1. INTRODUCTION

1.1 Detection and discrimination in the somatosensory system

Vibration has been used to probe the responses to touch of primate somatosensory receptors and afferent fibers in the peripheral sensory nerves. Several researchers have distinguished the different types of afferent fibers, their spatial and temporal receptive field properties, and their effects in somatosensory cortex; their work can be traced back to the classic studies of Vernon Mountcastle [1]. These researchers' findings relate to behavioral psychophysical detection and discrimination thresholds for tactile stimuli. In your lab report, we want you to briefly discuss whether there are comparable types of receptors and afferent fibers in the sensory periphery of other species, specifically, in the carapace ("the hard outer shell of an arthropod") of the cockroach.

1.2 Detection and discrimination to touch and vibration in the cockroach leg

The cockroach, like its peripheral nervous system, is exquisitely sensitive to mechanical perturbations; it can detect subtle changes in the air circulating around its carapace. This sensitivity is partly the reason that it is so difficult to catch a cockroach: if a hand moves quickly toward the roach, there are changes in the air currents surrounding the roach which are detected by the sensitive mechanoreceptors on the roach's legs and body. This detection compels the roach to escape. We can quantify the sensitivity of the afferent fibers that are connected to the touch receptors in the leg of the roach, and relate these fibers to the types of sensory receptors that are found in the glabrous skin of the finger of the primate. This is the goal of the second and third parts of the laboratory.

1.3 Anatomy of the cockroach leg

The carapace of the cockroach leg is spiny and hairy. Spines are hinged and can move in one direction. Hairs, or saete, are smaller and can move in all directions. Each spine or hair is innervated by sensory mechanoreceptive nerve endings. An individual sensory fiber usually innervates more than one spine or hair, and thus its receptive field spans a number of spines or hairs. The first goal of the experiments is to determine the receptive field of an individual fiber.
2. EXPERIMENTS

The objective of the experiments is to obtain single and multifiber recordings from the afferents that innervate spines and hairs in the leg of the cockroach. The set-up uses an extracellular amplifier (SpikerBox from Backyard Brains) to record from "insect pin" electrodes that are inserted through the carapace of the leg into, or close to, the afferent nerve bundle. The output of the amplifier is displayed on an oscilloscope, acquired using Matlab, and/or feed into an audio amplifier and played over a speaker or headphones.

Vibration of the hairs on the leg is made using a glass rod attached to a "stimulating" speaker that is linked, via an amplifier, to the desktop iMac; we will use Matlab to generate functions and play those functions, via the headphone jack, on the stimulating speaker. Matlab's function generator can be set to deliver sinusoidal stimulus trains of different frequencies. The amplitude of the vibration is controlled by the output of the audio amplifier. Using this set of equipment a range of amplitudes and vibration frequencies can be tested for each sensory fiber the is encountered with the electrode.

There are four objectives for from this lab class:

1. Set up the electrophysiological recording and stimulation system.

2. Obtain receptive field maps of individual sensory fibers, or multi-unit responses if you have difficulty isolating a single fiber. Design an experiment allowing you to probe the spatial organization of the receptive field. Before you start, decide how to best represent your data, and discuss your design with other groups. How will you represent your class results?

3. Using Matlab, obtain a response-versus-vibration frequency function for a single fiber, or small number of fibers. As in the first experiment, first decide how to represent your data and predict your results. Plot this function for one or more fibers. How could you characterize the function that you have recorded?

4. Obtain a response-versus-vibration amplitude function for a single fiber or small number of fibers. Plot this function for one or, if time permits, several fibers and then average. Is the response-versus-vibration amplitude relationship linear? If you had to make an estimate of the threshold for response, how would you go about this?

5. Can you design an experiment to measure the responses to signals below, near and just above threshold? If you can implement such an experiment then how would you analyze the results of the experiment to find a level of response that was above the response when no signal was present? Can you apply a statistical analysis to the results to justify that the measured threshold was significantly above the noise?
A brief description of the set-up of equipment for recording and stimulation -- especially parts 3 and 4 above -- appears in the **APPENDIX**.

### 3. ANALYSIS OF RESULTS

*Threshold vs stimulus frequency.* Use Matlab to graph your results for each fiber; estimate the best frequency for each fiber, and if possible estimate the bandwidth of the tuning function; estimate the high frequency cutoff for each fiber.

*Response vs stimulus amplitude:* Use Matlab to graph your results for each fiber; normalize the responses and re-plot the data for each fiber; average the normalized responses that you have obtained for all the fibers. Collect the data from the other members of the class and make a group average of the normalized responses.

### 4. WRITE-UP

Write a paragraph motivating your experiments. Give a synopsis of the procedures you used. Present data that you recorded, and then present summarized class data. Use graphs and tables as required to support any claims. Discuss whether there is there a relationship between the the tuning characteristics you obtained for the cockroach afferents and the different types of afferents that innervate the primate finger?

Keep it short. Five pages is about right.

Date due: To be announced.

### 5. REFERENCES

APPENDIX

1. To listen to the single/multi-unit activity, the set-up is as follows:

SpikerBox -> [3.5mm tip-ring-ring-sleeve to tip-ring-sleeve (a.k.a. the cable labeled "smart phone")]-> built-in audio jack on the iMac

One quick-and-easy way of monitoring the input to iMac's audio jack is to use the program GarageBand, which is installed as standard.

2. To record the spike signal on the iMac, set-up as in 1 (above).

2a. Open "System preferences" and then "Sound" on the iMac. Under "input", ensure that the iMac sees the incoming signal; System preferences|Sound provides an input meter would should be bouncing.

2b. Open Matlab and change directory as follows:

   >> cd Desktop/NS2_somatosensory

Run the following script:

   >> run NS2RoachTestAcquisition

That script records a 3-second signal, and then plots that signal in a new figure window. By zooming in, you should be able to clearly identify spikes. By examining the spike amplitude, decide on a threshold that you can use to count spikes (or at least to count threshold crossings).

3. To drive the stimulating speaker, plug the Syba audio adapter into a USB port and ensure that System preferences|Sound sees it as an output. Make the following set-up:

Syba "Headphones" jack on the iMac -> (small audio jack to BNC to RCA) -> "Stimulating" audio amplifier "aux" input -> (speaker wire) -> speaker

3a. Set the volume on the "Stimulating" audio amp to low.

3b. Open Matlab and change directory as follows:

   >> cd Desktop/NS2_somatosensory

To get started, run the following function, which will drive the speaker at 100 Hz, trial after trial:

   >> [resp, sigs] = NS2RoachRespVersusFreq([100 100 100 100], 100, 0.1)
From the function itself:

```matlab
% This function coordinates the experiment -- stimulation and data
% recording/basic analysis. It receives 3 inputs:
% 1. a vector of test frequencies;
% 2. the number of repeats, i.e., the number of trials at each frequency;
% 3. a threshold to use for counting spikes.
% In addition to the test frequencies, there's also a "blank" trial. Therefore,
% if 5 test frequencies are passed as arguments, say, [2 4 8 16 32], then the
% experiment will involve 6 trial types.
% The function returns a matrix of spike counts which can be saved and used
% later to (re)plot responses. It also returns a cell array of signals -- in
% fact, each input signal that was recorded on each trial. This cell can be
% used if you want to do further processing offline.
% Example usage:
% >> [resp, sigs] = NS2RoachRespVersusFreq([10 20 30 40], 5, 0.1)
```

While running the function, slowly increase the amplitude on the "Stimulating" audio amplifier as necessary.

3c. After having set the volume of the "Stimulating" audio amplifier, run the experiment for real choosing appropriate stimulation frequencies. For example:

```matlab
>> [resp, sigs] = NS2RoachRespVersusFreq([1 2 4 8 16 32], 10, 0.1)
```