Homework Assignment 3

*** Due Thursday 10/30/14 by noon on Sakai under HW3. Upload your submission as a Word document. ***

Please reaffirm the Duke Community Standard at the top of your assignment:
"I have adhered to the Duke Community Standard in completing this assignment." [Electronic Student Signature]

Please explain your methods, show all work, explain your results, include units, and label your axes.
Upload your .hoc and .m files with clear filenames.

TOTAL: 105pts

The objective of this assignment is to determine the effect of electrode geometry on the recorded compound nerve action potential (CNAP) and electroneurographic (ENG) signals. You are using a tripolar recording electrode with interelectrode spacing of *k* and the first and third (end) electrodes shorted together (averages potentials at these points) and acting as the reference. You will construct a virtual nerve bundle and use this to study the recorded signal. For simplification consider that your recording electrodes are simply point sources, that all fibers in your bundle are at the same "vertical" distance (r=1 mm) from the electrodes.

CNAP (compound nerve action potential) is an umbrella term that includes ECAP (evoked compound action potential) and ENG (electroneurogram). ENG is recording the intrinsic/natural activity of a nerve, whereas ECAP is recording the nerve's response to a stimulus (so you "evoked" the compound AP).

Part 1 – The Source: Transmembrane Current during an Action Potential

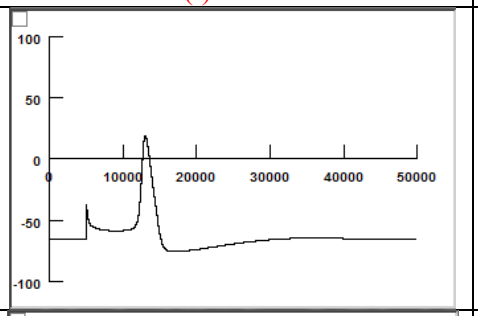
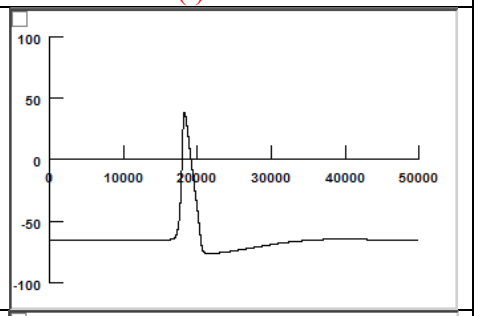
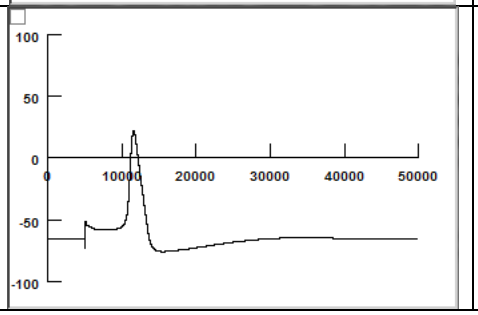
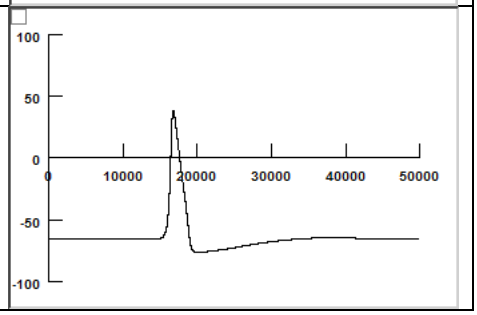
Start with your code from Homework 2, Part 5. I suggest using dt=20us to reduce the computational demands, particularly for later parts. We will record the total transmembrane current at a node during propagation of an action potential.

ANSWER: [20pts]

a. How can you do this so that your recorded currents are not influenced by the stimulation current applied to initiate the action potential? Explain. Show plots to demonstrate that your recording does not have stimulation artifact.

Multiple ways to reduce stimulation artifact: [3pts for explanation and 2pts for plots; 5pts total]

- Staying near threshold. I used my threshold values as per HW2, Part 5.
- Record "far enough" away from stimulating electrode. But how far is far enough? Could estimate required distance analytically (knowing RC time constant, etc.), or instead, ensure that chosen recording site exhibits no passive response. Thus, I stimulated above node 5, and then plotted Vm(t) at nodes 6 and 48 for both the smallest and largest diameters (although the former should be a stricter condition, since I'm fixing the number of nodes between stimulation and recording, rather than fixing the distance). Note that I did not record at the end node (node 50) to avoid end effects (Vm(t) driven by first difference in Ve rather than second difference in Ve at the end).

	Vm(t) at node 6	Vm(t) at node 48
D=2um		
D=20um		

b. Briefly explain how you recorded Im(t) in NEURON.

Recording Im(t) [5pts]

There are at least three ways to record Im(t) in NEURON: **[3pts for explanation and 2pts for area; 5pts total]**

1. Record i_membrane (mA/cm^2) of the extracellular mechanism.
2. Record i_na, i_k, i_leak, i_cap.
3. Record m_hh, h_hh and n_hh to compute the sodium and potassium currents, and compute the capacitive current using the time derivative of Vm(t).

I'll use the first method. The extracellular mechanism's i_membrane is in mA/cm^2 (as given in the Programmer's Reference). But we want total current. So before saving the currents to a file, I'm going to scale everything by the area of node 48 (i.e. the node where I'm recording i_membrane). Again, there are two possible ways to compute this area:

1. Manually (pi*node_diam*node_length).
2. Using the area function:

```
access node[48]
area(0.5)
```

I used the latter method, and converted the units of current to nA before printing the results to file. I then loaded the data and created the required plots using Matlab.

c. Plot the <u>total</u> transmembrane current for a node as a function of time for <u>2 ≤ D ≤ 20µm</u> in steps of 2µm with all curves on the same plot.

Im(t) plots for 10 D values: **[5pts]**

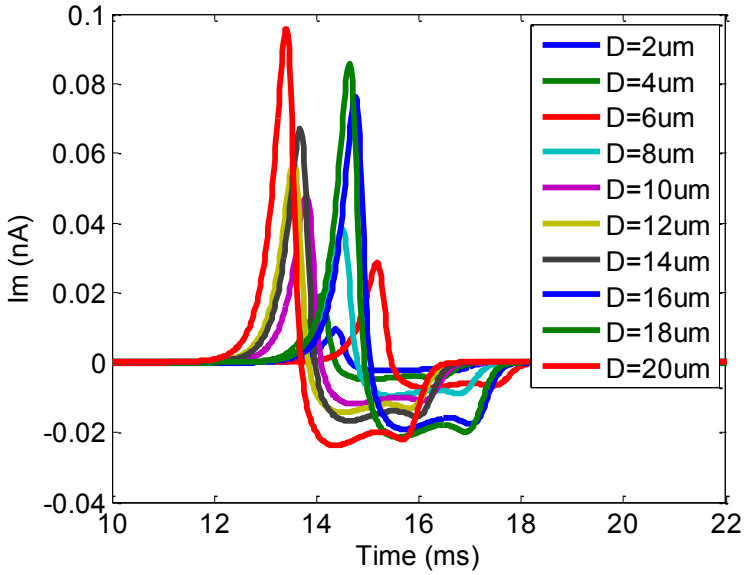


Figure 1 - Recorded at node 48 with stim.amp=threshold.

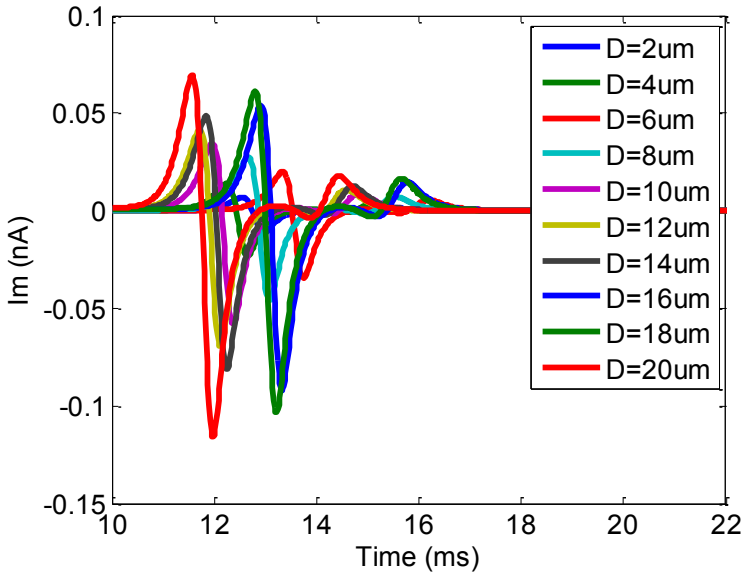


Figure 2 - Recorded at node int(num_nodes/2) with stim.amp=threshold.

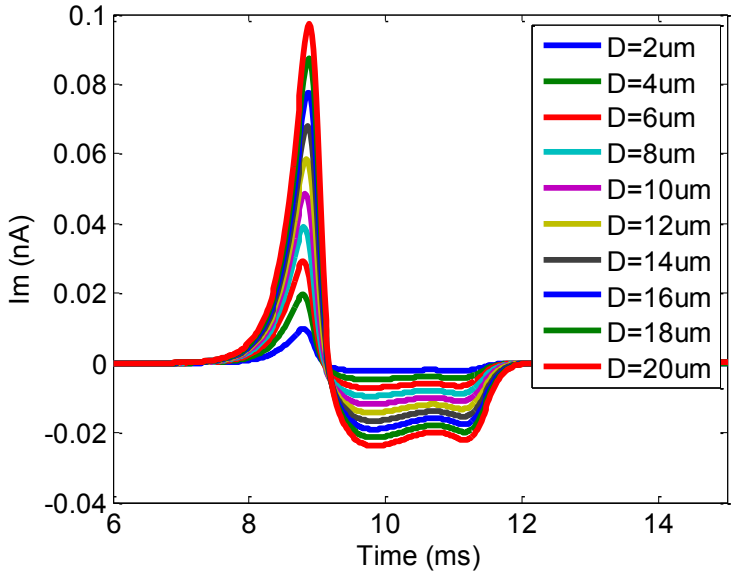


*Figure 3 - Recorded at node 48 with stim.amp=2*threshold.*

Note that the currents are time-aligned when using a sufficiently suprathreshold stimulus (2*thresh in this case), whereas when you're right at threshold, the AP initiation time is inconsistent with respect to the stimulus start/end time.

d. Plot the peak positive current as a function of diameter D for the range. On your plot, include a trendline and the trendline's equation.

max(Im) plot: **[1pt for showing trendline, 1pt for trendline equation, 3pts for data; 5pts total]**

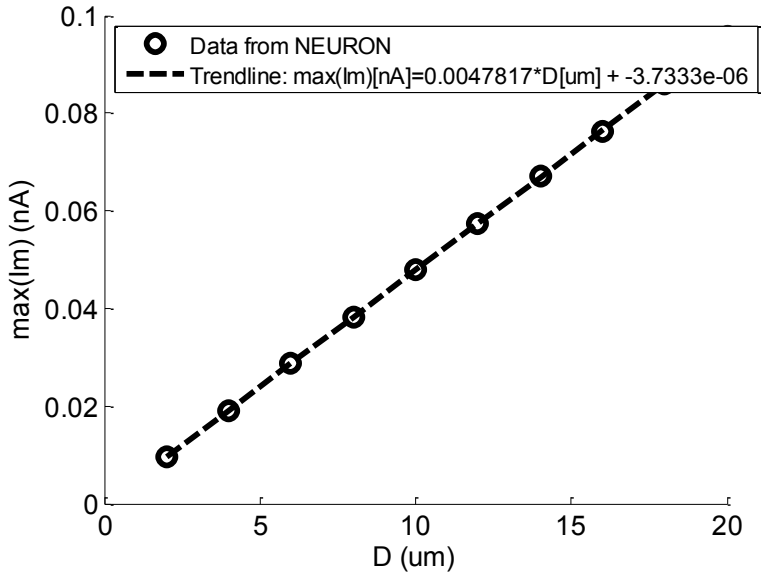


Figure 4 - Recorded at node 48 with stim.amp=threshold.

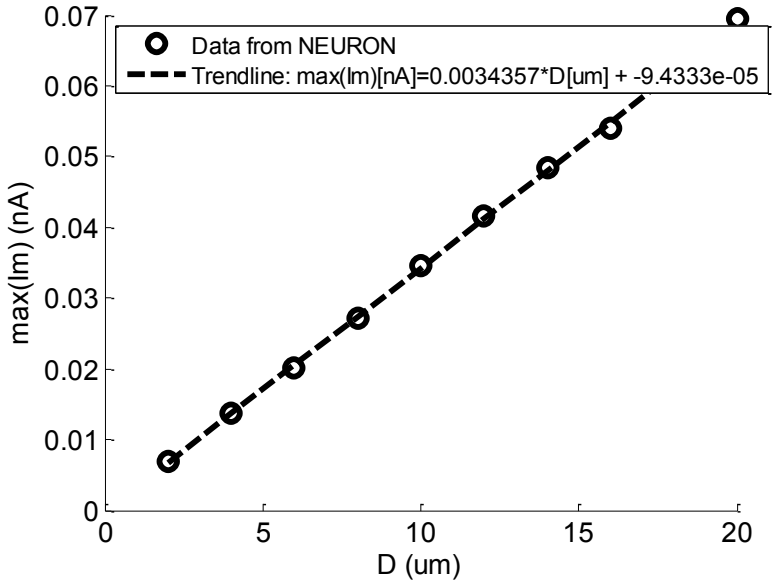


Figure 5 - Recorded at node int(num_nodes/2) with stim.amp=threshold.

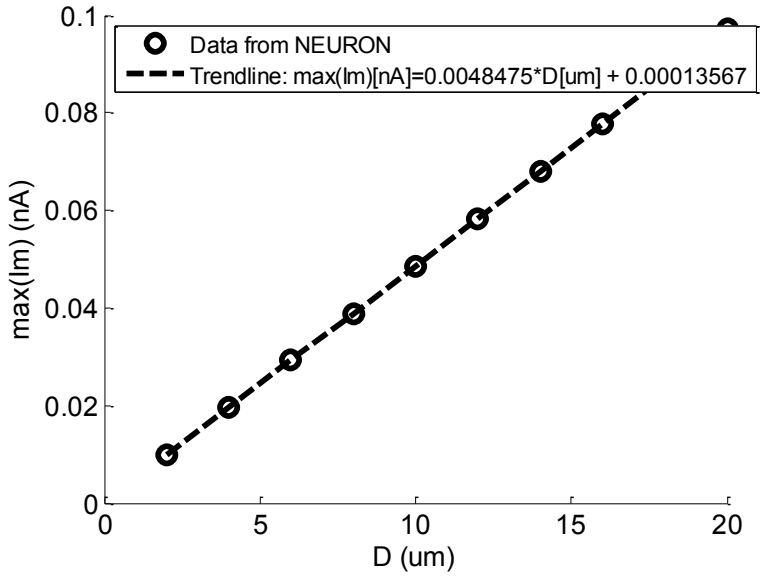


*Figure 6 - Recorded at node 48 with stim.amp=2*threshold.*

Part 2 – Conduction Speed

We're going to use NEURON's NetCon ("network connection") objects to monitor spike times. The following code will monitor spike times at all nodes:

```
objref nc[num_nodes], spiketimes[num_nodes], nil
for i = 0, num_nodes - 1 {
        axon[i] nc[i] = new NetCon(&v(0.5), nil)
        nc.threshold = ap_thresh
        spiketimes[i] = new Vector()
```

```
        nc[i].record(spiketimes[i])
}
```

Note that NetCon's are usually used for synapses, but here, we're just using their functionality to record spike times. Some other useful lines of code:

```
spiketimes[<insert node number>].size()
spiketimes[<insert node number>].x[<insert spike number>]
```

Determine the conduction speed for each of fiber diameter: D=2 to 20um with Δ=2um. I suggest placing your electrode closer to one end, and recording far enough away from your stimulating electrode; otherwise the passive response will compromise the accuracy of your spike times for the purpose of conduction speed calculation.

ANSWER: [20pts total]

a. Briefly explain how you determined the conduction speed (e.g. method in NEURON, method in Matlab after saving the spike times to a text file, etc.; explain what equation you used…).

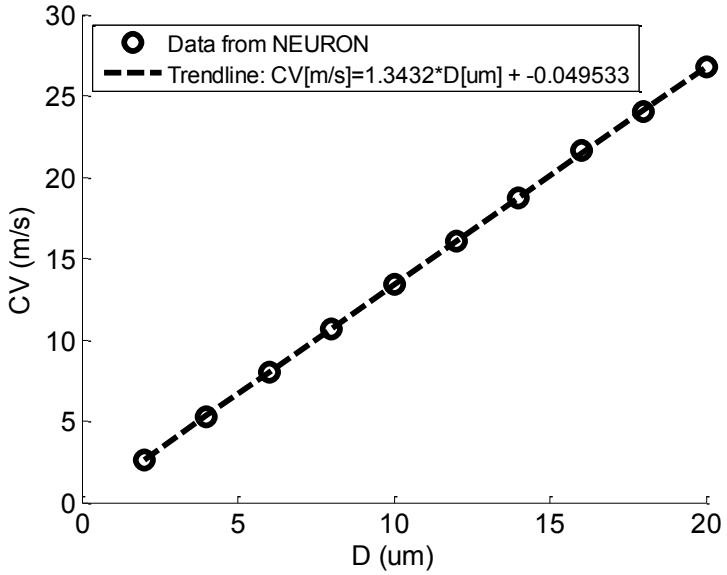
Explanation [4pts]
I computed the conduction speed in NEURON by taking the difference between the x coordinates of nodes 25 and 48, and dividing by the difference in spike times between the same nodes. I then manually entered the results into Matlab and plotted the results. I used Matlab's "polyfit" to obtain the trendline. I stimulated above node 5, thereby avoiding having the passive response compromise the accuracy of my conduction speed calculations.

b. Provide a table with your results.
c. Plot your results.
d. Fit a trendline to your data. Provide the trendline's equation and show it on your plot.

Results

- Correct values [10pts]
- Providing table [2pts]
- Providing plot [2pts]
- Trendline on plot + equation [2pt]

D (um)	CV (m/s)
2	2.644
4	5.287
6	8.023
8	10.698
10	13.372
12	16.047
14	18.721
16	21.647
18	24.070
20	26.744



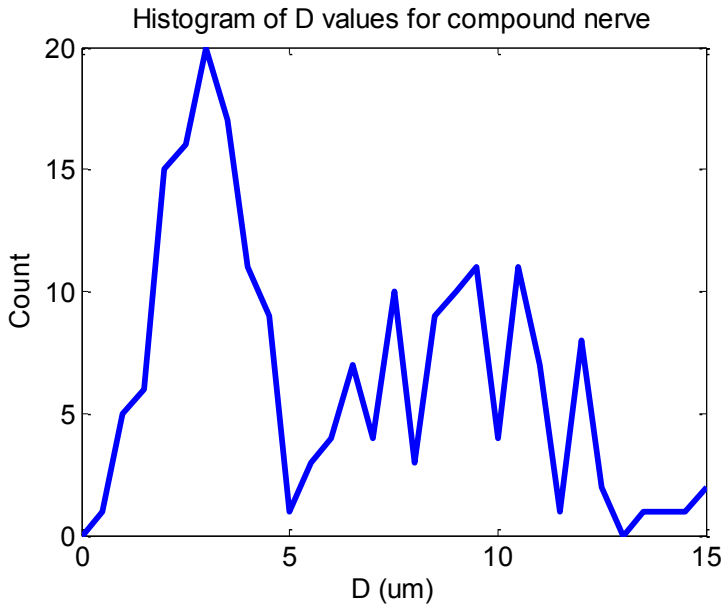
Part 3 – Compound Nerve Action Potential (CNAP)

In this problem, you will construct a virtual nerve bundle in Matlab and quantify how the spacing between your recording electrodes influences the evoked CNAPs. Create a bundle of at least 100 virtual axons, distributed among two different sub-populations of axon diameters (each with a distribution of diameters D, with a mean±s.d. that you select to represent two different types of nerve fibers present in peripheral nerve bundles). Note that superposition holds, so you can generate the recorded signals by summation of scaled (by distance) and time shifted (due to propagation) sources.

Part 3.A Provide a reference and histogram for your chosen axon diameters.

ANSWER: [5pts]

I chose two populations of fiber diameters. As shown on p. 193 in Purves et al. (Purves, D. et al. (Eds) (2012). Neuroscience. Sinauer Associates, Inc.), Aβ fiber diameters range from 6 to 12um and Aδ fiber diameters range from 1 to 5um. I generated a distribution of fiber diameters, with 100 values in each population. For the Aβ population, I generated D values with a mean of 9um and a standard deviation of 2um. For the Aδ population, I generated D values with a mean of 3um and a standard deviation of 1um.

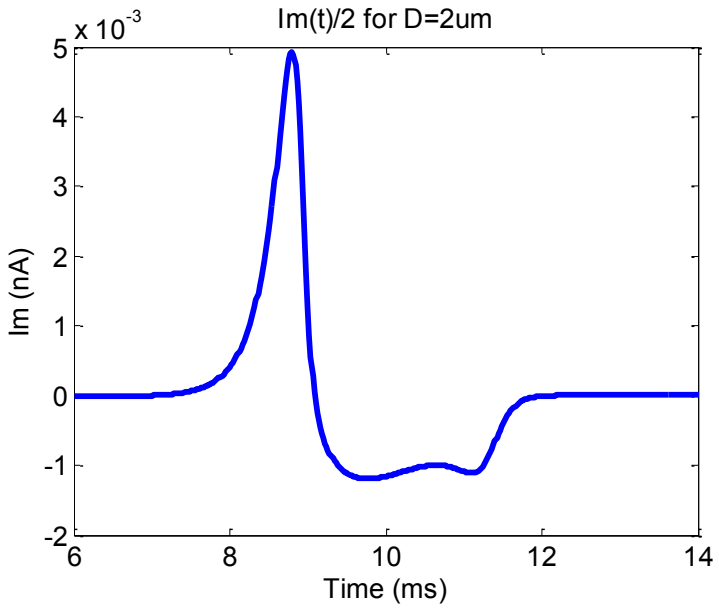


Part 3.B Initiate an action potential at one end of your nerve bundle and record the evoked signals with a tripolar recording electrode with *k*=10mm interelectrode spacings. Assume an extracellular resistivity of 500ohm-cm and a perpendicular electrode-fiber distance of 1mm.

a. What is the relationship between the current time course (Im(t)) and the fiber diameter?

Question (a) [5pts]

Im(t) is proportional to fiber diameter, as illustrated partially by the linear relationship between max(Im) and D in Part 1. For example, when the fiber diameter doubles, Im(t) doubles – albeit slightly time-shifted if using a stimulus amplitude near threshold, but I'm going to neglect this effect. Thus, I took Im(t) for D=2um, divided by 2, thereby producing the Im(t) time course for a "unit" axon (i.e. D=1um). I could them simply multiply this time course by any D value of interest. I used the time course from t=15 to 22ms.



b. Consider Im(t=t_o) for a given node N of your axon. What is the equation for the potential at a recording electrode some distance r from N due to Im(t=t_o) at node N? Define all your variables.

Question (b) [2.5pts]

$$\phi = \frac{I_m(t = t_o)}{4\pi\sigma_e r}$$

ϕ: Potential at the recording electrode
$I_m(t=t_o)$: Transmembrane potential at time t_o (for a given node of Ranvier N)
σ_e: Extracellular conductivity
r: Distance between node N and the recording electrode

c. Given the potentials V1, V2, and V3 at each contact in a tripolar electrode (left, middle, and right, respectively), what is the equation for the net recorded voltage in response to Im(t=t_o) from a given node N? Define all your variables.

Question (c) [2.5pts]

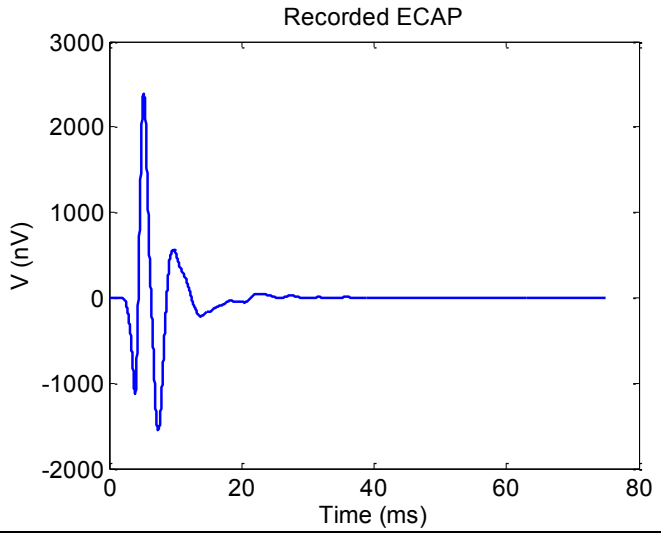
$$V_{rec} = V_2 - \frac{V_1 + V_3}{2}$$

Vrec: Net recorded voltage
Vi: Voltage recorded at contact i

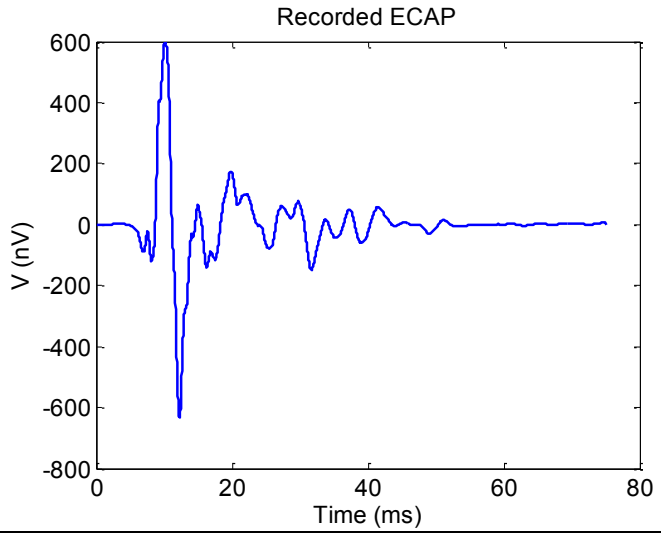
d. Plot the recorded ECAP for two different distances between the axons' proximal end (where the action potentials initiate) and the middle recording electrode. Include one distance that is quite close to the proximal end. Briefly comment on the effect of changing this distance and the underlying cause for this effect.

Question (d) [10pts for two plots, 5pts for observation; 15pts total]

x_elec1 = 10000um
Middle electrode position (x_elec2) = 10000um+10000um=20000um
All fiber diameters



x_elec1 = 50000um
Middle electrode position (x_elec2) = 50000um+10000um=60000um
All fiber diameters

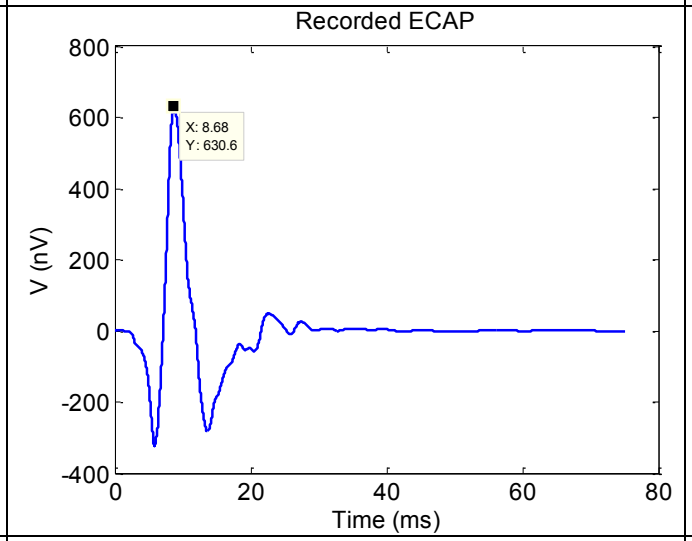
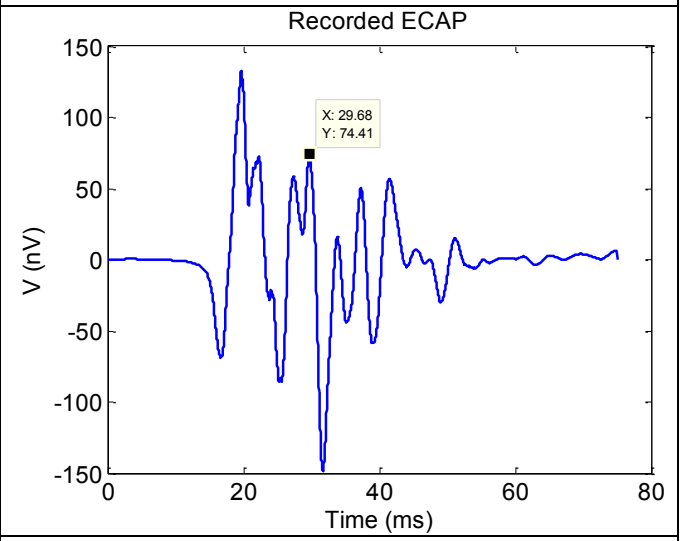
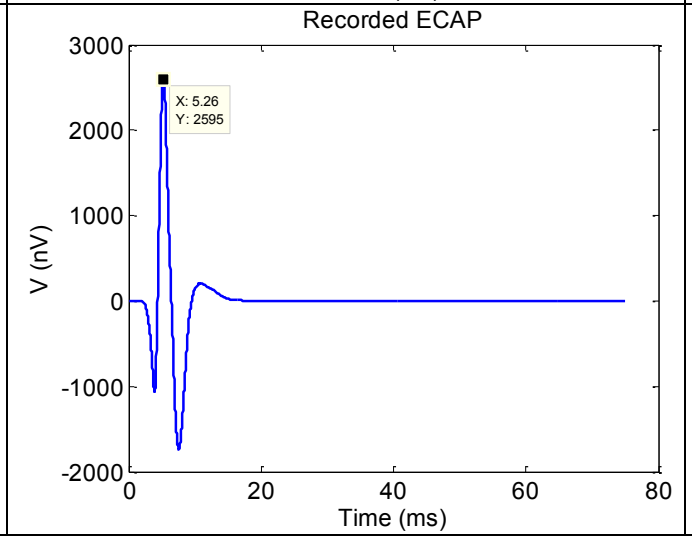
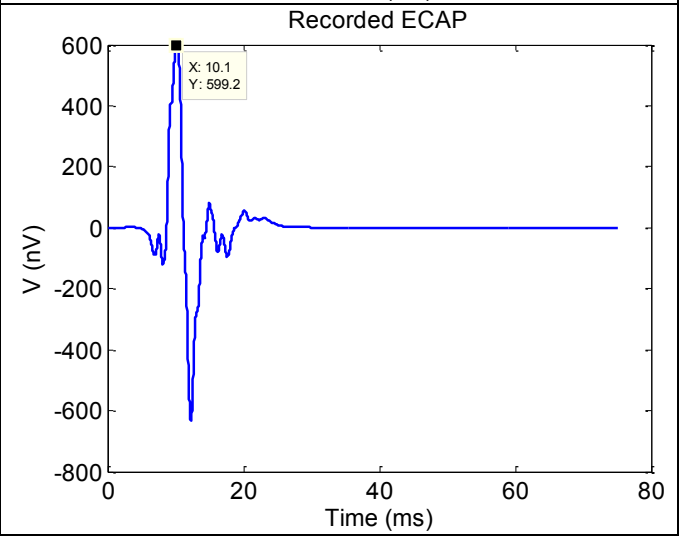


Main observations:

- ECAP effects when recording further from the point of stimulation: (1) becomes more spread out, "messier", and (2) lower amplitude.
- Cause? Temporal dispersion due to the different conduction speeds of the different fiber diameters.

e. Using your ECAPs from different stimulation-recording distances, determine the conduction velocity for the contribution to the CNAP from each fiber subpopulation. Explain your method and show your calculations. Are your results in keeping with your expected values? Explain.

Question (e) [2pts for method, 5pts for values/calculations, 3pts for expectations; 10pts total]

	x_elec1 = 10000um Middle electrode position (x_elec2) = 10000um+10000um=20000um	x_elec1 = 50000um Middle electrode position (x_elec2) = 50000um+10000um=60000um
Dvalues(1:100)		
Dvalues(101:200)		

Expected results:

The conduction speed was found to be 0.8m/s per um of fiber diameter from Part 2. My populations of fibers have mean diameters of 3um and 9um. Therefore, I expect the smaller fibers (Dvalues(1:100)) to have a conduction speed of approximately 0.8m/s*3 = 2.4m/s, while I expect the larger fibers (Dvalues(101:200)) to have a conduction speed of approximately 0.8m/s*9=7.2m/s.

Actual results:
My recording locations were 40mm apart. How to select a single ECAP time for each recording location and each fiber population? As shown in the figures above, the ECAP has a distinct positive peak for x_elec1=10mm for both fiber populations. However, ECAP recorded more distally has a less distinct shape. In order to get an approximate typical conduction speed value, I selected a time point for a positive peak around the middle of each ECAP.

$$\Delta d = 40mm$$
$$\Delta t_1 = 29.68 - 8.68 = 21ms$$
$$CV_1 = \frac{40mm}{21ms} = \frac{1.9m}{s}$$
$$\Delta t_2 = 10.1 - 5.26 = 4.84ms$$
$$CV_2 = \frac{40mm}{4.84ms} = \frac{8.26m}{s}$$

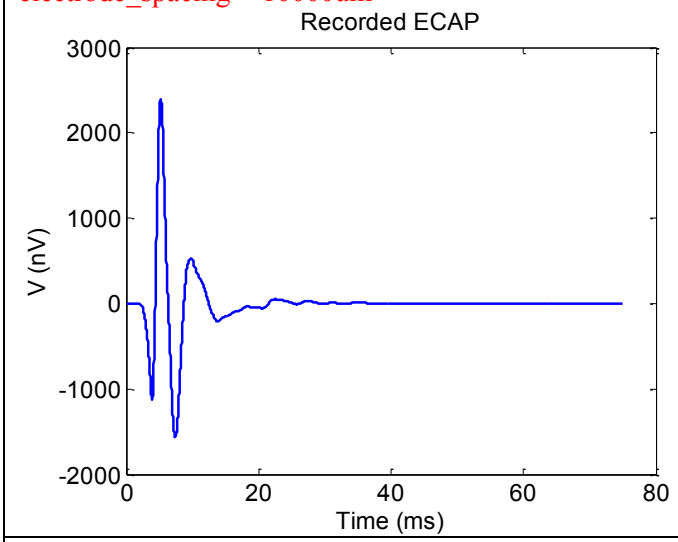
These are close to my expected values. Due to temporal dispersion, random fiber size distribution, and unclear "peak times", among other effects, they don't match exactly.

f. Plot the recorded ECAP for three different interelectrode spacings (k). How do the recorded signals change?

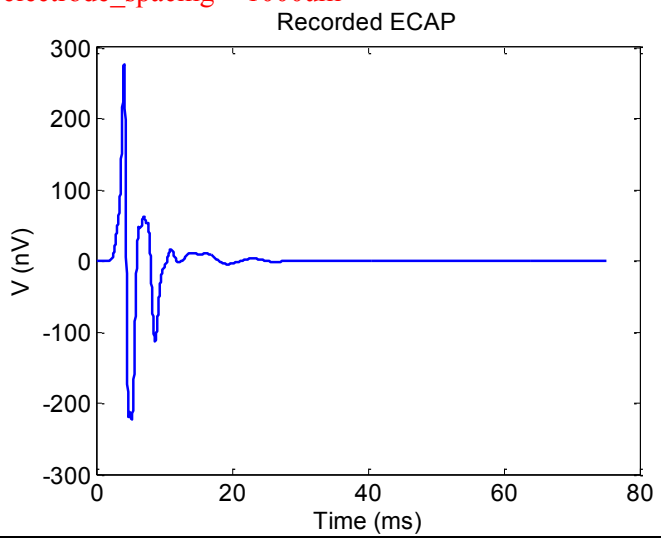
Question (f) [5pts for plots, 5pts for explanation; 10pts total]

x_elec1 = 10000um

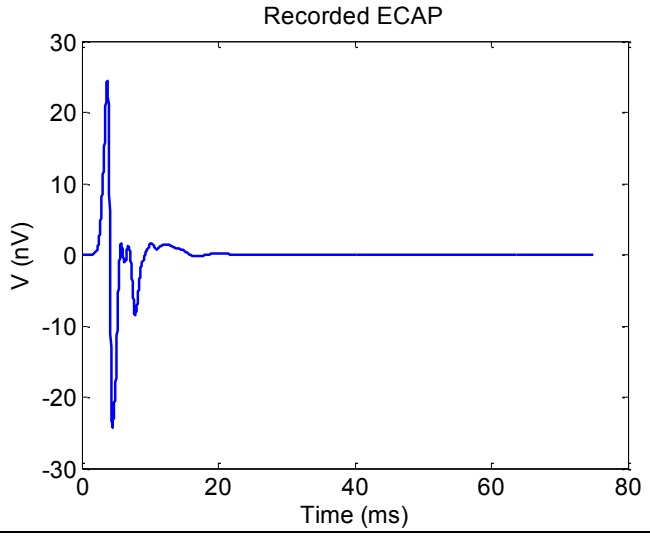
electrode_spacing = 10000um



electrode_spacing = 1000um



electrode_spacing = 100um



The primary effect of reduced electrode spacing is reduced ECAP amplitude. Given the equation for computing the tripolar recording, when the electrodes are closer together, V1, V2, and V3 will be more similar, resulting in a smaller V2-avg(V1, V3). However, smaller electrode spacing will also result in a more accurate resolving of the signal at the closest codes.

Part 3.C Recording of CNAPs is used frequently to diagnose peripheral nerve dysfunction, but clearly cuff electrodes cannot be implanted for diagnostic purposes. Do some research to determine how CNAPs are recorded for clinical diagnostic purposes and summarize briefly (<100 words). Provide your reference(s).

ANSWER: [5pts]
Clinically, recording of CNAPs is usually done using surface electrodes. The nerve is stimulated proximally, and the recording electrode is placed at a more distal area of the nerve, where the nerve is more superficial. It may be necessary to average over multiple measurements to have a sufficiently high signal-to-noise ratio. The amplitude and latency of the recorded ECAP are used to diagnose the neuropathy, where a reduction in the ECAP amplitude indicates axonal degeneration while a reduction in the ECAP latency indicates axon demyelination.

References:

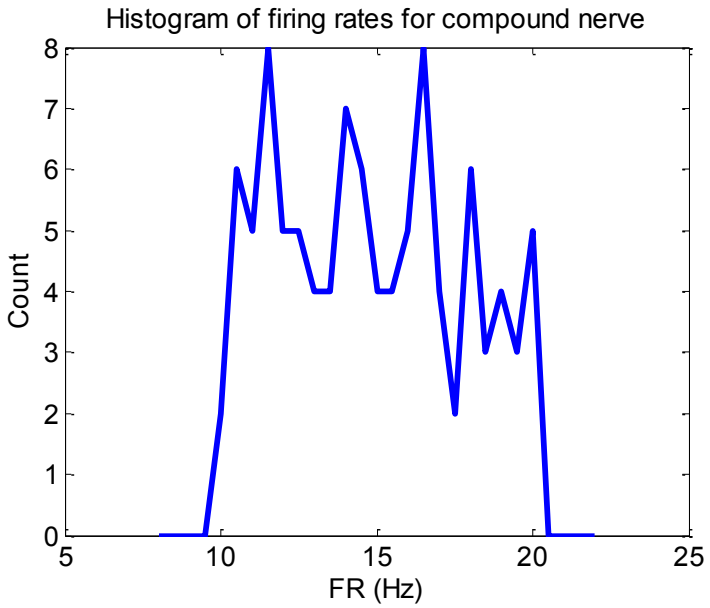
- Oh, S.J. (2002). *Clinical Electromyography : Nerve Conduction Studies*. Lippincott Williams & Wilkins. p. 16.
- Alport, A.R. and Howard, W. (2012). Clinical Approach to Peripheral Neuropathy: Anatomic Localization and Diagnostic Testing. *CONTINUUM: Lifelong Learning In Neurology.* 18(1):13-38.
- S. M. Mason, "Evoked potentials and their clinical application," *Current Anaesthesia & Critical Care*, vol. 15, no. 6, pp. 392–399, Dec. 2004.
- http://www.ninds.nih.gov/disorders/peripheralneuropathy/peripheralneuropathy.htm

Part 4 – Electroneurogram (ENG)
Use the virtual nerve bundle you developed in Part 3 to simulate spontaneous electroneurographic (ENG) signals and ENG signals evoked by natural (asynchronous) stimulation. Give each axon a spontaneous basal firing rate drawn from a uniform distribution of firing frequencies between 10 Hz and 20 Hz.

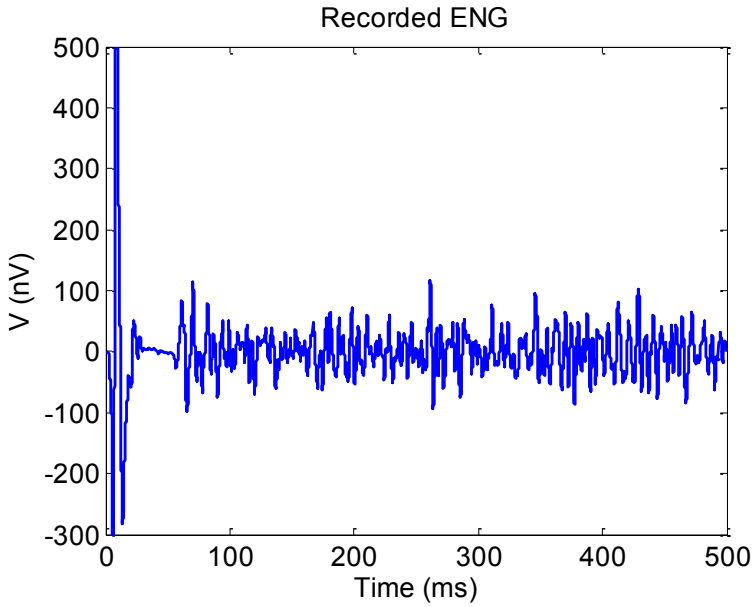
a. Show a histogram of your firing rates.
b. Explain your coding algorithm.
c. Plot your ENG for each fiber distribution separately. What is the primary difference between your two plots? Why is this the case?

ANSWER: [10pts]

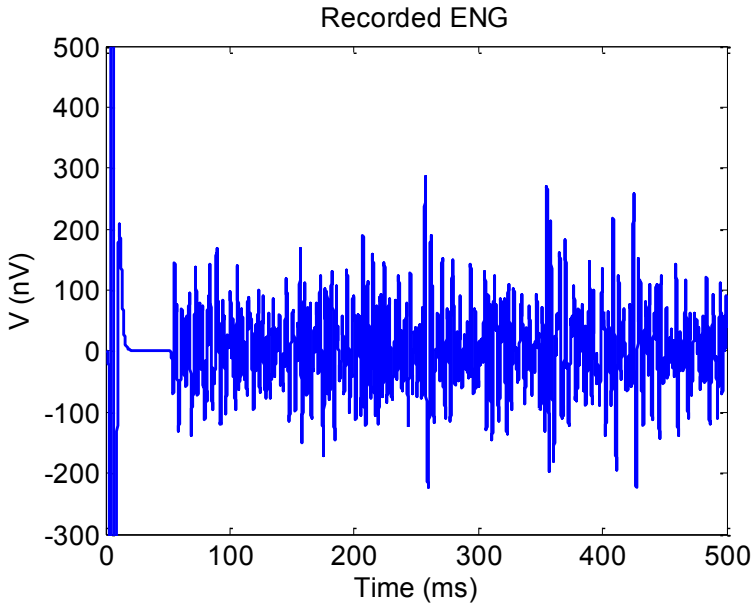
- Histogram [2pts]
- Algorithm [2pts]
- ENG plots [5pts]
- Explanation [3pts]



Smaller fibers:



Larger fibers:



Recorded ENG has much larger amplitude for larger fibers because their Im(t) has a larger amplitude, as seen in Part 1.