

BME 301A Quantitative Physiology

Physical Lab #5:
Locust Olfactory

11/13/2013

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Introduction:

Chemical sensing, like many processes occurs via a complex pathway. At the start of that pathway is the olfactory receptor neuron(ORN) which can convert a chemical stimuli (odor) to an electrical signal.

Although there is much more than initial sensation involved in the pathway of processing and interpretation, the phenomenon of ORNs sensing odors and producing response signals is important in recording and analyzing chemical sensation.

In insects, the ORNs reside in the antennae. In this lab, electroantennogram (EAG) responses from locust antennae will be carried out under different odor presentation. By recording the responses during complex odor presentations, a transformation function that converts input to output can be designed. Such a transform or filter would allow the prediction of a response for a given odor stimulus. This is a phenomenological approach to creating a filter, meaning an empirical observation process will be used to create a mathematical expression or model of the signal transduction without noting the significance of any single observation itself.

Lucrative benefits from this sort of filter could be found in combination with neural prosthesis. Consider a person without a working sense of smell, ranging from birth defect, injury, poor gene expression or aging. If the science of neural prosthesis continues to progress to a point where direct stimulation of sensory nerves is viable, then the possibility of innervating such a nerve for the olfactory sense presents an opportunity to use the filter design conducted in this lab. If a sensory machine was developed to obtain the stimulus of a given odor, then the filter design could be used to transform that stimulus into neuronal input for the sensory nerve. An artificial nose could be crafted.

Materials & Methods:

1. Locust	9. 5 ml syringe (2)	17. Ring Stand	25.BNC-to-minigrabber converter
2. Petri dish	10.Cotton balls	18.Ring stand clamps	26.BNC-to-banana converter
3. Clay	11.Activated charcoal	19.Tubing, various	27.BNC-to-DIN8 converter (2)
4. Wax	12.Needle, 18 gauge	20.Dissection scissors	28.Solenoid valve, 3-way, with plastic connections
5. Wooden applicator stick	13.Electrical tape	21.Tissue adhesive	29.Breadboard with power supply & Pushbutton
6. Plastic cuvette	14.Waterproof tape	22.Odor bottles (2)	
7. Silver wire	15.Double-sided tape	23.Rubber stopper, 2 holes	
8. Chlorine bleach	16.Aquarium pump	24.BNC cable (2)	

Prepare 2 filters by placing cotton at the ends of two syringes, filling the remainder with activated charcoal and sealing the two together with tape. Attach a needle to the end of 1 filter and place the other end of the needle into the odor jar. Insert tubing into the other hole in the odor jar and seal the junction with clay. A setup was prepared with tubing, ring stand, air pump and odor bottle with a filter to puff odor at an antennae.

The locust antennae was prepared on a glass slide. To do this an antennae was clipped from a locust, trimmed on its distal end and placed on sticky tape on a slide. Two pieces of silver wire were placed in contact with either end of the antennae for the alligator clips to grab. The antennae slide was placed into the dish on the ring stand such that the odor would blow directly onto the antennae.

Next the circuit and recording software were properly setup. This included wiring in a pushbutton, a solenoid valve and testing the circuit to see that data was being properly recorded.

Next, various frequencies of two odors were applied to the antennae by activating the solenoid valve with the push button. For both odors the following experiments were recorded: 4second long pulses of odor with a minute ISI, train of 10 ~1s pulses with ~2s ISI, train of 10 ~1s pulses with ~1s ISI, train of 10 ~1s pulses with ~500ms ISI, train of 10 ~1s pulses with ~250ms ISI, train of many randomly spaced pulses for ~16 seconds.

Results:

Figures 1-5 show the antennae response to trains of various frequency stimuli using puffs of 1% Benzaldehyde. Figure 1 shows the raw EAG response using a 2 second inter-stimulus interval. Figures 2-5 show filtered response (via 200 pt. smoothing) with inter-stimulus intervals of 2s, 1s, 500ms, and 250ms respectively.

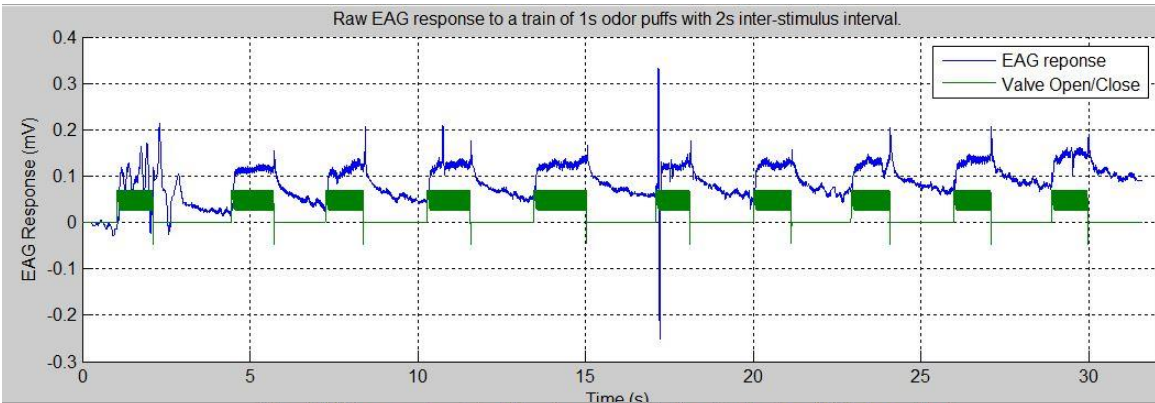


Figure 1: Raw EAG response for Benzaldehyde odor



Figure 2: Smoothed EAG response for Benzaldehyde odor

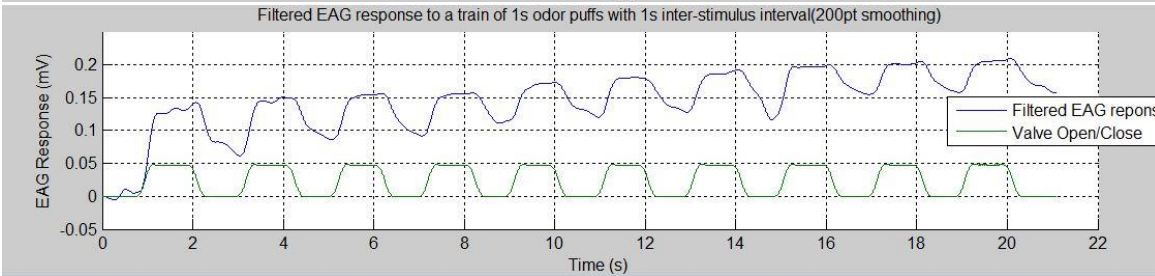


Figure 3: Smoothed EAG response for Benzaldehyde odor

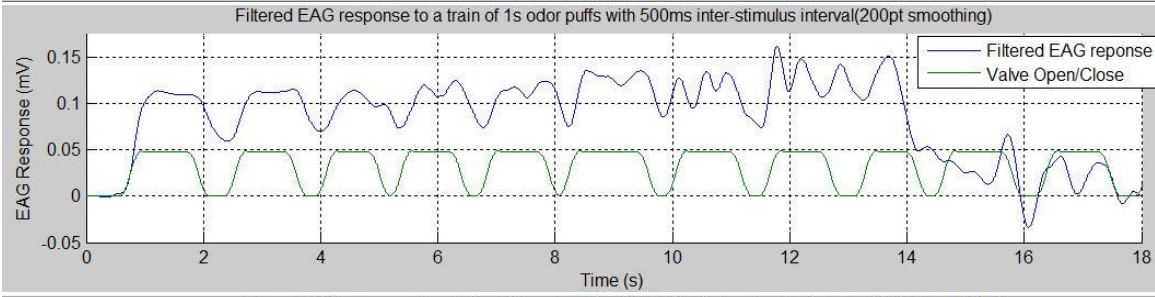


Figure 4: Smoothed EAG response for Benzaldehyde odor

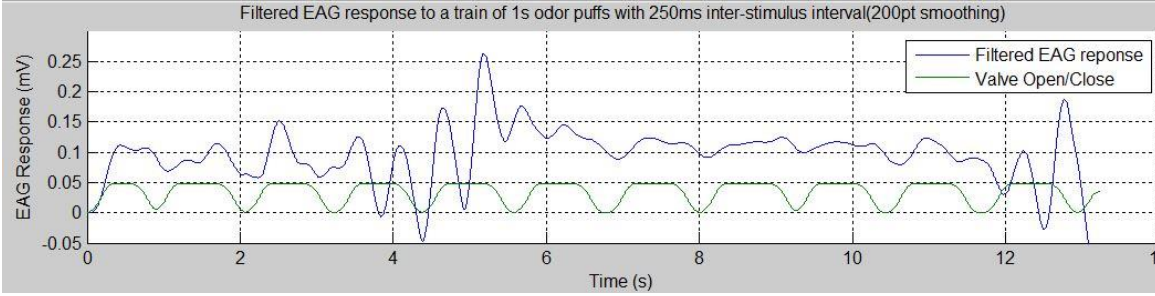


Figure 5: Smoothed EAG response for Benzaldehyde odor

Figures 6-10 show the antennae response to trains of various frequency stimuli using puffs of 1% Hexanol. (Citation #2). Figure 6 shows the raw EAG response using a 2 second inter-stimulus interval. Figures 7-10 show filtered response (via 200 pt. smoothing) with inter-stimulus intervals of 2s, 1s, 500ms, and 250ms respectively. Note, because the antennae used for the Benzaldehyde trials was exhausted and because the filter was saturated with Benzaldehyde odor, the data for the Hexanol trials originated from a different antennae. This partially accounts for the variance in behavior.



Figure 6: Raw EAG response for Hexanol odor

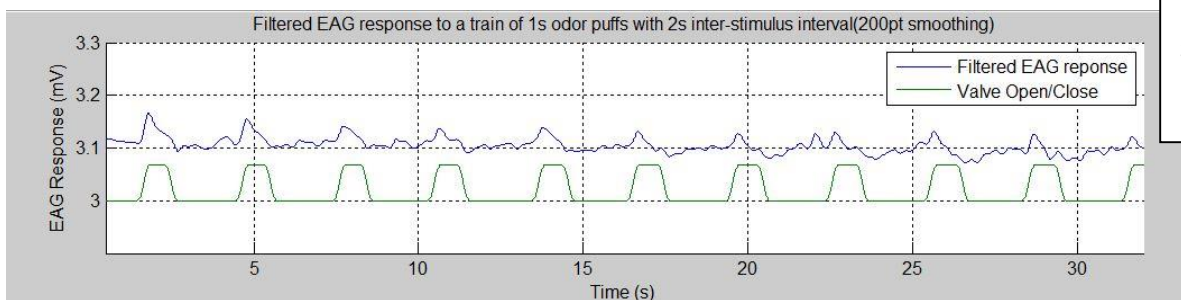


Figure 7: Smoothed EAG response for Hexanol odor

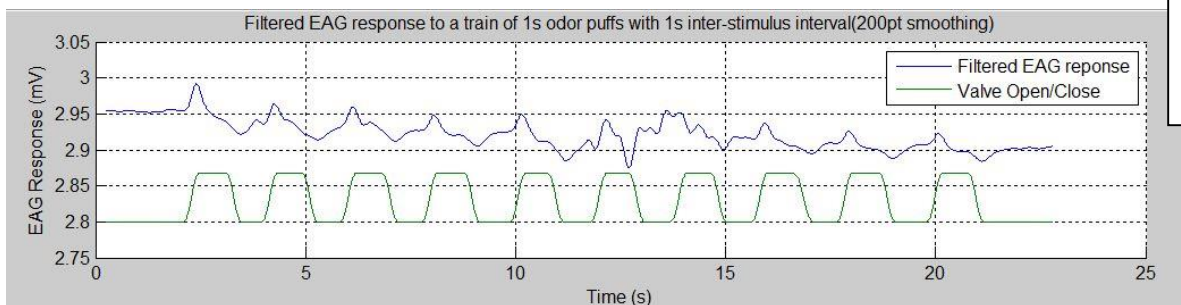


Figure 8: Smoothed EAG response for Hexanol odor

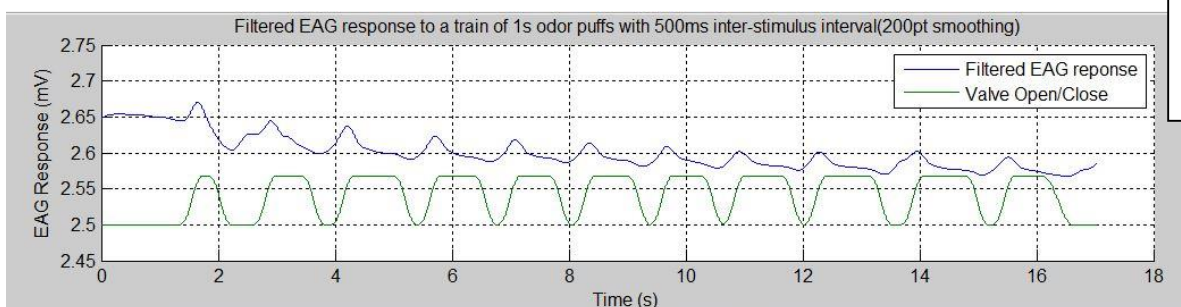


Figure 9: Smoothed EAG response for Hexanol odor

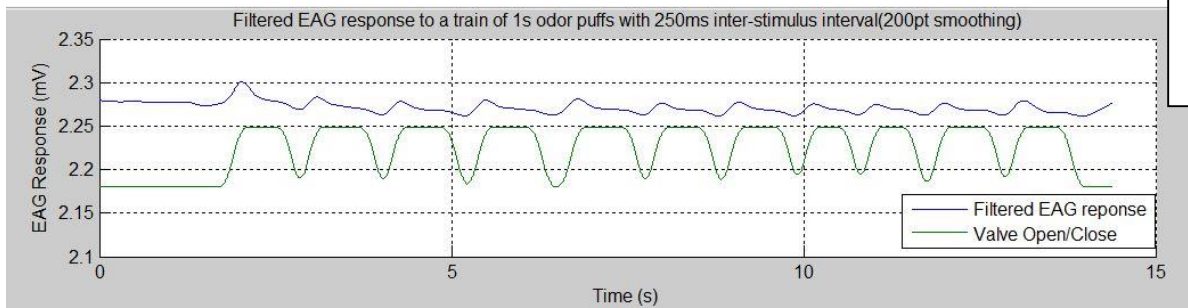


Figure 10: Smoothed EAG response for Hexanol odor

Figures 11 and 12 compare the response to individual pulses by plotting the response amplitude as a function of pulse frequency. For versatility, increasing frequency has been shown in both Hz (figure 12) and as decreasing inter stimulus interval (figure 13). Note again that the antennae used for the different odors was not the same.

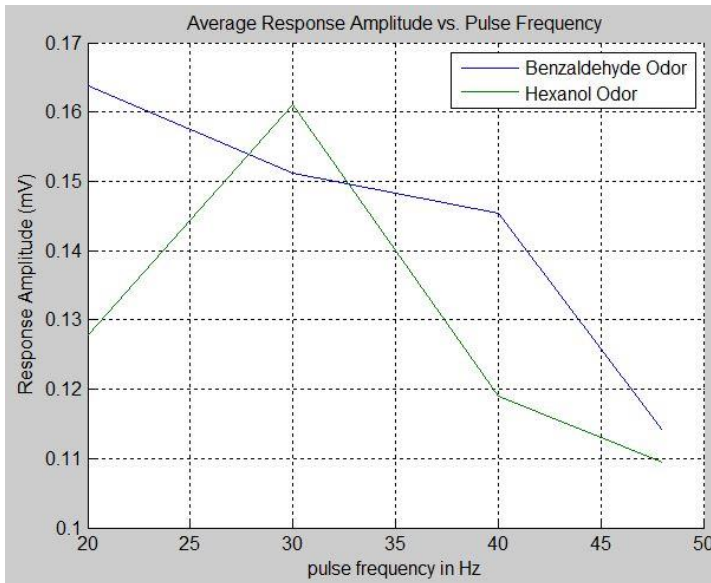


Figure 11: Amplitude of antennae response as a function of pulse frequency

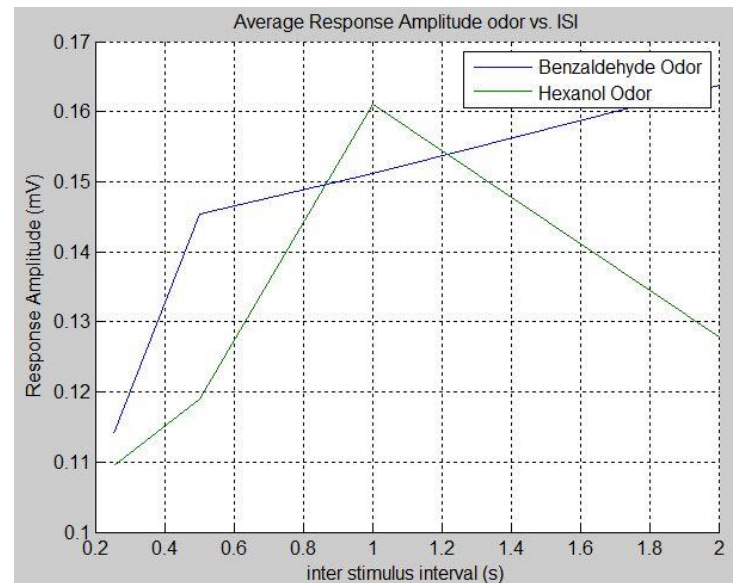
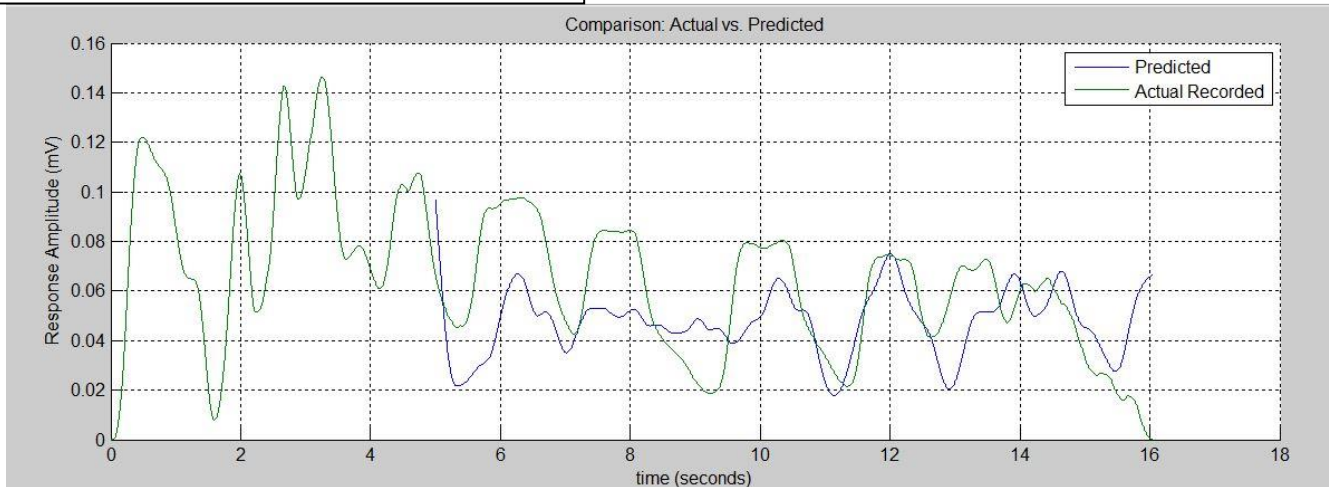


Figure 12: Amplitude of antennae response as a function of ISI

After designing and applying the filter included in the appendix, the recorded response from the random stimulation portion of the Benzaldehyde experiment was plotted along with the predicted response for the second portion of the data. Figure 13 shows visually the relative fit between the actual and predicted responses.

Figure 13: Comparison between recorded response and predicted response created from filter (for 5-16 seconds)



Discussion:

Although the antennae change between odors could account for variance in the responses, there are some similarities and differences worth noting. First off, both odors produce EAG responses that increase upon stimuli. For the Benzaldehyde odor, the EAG recording is somewhat like a square wave. It increases upon onset of stimulus to an amplitude at which it stays until the stimulus is removed. Upon removal of the stimulus the response returns to baseline. The Hexanol odor response however has a different behavior that seems to ramp up and then ramp down instead of forming a square response. Ramp down of the response begins immediately after onset of stimulus and the consequent spike in response.

Rise time for the Benzaldehyde odor was 73ms and fall time was 3894ms. Rise time for the Hexanol odor was 82ms and fall time was 2146ms. Although rise times seem to be relatively similar (10.98% difference), the fall time for the Benzaldehyde odor response was 81.45% longer than for the Hexanol. This slower drop off rate coincides with the square response form described previously for the Benzaldehyde odor and the ramped decay for the Hexanol odor. From this comparison I can infer that under stimulus of the Benzaldehyde odor, the antennae remains active and stimulated for a longer period of time. However this behavior does not seem appropriate because a locust antennae should not be able to sense odor when it is no longer present. Therefore it is more likely that the system used for experimentation was variable in its

effectiveness at removing odor under vacuum. Its ability to present odor quickly was probably much more precise than its ability to stop delivering odor.

The amplitude of the odor response for Benzaldehyde was 1.0448mV, whereas for Hexanol the response amplitude was .1719mV. This makes sense given Benzaldehyde has a shorter rise time, indicating a more potent odor that induced a quicker and stronger response (in speed and amplitude). Given that the amplitude variation was much larger relative to the rise and fall time variations, the amplitude response feature provides better discrimination between the two odors.

The overall EAG response mirrors the temporal dynamics of the stimulus quite closely for the Benzaldehyde odor as shown in figures 1-7. The responses tend to increase when the stimulus is applied and decrease when it is removed. The Hexanol data however is less in line with the temporal dynamics of the stimulus, mainly in its ramped decrease compared to the immediate removal of stimulus.

As the inter stimulus interval decreases, the amplitude of the response also decreases. The antennae may work by sensing change in odor, so a possible reason for this behavior might be the antennae becoming used to the odor when it begins to saturate the air surrounding the antennae.

The proper input output filter shape is similar to a bell curve. It looks like a band pass filter in a frequency domain. Or possibly a sinc function in the time domain. This makes sense given it operates as a low pass or low positioned band pass filter.

References:

1. Enderle, John D., and Joseph D. Bronzino. *Introduction to Biomedical Engineering*. Amsterdam: Elsevier/Academic, 2012. Print.
2. Lab Data from fellow lab group comprised of Paras Vora, Matt Everett, Karthik Karishnan

Unofficial Sources:

- Physical Lab Manual for Quantitative Physiology
- Computational Lab Manual for Quantitative Physiology
- Nerve II Module Lecture Slides
- Blackboard Discussions
- Discussants: Lauren Bedell, Maeve Woeltje, Paras Vora, Karthic, Matt Everett

APPENDIX

Matlab Code for the filter

```

% Load data for the stimulus and the response
S = load('pulseData.txt'); %stimulus data
S2=load('fxnData.txt'); %response data

t=S(:,1).'; %vector of time values for the entire trial in ms increments

stimAmp=S(:,2).'; %vector of stimulus amplitude values for the entire trial data in ms
increments

fxnAmp=S2(:,2).'; %vector of response amplitude values for entire trial
fxnAmp=filtfilt(ones(1,200)/200,1,fxnAmp);
fxnAmp=fxnAmp-[0:(fxnAmp(end)-fxnAmp(1))/(length(fxnAmp)-1):(fxnAmp(end)-fxnAmp(1))];
fxnAmp=fxnAmp-min(fxnAmp);

% Turn the stimulus vector into a vector of effective booleans (1 for on, 0
% for off)
stim=zeros(1,length(stimAmp)); %instantiate a vector of zeros the same size as the vector
of stim values
for i=1:length(stim)
    if (stimAmp(i)>=0.01)
        stim(i)=1;
    end
end

%slide a window 5000 values large (5s in ms increments) across the fxnAmp
%vector to and record the values
slideWindowStart=0:100:(1000*t(length(t))-5000);
slidesMatrix=zeros(length(slideWindowStart),5000);

rVec=zeros(1,length(slideWindowStart));
for i= 1:length(slideWindowStart)
    ampVec=zeros(1,5000);
    for j=1:5000
        ampVec(j)=stim(i+j);
    end
    rVec(i)=fxnAmp(slideWindowStart(i)+5000);
    slidesMatrix(i,:)=ampVec;
end

%define k, the coefficient matrix to get from stimulus to response

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k=pinv(slidesMatrix)*rVec.';

%PREDICT A RESPONSE

index = find(t==5);
for i=index:length(stim)
    answer(i-5000,:)=stim(i-4999:i)*k; %answer is the predicted response amplitude vector
end

answerFiltered=filtfilt(ones(1,200)/200,1,answer);

%plot the production and the original on the same graph
figure
hold all
plot(5:.001:4.999+0.001*length(answer),answer)
% plot(5:.001:4.999+0.001*length(answerFiltered),answerFiltered+0.03);
plot(t,fxnAmp)
grid on;
title('Comparison: Actual vs. Predicted');
xlabel('time (seconds)');
ylabel('Response Amplitude (mV)');
legend('Predicted','Actual Recorded');

%plot k
k2=filtfilt(ones(1,200)/200,1,k);
figure
hold all
grid on;
plot(1:length(k2),k2);
title('Weighting of Stimulus over Time');
xlabel('relative weighting of stimulus');
ylabel('time referenced to 0 in ms');

```