were missed.
2. *Classification*: For each of the units assigned to your group, construct PST and interspike interval histograms. Use the lecture hand-outs to classify the units according to histogram shape. Classification is generally done at sound levels 30 dB above threshold. Please specify the unit type and write a sentence or two why you chose that particular unit type. For phase locked units, if the phase locking is so strong that it obscures the PST, it is OK to write "Phase-locked", but if there are some clues and you can make a guess at the unit type, it is better to write "Phase-locked, probably a XXX Unit".
3. *Sound Level:* On one of your units that is not a "Primarylike" unit, study the effect of sound level on PST shape.

Questions to answer in your write-up (READ 2-5 WHEN DOING THE LAB):

1. Alongside your unit classifications in your write-up, state the anatomical cell types that are expected for each of your units.
2. There are several units that are difficult to classify. In rare cases, this is because the recording is made simultaneously from two units. Sketch a PST that might be expected if the recording is simultaneously a Chopper unit and an Onset unit. By looking at the individual spikes on the oscilloscope, how might you know that you were recording simultaneously from two different units?
3. Let's consider a special case of a unit with CF equal to 1 kHz that you suspect is a Pri-notch unit, but which has such strong phase locking that the notch is obscured. How might you specify the bin width so that the notch becomes more visible?
4. From looking at your histograms, how might you determine that there were false triggers?
5. What problems do you encounter in using the runs where the tone bursts were presented at high sound levels?
6. What is the difference between a Pri-like unit and a Pri-like PST? Hint: Use your study of the effects of sound level on PST shape.
7. The recordings from your units were made with large-tipped, gelatin-filled pipette electrodes. These may record pre-potentials. If you were searching for a prepotential unit, how would you adjust the triggering to find it? When you found a prepotential unit, what type of PST histogram would you expect it to produce?
8. In the entire data set, there are about 7 Choppers, 8 Primarylike, 2 Onset, and 1 Pauser Units. Suggest the subdivision (AVCN, PVCN, or DCN) of the cochlear nucleus in which most of the units were recorded.