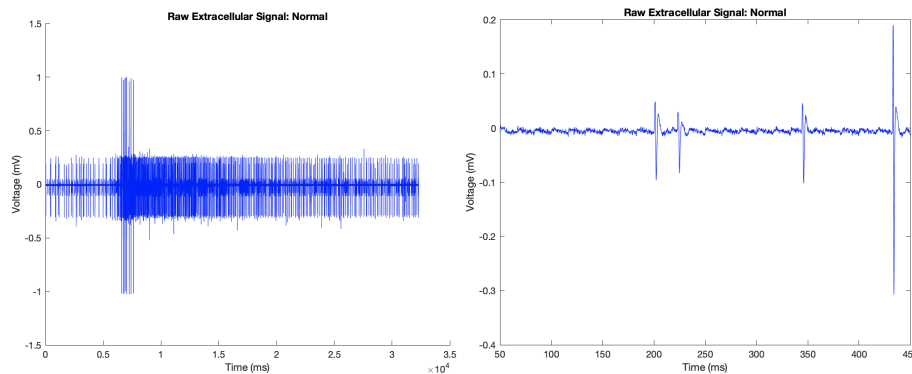


## Results

### *Extracellular recording*

#### *Extracellular Normal Analysis*

The depiction of the raw extracellular image in millivolts (mV) was plotted corresponding to sampling frequency (10,000 samples/s) and plotted against a millisecond (ms) time scale (Figure 1).



*Figure 1: The first (left) plot depicts the raw Normal signal in milliVolts (mV) in relation to time in milliseconds (ms). The second plot (right) depicts a zoomed-in interval of the previous plot.*

In preparation for action potential classification, the raw signal was slightly smoothed using a Savitzky-Golay filter. The “findpeaks()” library in MATLAB was used to identify both the largest positive and negative peaks of the action potential. The closest time interval between positive and negative peaks were used to calculate the peak-peak action potential width, and then the amplitudes of these same pairs were added to define an action potential’s amplitude.

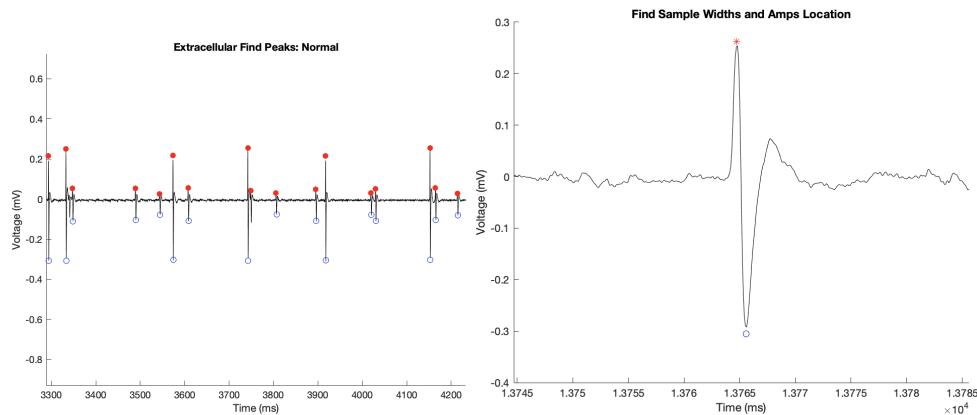


Figure 2: The first plot (left) depicts the zoomed in Normal signal in milliVolts (mV) in relation to time in milliseconds (ms) with red positive peaks identified, and blue negative peaks identified. The second plot (right) depicts a zoomed-in interval of the previous plot.

The above features extracted (width and amplitudes) were grouped into three distinct groupings, with respect to the width vs. amplitude scatter plots and the (three) distinct means visualized by the histogram of action potential amplitudes (Figure 3.). Thresholds were generated to about 1.5 standard deviations from distinct means (visually). The same protocol was followed and resulted in the same thresholds for the subsequent locations (Figures 3, 6, 9).

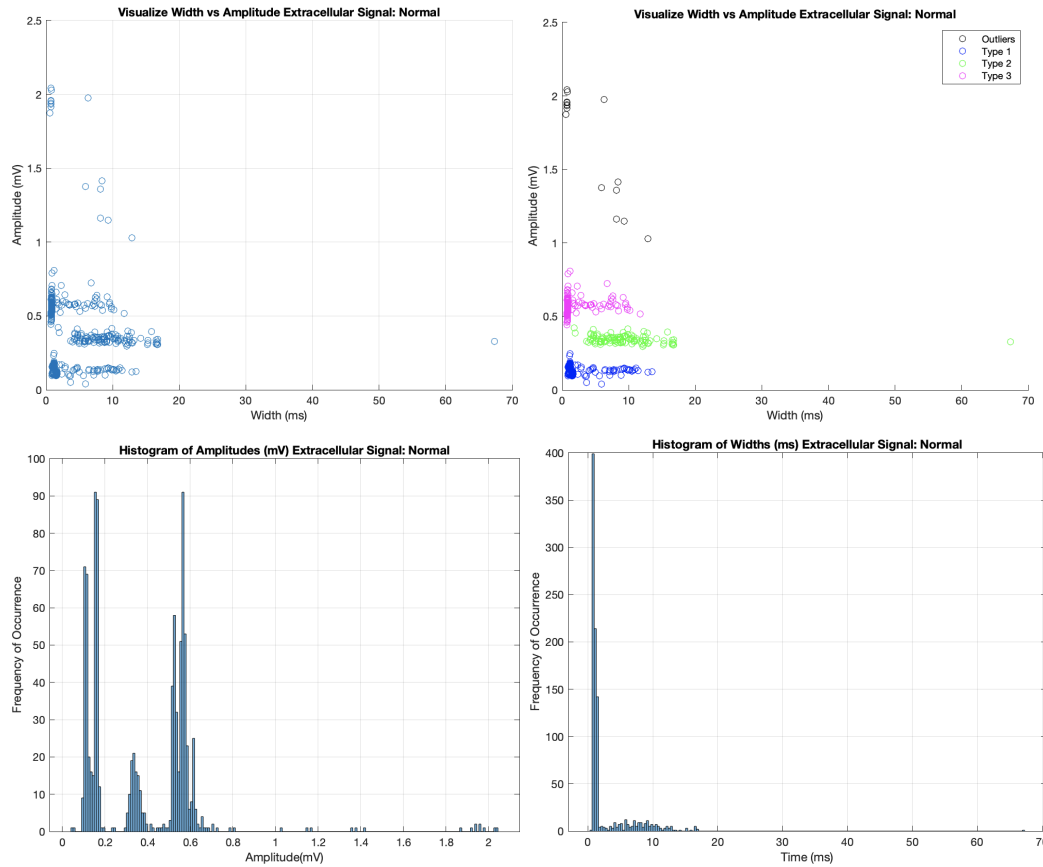


Figure 3: The first plot (top left) displays the width (ms) vs amplitude (mV) of each action potential in uncategorized form. The second plot (top right) displays the categorized form of the top left plot. The bottom left and bottom right plots display the distribution of amplitude and width, respectively.

The following depicts the original and scaled raw signal presented in Figure 1, categorized by the three signal types.

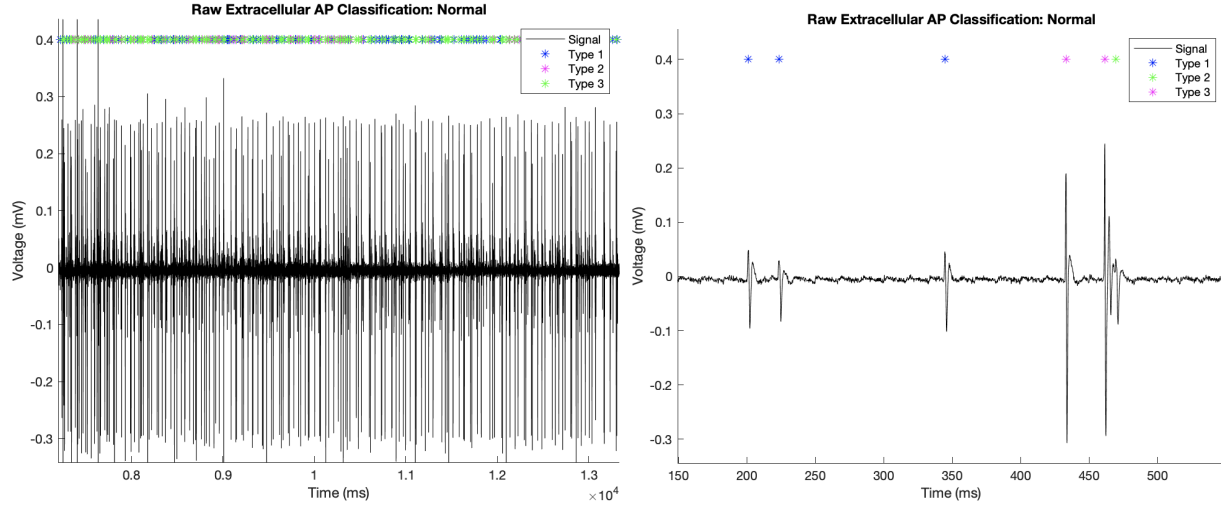


Figure 4: The first (left) plot depicts the raw Normal signal in milliVolts (mV) in relation to time in milliseconds (ms); the second plot (right) depicts a zoomed-in interval of the previous plot, both with three labelled types of action potentials above-head.

The following plots for additional locations (Rec Tail and Swimmeret) were generated using the same specifications as previously stated, including thresholds.

### Extracellular Rec Tail Analysis

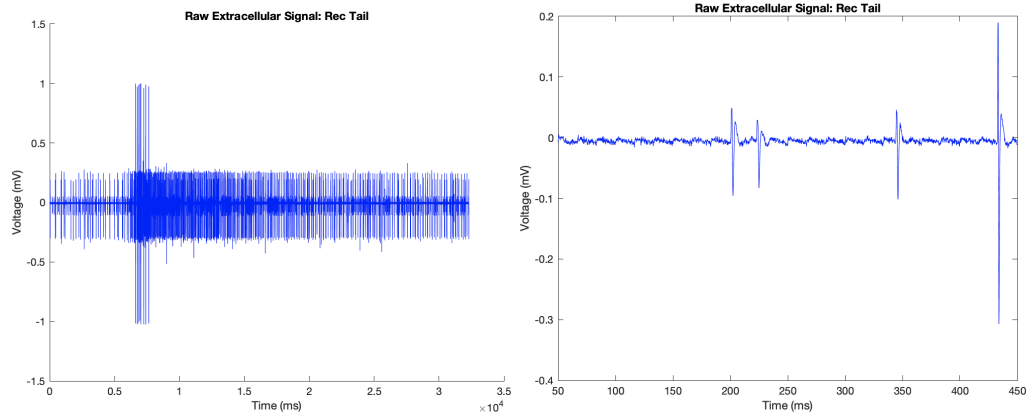


Figure 5: The first (left) plot depicts the raw rec tail signal in milliVolts (mV) in relation to time in milliseconds (ms); the second plot (right) depicts a zoomed-in interval of the previous plot, both with three labelled types of action potentials above-head.

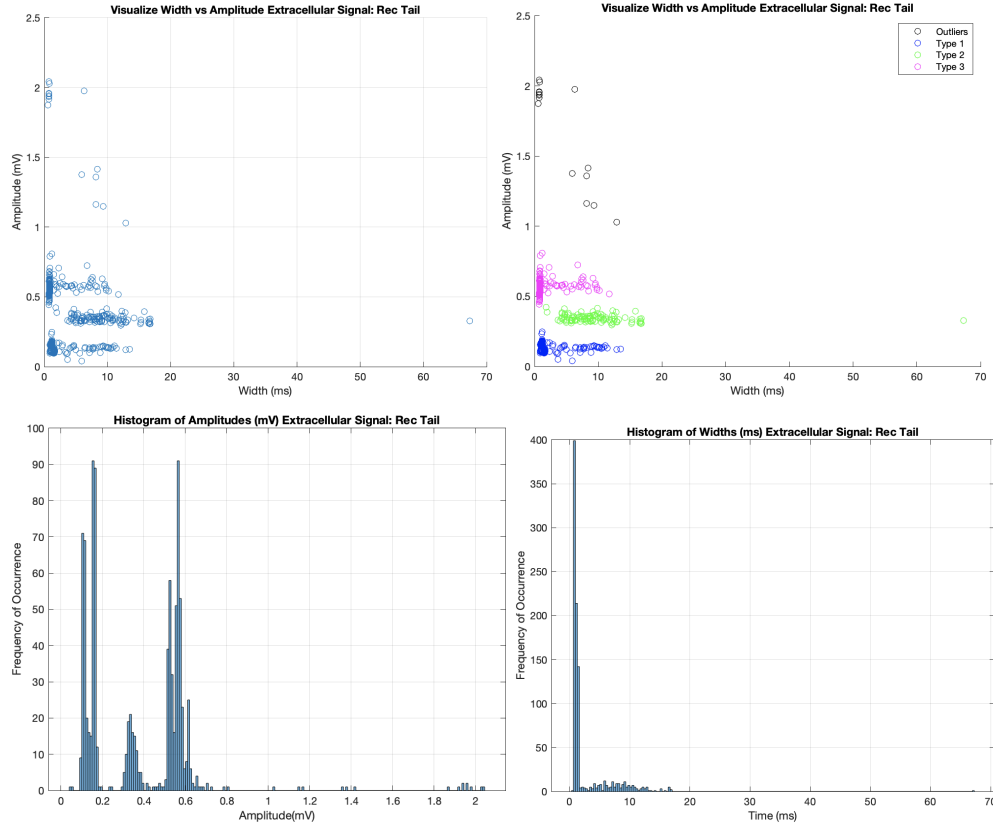


Figure 6: The first plot (top left) displays the width (ms) vs amplitude (mV) of each action potential in uncategorized form. The second plot (top right) displays the categorized form of the top left plot. The bottom left and bottom right plots display the distribution of amplitude and width, respectively.

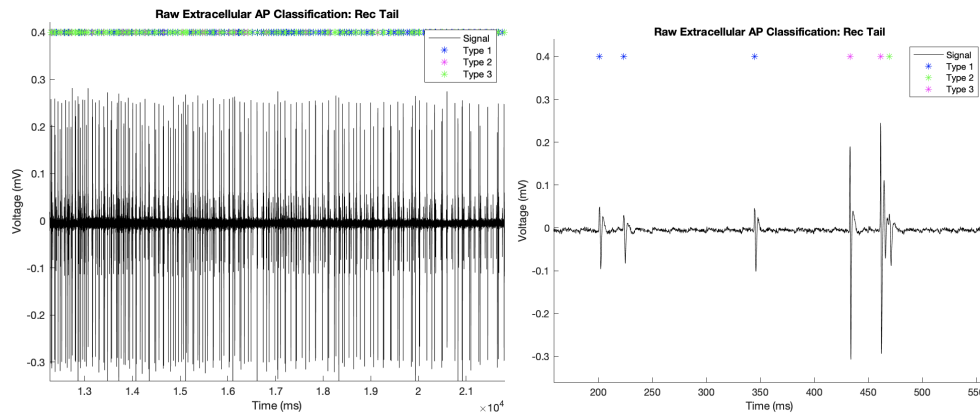


Figure 7: The first (left) plot depicts the raw Rec Tail signal in milliVolts (mV) in relation to time in milliseconds (ms); the second plot (right) depicts a zoomed-in interval of the previous plot, both with three labelled types of action potentials above-head.

## Extracellular Swimmeret Analysis

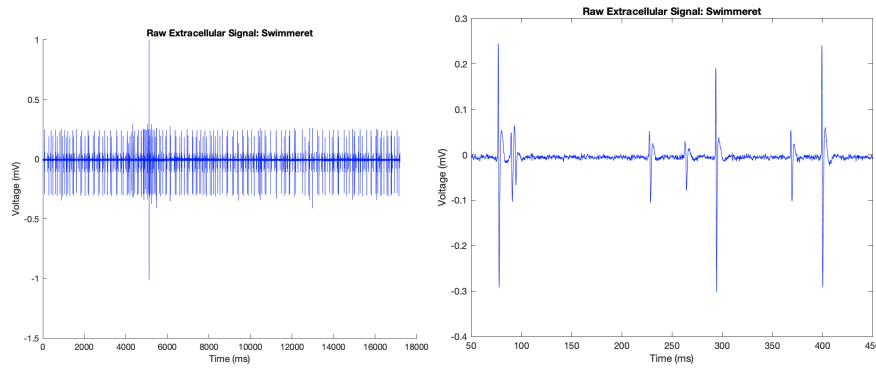


Figure 8: The first (left) plot depicts the raw Swimmeret signal in milliVolts (mV) in relation to time in milliseconds (ms); the second plot (right) depicts a zoomed-in interval of the previous plot, both with three labelled types of action potentials above-head.

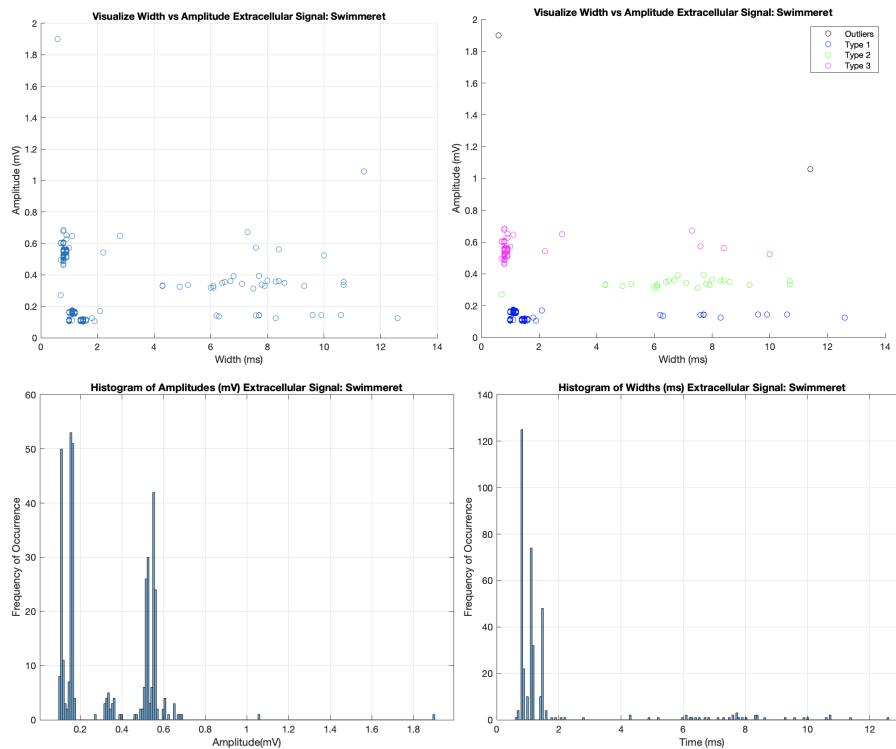


Figure 9: The first plot (top left) displays the width (ms) vs amplitude (mV) of each action potential in uncategorized form. The second plot (top right) displays the categorized form of the top left plot. The bottom left and bottom right plots display the distribution of amplitude and width, respectively.

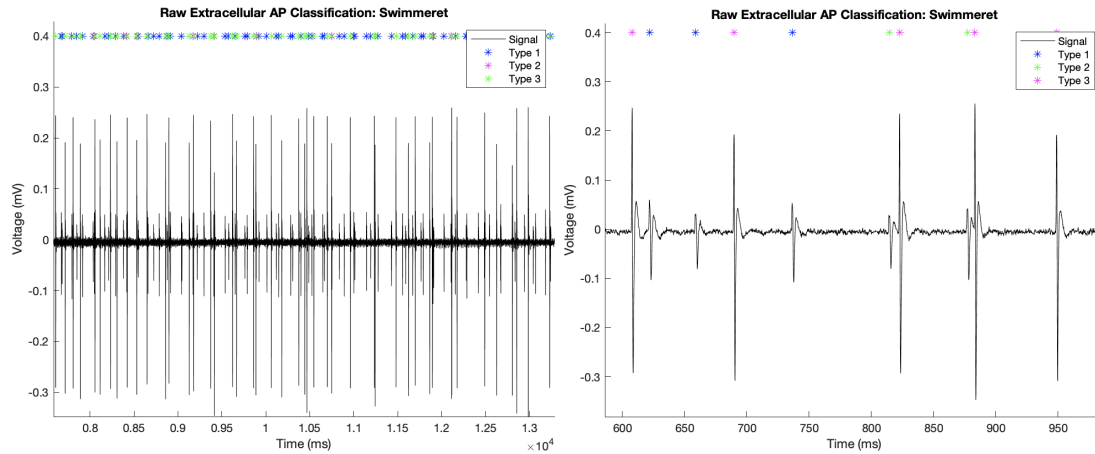


Figure 10: The first (left) plot depicts the raw Swimmeret signal in milliVolts (mV) in relation to time in milliseconds (ms); the second plot (right) depicts a zoomed-in interval of the previous plot, both with three labelled types of action potentials above-head.

Table 1: The following table depicts the frequencies (in Hz) detected per axon type for each cell condition/location (Normal, Rec Tail, and Swimmeret). Three axonal types were distinguished per each location. Axonal types were segregated by action potential amplitude via the following ranges in mV: Type 1 [0, 0.25); Type 2 [0.25, 0.435); Type 3 [0.435, 0.85).

	Frequency By Action Potential Type (Hz)		
Condition	Type 1	Type 2	Type 3
Normal	12.4	3.5	13.4
Rec Tail	12.4	3.5	13.4
Swimmeret	11.0	1.4	9.2

## Appendix Code:

### Extracellular

```

%% Visualize the existing data
%% Assuming tickrate and sample rate are in 10000 Hz
clc
clear all
% Load data
x = load('Crayfish_Extracellular_Rec_Tail_Stim.mat') %interchanged with the corresponding data
Fs = x.samplerate; %create sample frequency
ts = 0:1/x.samplerate(1):(length(x.data)-1)/x.samplerate(1); %time in s
ts = ts.*1000; %time in ms
ts = transpose(ts);

%length(ts) //for debugging
%length(x.data) //for debugging

```

```

V = transpose(x.data); %%Voltage

close all

%raw
hold on
figure(1)
plot(ts,V,'b')
title(sprintf('Raw %s Signal: %s',x.title, "Normal"))
xlabel('Time (ms)')
ylabel('Voltage (mV)')
hold off

%Zoomed plot
hold on
figure(2)
plot(ts,V,'b')
title(sprintf('Raw %s Signal: %s',x.title, "Normal"))
xlabel('Time (ms)')
ylabel('Voltage (mV)')
xlim([50,450])
%xlim([50,950])
hold off

%% Peak Analysis
%index = [58571];% find a sample of the peaks
index = 1;
%index = [296000];

%Avoid outlier

ts = ts(index:end);

V = V(index:end);

t = ts;
V = V;

V_processed = sgolayfilt(V,7,21); %data filter

[Vpeak,tpeak]=findpeaks(V_processed,t,'MinPeakHeight',.01,'MinPeakProminence',.03,'MinPeakDistance',5);
[Vpeak2,tpeak2]=findpeaks(-(V_processed),t,'MinPeakHeight',.03,'MinPeakProminence',.03,'MinPeakDistance',10);

%% Test peak ID
figure()
hold on
plot(t,V,'k');
scatter(tpeak,Vpeak,'r',''); %max points
scatter(tpeak2,-Vpeak2,'b','o'); %min points
xlabel('Time (ms)')
ylabel('Voltage (mV)')
title(sprintf('%s Find Peaks: %s',x.title, "Normal"))
xlim([50,450])
hold off

%% Compute amplitudes and Widths accurately
%ID the corresponding peaks
paired_peak = tpeak2;
paired_width = tpeak2;
Amplitude = [];
Width = [];

```

```

Original_Index = [];
for i = 1:length(tpeak) %find the pair to each peak - calculate amplitude
    a = abs(paired_peak-tpeak(i)); %find paired amplitudes
    index = find(a == min(a)); %return location of the peak in tpeak2

    if(i == round(length(tpeak)/2))%To test one point
        tpeak_val = i; %pick a tpeak value index
        matching_tpeak2 = index; %pick the chosen tpeak2 val index
    end

    Original_Index(i) = tpeak(i); %reference the top peak used; holds time
    Amplitude(i) = Vpeak(i)+Vpeak2(index(1));%set amplitude i
    Width(i) = a(index(1));
end
%% Verify Ampl. Difference (test one pair)
figure()
hold on
plot(t,V,'k');
scatter(tpeak(tpeak_val),Vpeak(tpeak_val),'r','*'); %time 1
scatter(tpeak2(matching_tpeak2),-Vpeak2(matching_tpeak2),'b','o'); %time 2
xlabel('Time (ms)')
ylabel('Voltage (mV)')
title('Find Sample Widths and Amps Location');
Min = min(tpeak(tpeak_val),tpeak2(matching_tpeak2));
Max = max(tpeak(tpeak_val),tpeak2(matching_tpeak2));
xlim([Min-20,Max+20]);
hold off
%% Find histograms
figure()
%hist(Vpeak,100)
histogram(Amplitude,200)
grid on
xlabel('Amplitude(mV)')
ylabel('Frequency of Occurrence')
title(sprintf('Histogram of Amplitudes (mV) %s Signal: %s',x,titles,"Normal"))

figure()
histogram(Width,200)
grid on
xlabel('Time (ms)')
ylabel('Frequency of Occurrence')
title(sprintf('Histogram of Widths (ms) %s Signal: %s',x,titles,"Normal"))

%% Plot separations (Visualize)
figure()
hold on
grid on
%G = findgroups(Width, Amplitude);
scatter(Width,Amplitude);
xlabel('Width (ms)')
ylabel('Amplitude (mV)')
title(sprintf('Visualize Width vs Amplitude %s Signal: %s',x,titles,"Normal"))

hold off
pause
close all
%% Set Thresholds, find average time differences for frequency
%find(threshold) where threshold<= && >=; return the index; set these
thresh1 = find(Amplitude>=0 & Amplitude<0.25);
thresh2 = find(Amplitude>=.25 & Amplitude<0.435);
thresh3 = find(Amplitude>=.435 & Amplitude<0.85);

%find their mean
Amplitude = transpose(Amplitude);

```



```

Width = transpose(Width);
thresh1 = transpose(thresh1);
thresh2 = transpose(thresh2);
thresh3 = transpose(thresh3);
X = [tpeak,Amplitude,Width];

X1 = X(thresh1,:);
X2 = X(thresh2,:);
X3 = X(thresh3,:);

mean(X1(:,2));
mean(X2(:,2));
mean(X3(:,2));

%find their time occurrence
Group1 = mean(diff(X1(:,1)))/2; %calc average time difference
Group2 = mean(diff(X2(:,1)))/2;
Group3 = mean(diff(X3(:,1)))/2;

%Find frequency 1/mean timedifference (in kHz)
sprintf("Frequency per Group in kHz")
Group1 = 1/Group1 %calc average time difference
Group2 = 1/Group2
Group3 = 1/Group3

%% Plot the grouped scatter plot
figure()
hold on
scatter(X(:,3),X(:,2),'k','o')
scatter(X1(:,3),X1(:,2),'b','o')
scatter(X2(:,3),X2(:,2),'g','o')
scatter(X3(:,3),X3(:,2),'m','o')
legend('Outliers','Type 1','Type 2','Type 3')
xlabel('Width (ms)')
ylabel('Amplitude (mV)')
title(sprintf("Visualize Width vs Amplitude %s Signal: %s",x,titles,"Normal"))
hold off

%% Identify Raw Signal APs

figure()

hold on
plot(ts,V,'k')

AP = ones(length(X1),1)*.4; %indicate APs at this height
scatter(X1(:,1),AP,'b','*');

AP = ones(length(X2),1)*.4; %indicate APs at this height
scatter(X2(:,1),AP,'m','*');

AP = ones(length(X3),1)*.4; %indicate APs at this height
scatter(X3(:,1),AP,'g','*');

legend('Signal','Type 1','Type 2','Type 3')

title(sprintf("Raw %s AP Classification: %s",x,titles,"Normal"))
xlabel('Time (ms)')
ylabel('Voltage (mV)')
hold off

%Zoomed plot

figure()
hold on

```

```

plot(ts,V,'k')

AP = ones(length(X1),1)*.4; %indicate APs at this height
scatter(X1(:,1),AP,'b','*');

AP = ones(length(X2),1)*.4; %indicate APs at this height
scatter(X2(:,1),AP,'g','*');

AP = ones(length(X3),1)*.4; %indicate APs at this height
scatter(X3(:,1),AP,'m','*');

title(sprintf('Raw %s AP Classification: %s',x,titles,"Normal"))
xlabel('Time (ms)')
ylabel('Voltage (mV)')
legend('Signal','Type 1','Type 2','Type 3')
xlim([50,450])
%xlim([50,950])
hold off

```