3. Practical content

The practical consists of an introductory lecture coupled with preparatory reading material, performing the practical recordings on a live specimen according to the Practical guide (Matlab live script), the Practical introduction document, and finally writing the report. Each element is detailed in this section. In addition there is a Matlab tutorial document taken from another course to support deep learning of Matlab programming, which is not included here. Also a folder of reference literature [2–7] was curated for students to enable a deep dive into the classic studies and so they can easily find references for their reports.

In brief, the students select 2-3 research questions, such as:

-How many nerve fibers are likely to be in an extracellular recording from the coxa/femur?

-What are the sensory coding principles used by tibial tactile spines?

-How is the femoro-tibial joint angle encoded in the peripheral nervous system?

-Or a research question formulated by themselves.

Then they receive a metathoracic leg dissected from an orange-spotted roach, place extracellular electrodes into it and record:

-spontaneous action potentials

-sensory evoked action potentials in response to:

- -joint angle manipulation (proprioception)
- -tactile spine stimulation (exteroception):
  - -map sensory responsive spines
  - -record orientation tuning
  - -record intensity tuning
  - -record response habituation

These are all plotted in a Matlab live script as they go, thus demonstrating the versatility of programming as a hybrid of acquisition and analysis workflows.

The students then write a report focused on their research questions, which also very briefly documents answers to 20 short questions interspersed in the Practical guide.

3.1 Introduction lecture PEL model

Lesson plan (PEL model) Practical preparation lecture

Learning Goals of this lesson:
1. Be able to describe and reflect on mechanosensors including proprioceptors and how their responses can be recorded with extracellular electrophysiology
2. Prepare to perform practical exercise in the lab next week

Phase	Time	Content/ assignments	Teacher activities (How am I trying to stimulate the learning of the students: teaching activities?) What will I be doing as a teacher?	Student learning activities (What do I really want the students to do: learning activities?) How do I want them to learn?	Organisation	Material
PREVIEW	1h	Revise proprioception and tactile sensory coding from textbook Purves et al ch9 pp 193-202.	Communicate the assignment on canvas.	Read the 10 pages and come to class with at least a rudimentary prior knowledge that: 1) Tactile sensation is based on cutaneous mechanoreceptors and hair follicle afferents. 2) Proprioception is based on muscle stretch receptors including muscle spindles and golgi tendon organs. 3) Mechanosensory responses are encoded as receptive fields of sensory neurons and stimulus intensity is encoded in firing rates of sensory neurons.	Communication on canvas.	Textbook, Purves et al ed5/6
ELABORATE/ EXPERIENCE/ ETC	2min	Ice breaker: turn to your right side neighbour and tell them how the brain detects joint angles. Explain why you think that based on your personal background.	Tell students this is their time to get to know a study partner for practicing using the vocabulary of motor neuroscience.	Sensitize students and create a safe learning environment.	Hybrid/online lecture with 122 students.	
	10min	Proprioception overview with emphasis on insect nervous system.	Explain how joint angles and other proprioceptive information is detected. Be clear about the learning goals and relate all material to them.	Grasp the diverse proprioceptive apparatuses.		Ppt slides.
	2min	Using vocabulary and recapping concepts: turn to your left side neighbour and tell them how the brain senses tactile stimuli.	Tell students this is their time to practice using the vocabulary from the previous lecture.	Sensitize to another nearby student.		
	15min	Somatosensory receptor overview with emphasis on insect tactile spines.	Explain sensory encoding in simple stimulus response systems. Detail cutaneous mechanosensors, receptive fields and rate coding.	Identify key concepts of sensory responses that they will record in the practical.		Ppt slides.
	15min	Break.				
	6min	Watch minutes 8:46-14:44 of a video about first intracellular recording of an action potential https://youtu.be/f8jxrxcLxiI?t=528	Explain key items in the video like the optical setup, oscilloscopes etc.	Get students excited about performing the practical on any specimen, be it squid giant axon or insect leg.		Youtube video
	15min	Intracellular and extracellular electrophysiology overview.	Explain how action potentials are recorded using extracellular electrodes.	Understand the scope and limitations of extracellular recordings they will use in the practical.		Ppt slides.
	30min	Preview practical.	Explain insect leg anatomy and how to do the practical effectively.	Understand the background and outline of the practical, so that the experience in the lab is more efficient.		Ppt slides.
	10min	Open for questions.	Remain available for discussions with students.	Resolve conflicts in understanding. Encourage deep learners to read beyond the textbook and start engaging with research through systematic reviews and contacting research groups.		
LOOK BACK AND FORWARD	10min	Feedback.	Request student feedback and monitor practical reports for performance.	Tune content based on feedback, e.g., not enough practical information → add a full demo run through of the experiment code.	Standard feedback form via email/canvas.	Feedback forms.

3.2 Practical

3.2.1 Practical session PEL model

Lesson plan (PEL model) Practical session

Learning Goals of this lesson.
1. Be able to describe and reflect on mechanosensors including proprioceptors and how their responses can be recorded with extracellular electrophysiology.
2. Internalize how experimental results are acquired, documented and interpreted in a logical, organized manner.

Phase	Time	Content/ assignments	Teacher activities (How am I trying to stimulate the learning of the students: teaching activities?) What will I be doing as a teacher?	Student learning activities (What do I really want the students to do: learning activities?) How do I want them to learn?	Organisation	Material
PREVIEW	1h	Revise proprioception and tactile sensory coding from textbook Purves et al ch9 pp 193-202.	Communicate the assignment on canvas.	Read the 10 pages in depth and know what the sensory apparatuses they record from are.	Communication on canvas.	Textbook, Purves et al ed5/6 Practical guide Lecture slides.
	1h	Study the practical guide.	Communicate the assignment on canvas.	Be prepared to use their time efficiently in the lab.		
	2h	Attend preparatory lecture.	Give the lecture.	Understand background knowledge required for the practical.		
ELABORATE/ EXPERIENCE/ ETC	10min	Listen to orientation by teaching assistants.	TAs explain what to do briefly and where to get equipment.	Create a safe learning environment. Orient students to the hands on exercise.	In person practical in teaching lab.	Electrophysiology recording setups with laptop and microscope . Lab consumables like tweezers, insect pins and probes. Cockroach legs. Practical guide.
	2-3h	Perform all 4 parts of the practical.	TAs go around and help students.	Learn to record sensory action potential trains.		
	Any time	Observe the living specimens.	TAs set up a terrarium with live behaving cockroaches.	Observe the leg in action and the coordination with other legs in the 3-by-3 leg alternating locomotor gait.		Terrarium with living specimens.
	2h	Write practical report.	Student activity.	Understand how to document and interpret results in a logical organized manner.	Prepare on own time and ask questions at the QnA session organized at the end of the week.	Practical report template.
LOOK BACK AND FORWARD	10min	Feedback.	Request student feedback and monitor practical reports for performance.		Standard feedback form via email/canvas.	Feedback forms.

3.2.2 Practical introduction document

Sensory coding practical Introduction

Action potentials and sensory coding in the cockroach leg

In this practical you will learn principles of sensory coding by action potentials. We will study these principles in the sensory neurons of the cockroach nervous system. Here you will learn to record action potentials using extracellular electrodes, stimulate sensory receptors and record their responses, map sensory responses, and understand how sensory coding works. Before the practical, you need to attend the "Practical Introduction" lecture and read this guide. It is also useful to review Ch 2 and Ch 9 from Purves textbook (editions 5 or 6).

Objectives:

Before doing this practical you should understand:

- the purpose and shape of an action potential
- the basics of electrophysiology
- how measuring action potentials from either inside or outside of a cell affects the data collected
- the basic anatomy of the cockroach leg and nervous system

After doing this practical you should be able to:

- distinguish between intra- and extracellular recordings
- perform basic analysis of electrophysiology data in Matlab
- design basic electrophysiology experiments to study the nervous system
- understand how sensory stimuli are encoded by peripheral sensory receptors as action potential trains which enter into the nervous system
- explain how an organism can differentiate between multiple types of sensory stimulations
- describe the concepts of rate coding and habituation

Materials

- SpikerBox
- Laptop
- Cockroach
- Ice
- Dissection scissors
- toothpicks
- plastic pipette

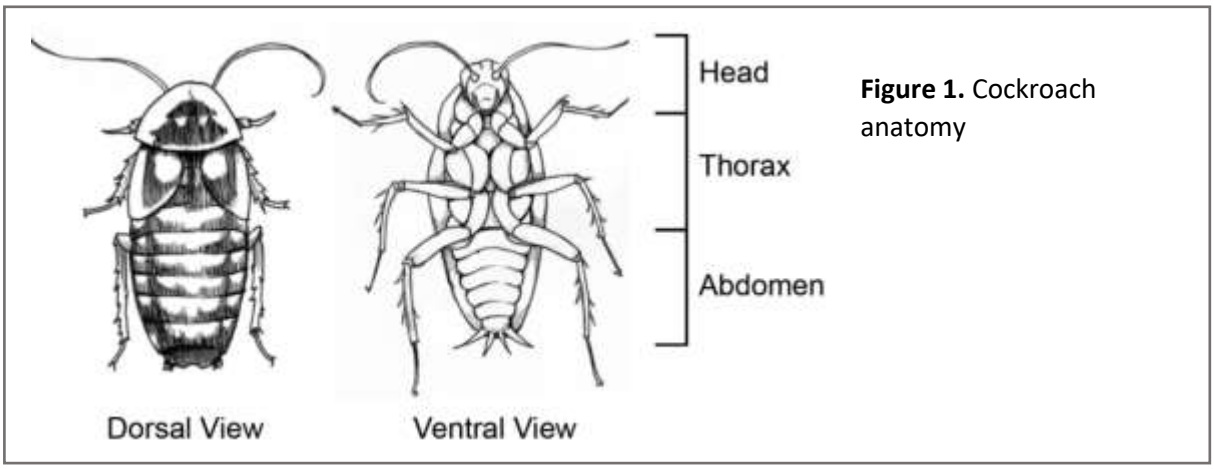


Figure 1. Cockroach anatomy

The cockroach leg as a sensorimotor model system for neurophysiology

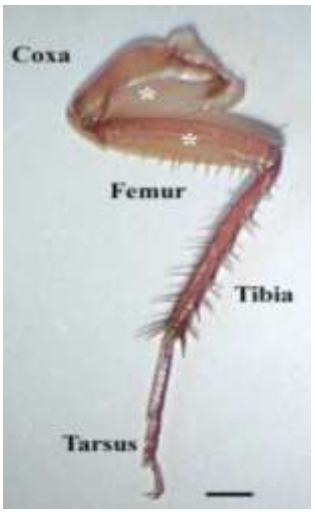


Figure 2. Cockroach leg. White asterisks indicate the placement of the recording and ground electrodes. (From Ramos et al 2007 JUNE)

For this practical, you will be recording action potentials (APs) in a leg from the orange-spotted roach *Blabtica Dubia* (Figures 1 & 2). Legs of large insects are useful preparations in neurophysiology as they contain many different sensory receptors and their large action potentials can be easily measured with simple equipment.

Like most arthropods, each segment of the cockroach contains a region of the Ventral Nerve Cord (VNC), which is analogous to the spinal cord in some chordates. The VNC neurons send information to the muscles of the body, while receiving information from the sensory organs of the periphery. This information is relayed to and from the brain using action potentials and synapses. The brain is located in the head of the cockroach, but it is not as important as the VNC for survival of the animal – after all, a decapitated cockroach can survive and remain mobile for weeks.

Because of the combined sensory and motor function of the leg, it has many sensory receptors aimed at proprioception (detection of leg position and load) and tactile sensation (detection of external tactile stimuli). When observed up close, you can see how the cockroach leg is covered with large tactile spines along the tibia and femur (Figure 3). Each spine has a sensory neuron at its flexible socket. When this apparatus moves due to deflections of the spine, the sensory neuron sends APs to the VNC and eventually the brain. The pattern and frequency of APs sent will allow the VNC to distinguish a strong external stimulus from a weak one. Which hair cells are being stimulated will determine where the cockroach perceives the stimulation is located. The duration of the stimulus is also *encoded* into the pattern of sensory APs, as is the direction of spine deflection! These are sophisticated sensors.

Figure 3. Tactile spines and their structure (from Tuthill and Wilson, 2016, Curr Biol)

The legs have load-sensitive sensory receptors on the cuticular surface, called campaniform sensilla (Figure 4). Campaniform sensilla are proprioceptors, which sense the force exerted on the leg, for

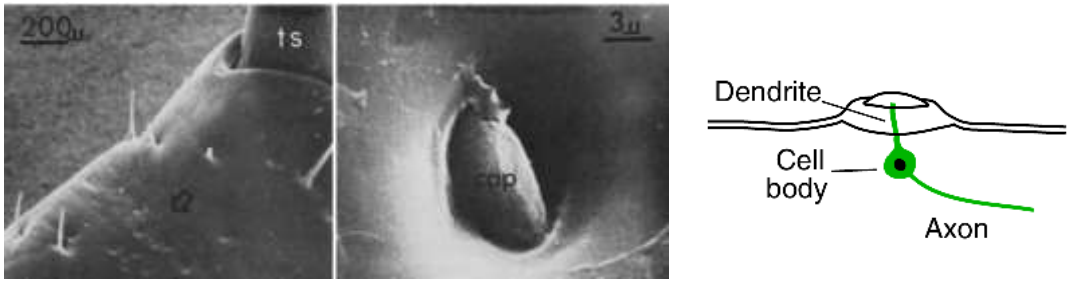


Figure 4. Scanning electron micrograph showing campaniform sensilla (arrow, left), its magnification (middle panel) and the schematic representation (from Moral et al, 1971, J of Cell Biol & Tuthill et al, 2016, Curr Biol)

example during locomotion. Like tactile spines, each campaniform sensillum contains one sensory neuron whose dendrite is coupled to a mechanical apparatus. This oval dome shaped cap flattens when the cuticle of the leg is under strain, causing AP firing in the sensory neuron. Their sensory feedback is thought to reinforce muscle activity during the stance phase and to contribute to inter-leg coordination, much like sensory feedback from mammalian Golgi tendon organs.

Both tactile spines and campaniform sensillae are mechanoreceptors that contain one neuronal cell body which sends an axon to the CNS. Finally, the femoral chordotonal organ (fCO, figure 5) located inside the femur is formed by hundreds of sensory neurons, some of which are sensitive to the angle of the femoral-tibial joint.

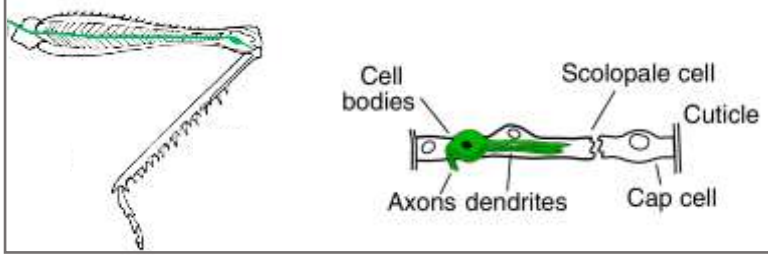


Figure 5. Femoral chordotonal organ located near the tibial-femoral joint (left), containing many sensory neurons (right). (from Tuthill and Wilson, 2016, Curr Biol)

These mechanosensory organs are sensitive detectors of kinetic energy. The deformation of a mechanical component (like the oval dome of a campaniform sensilla) induces a depolarizing receptor potential in the dendrites of a sensory neuron. This causes the neuron to fire APs, which are conducted along the axon to the CNS. These mechanosensors are distributed along all six legs of the cockroach in a stereotypical fashion, forming an overall sensory apparatus helpful in identifying external tactile stimuli as well as the self-motion when the animal walks.

Action Potentials

In this practical we will record action potentials (APs, Figure 6). An AP is an extremely fast (1-2 ms duration) voltage change across the cell membrane that acts as fast moving, long-range signal. The AP, once initiated, will spread along the membrane of a cell, thus allowing a cell to communicate with astonishing speed (80 m/s). Neurons can send APs in specific patterns along their axons to convey information to synapses, the point at which one neuron communicates with another through chemical signaling.

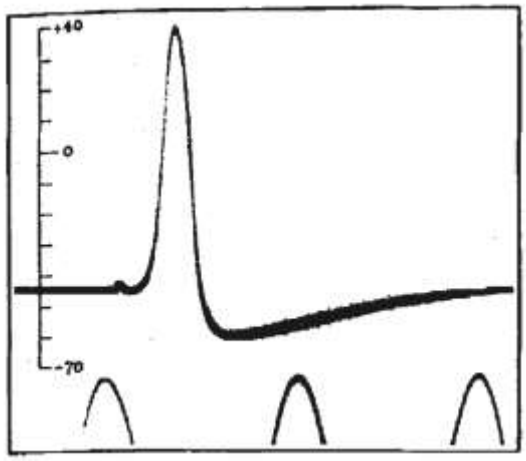


Figure 6. The first intracellular recording of an action potential in 1939 by Nobel laureates Hodgkin and Huxley, who are shown doing a recording on the cover page of this guide (from Hodgkin AL & Huxley AF,1939, Nature).

The main feature of an AP is the distinctive shape (Figure 6). This graph shows the charge across a section of membrane over a very short period of time. When a cell is at rest, or not sending an AP, it is typically at a resting membrane potential more negative than -40 mV. The resting membrane potential is the resulting voltage established by a cell concentrating various ions (Na+, K+, Cl-) on different sides of its membrane. This uneven distribution of ions creates a net negative charge inside the cell.

If a neuron is depolarized enough it will generate an AP. During the AP, the membrane potential has a fast rise and fall caused by the movement of Na+ and K+ across the membrane, respectively. The movement of these ions can be measured using electrophysiology.

Electrophysiology

Electrophysiology, the study of the electrical properties of cells, allows researchers to study how neurons communicate with each other and form complex neural networks. In particular, electrophysiology is used to measure the properties of APs in living neurons. Two of the most common approaches used to study APs are to record from either an intracellular or extracellular perspective (Figure 7).

Intracellular recordings allow someone to measure either the voltage or current (movement of ions) across the membrane of a single cell. This technique requires the insertion of a recording electrode, typically a glass pipette filled with a conductive solution, inside a neuron. A second electrode, the ground electrode, is placed extracellularly. The difference between these electrodes then records the voltage (or current, depending on the type of amplifier used) across the cell membrane directly. For instance, an AP measured using this technique will look like those described in textbooks, with an initial positive depolarization followed by a negative repolarization and after hyperpolarization. It is important to remember that intracellular recordings are measuring the change in electrical properties across the cell membrane from the perspective of being inside the cell.

Extracellular recording is another electrophysiological technique that measures the voltage and current across the membranes of neurons indirectly. However, rather than having to insert an electrode into a single cell, extracellular recordings can be made by simply placing a recording electrode adjacent to a cell membrane. For these experiments, measurements of charge and movement of ions across cell membranes will appear to be inverted compared to intracellular recordings. This is because the recording electrode is measuring ions entering and leaving the extracellular space. This is the technique that we will use in this practical by placing the recording electrode in the vicinity of sensory nerve axons.

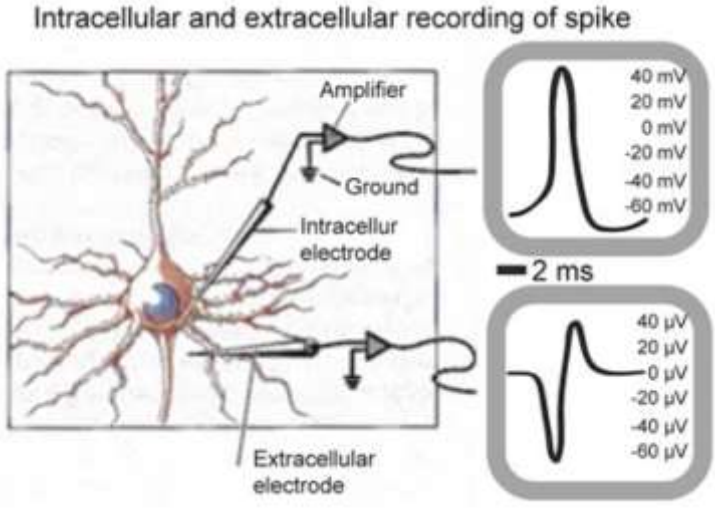


Figure 7. Examples of an intracellular and extracellular recording of action potentials. Do you see what the main differences are?

Another important way in which data collected from intra- or extracellular recordings differ is the amplitude of APs collected. APs collected from intracellular recordings are consistent in their amplitude during an experiment. This is because there is little difference in how APs look once they are initiated. However, the amplitude of APs collected from extracellular electrodes can be of varying sizes for several different reasons. The first is that extracellular recordings may be measuring electrical activity from multiple cells at one time. If multiple axons fire an AP at the same time, these can add up to one waveform on the recording electrode. However, keep in mind that the AP recorded by an extracellular electrode will get smaller the farther away the axon is from the point of measure.

Rate Coding

How do your neurons code the difference between a finger lightly touching your arm and a more forceful poke? There are several reasons for why you differentiate the two stimuli. First, the light touch likely stimulates nerves from a very small area (within the *receptive fields* of a small number of sensory neurons) while the more forceful poke may stimulate neurons from much more of your arm. Second, the light touch may only stimulate neurons that respond to being compressed. These neurons, among them Merkel cells, send action potentials to the spinal cord and brain in response to light touch. A light touch increases their firing rate slightly, which is interpreted by your spinal cord and brain as a light touch. With the more forceful poke, the Merkel cells fire much faster. This form of rate coding is a common way to encode information about sensory stimuli and is also used by motor neurons to control the force of muscle contraction. In this practical we will study several ways the cockroach sensory system uses rate coding to convey sensory information. Besides rate coding, neural networks also use other forms of temporal coding and ensemble coding to encode information.

Steps of the practical

The practical will consist of 4 parts:

Part 1: Record spontaneous action potentials
- Obtaining a recording
- Isolating units
- Analysing the spike shape

Part 2: Sensory stimulation – joint angle
- Encoding of leg flexion

Part 3: Sensory stimulation – mechanosensory spines
- Encoding of spine deflection direction and intensity

Part 4: Sensory stimulation – response habituation
- Encoding of stimulus duration

Figure 8. The cockroach leg on SpikerBox

Preparation of the cockroach leg

The preparation will be done with the help of the practical assistants. Animals are first placed on ice and cooled until immobile (three to five minutes). Roaches are then placed ventral side-up and small scissors are used to remove the legs at the level where the coxa is connected to the thorax, leaving the coxa, femur, tibia, and tarsus intact. You will place the Individual legs onto a piece of corkboard on the SpikerBox. One electrode is inserted into the coxa and another into the femur. Figure 8 illustrates the correct placement of electrodes.

You will use a step-by-step guide in Matlab designed for this practical to guide the recordings and analysis of the data. After the practical you will use the results of your analysis and generated figures to write a practical report.

Good luck

and lots of fun with your recordings!

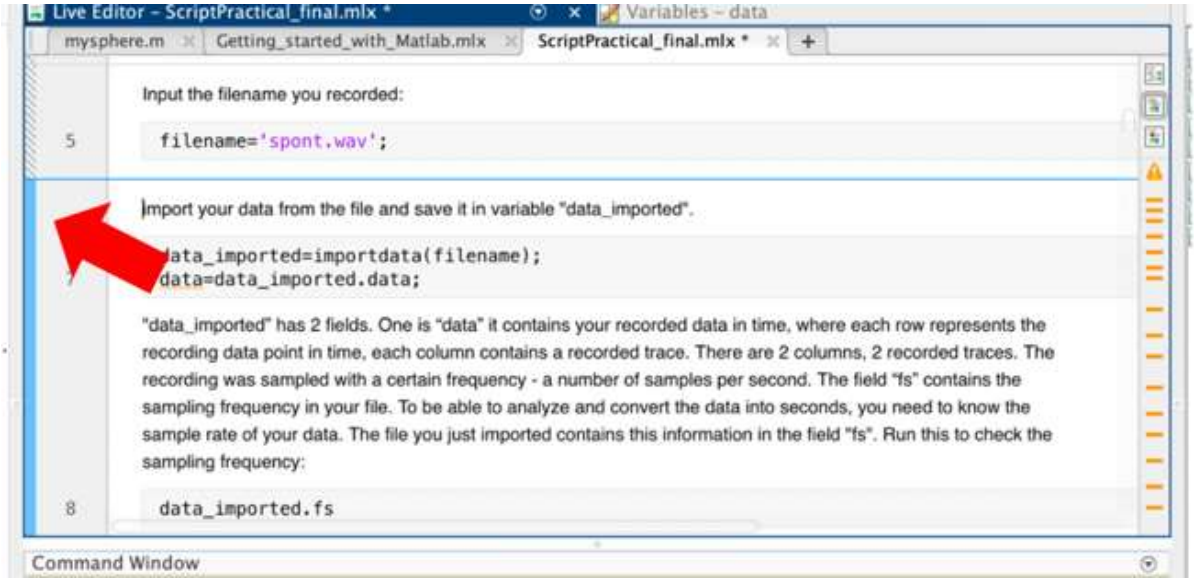
3.2.3 Practical guide

Sensory coding practical

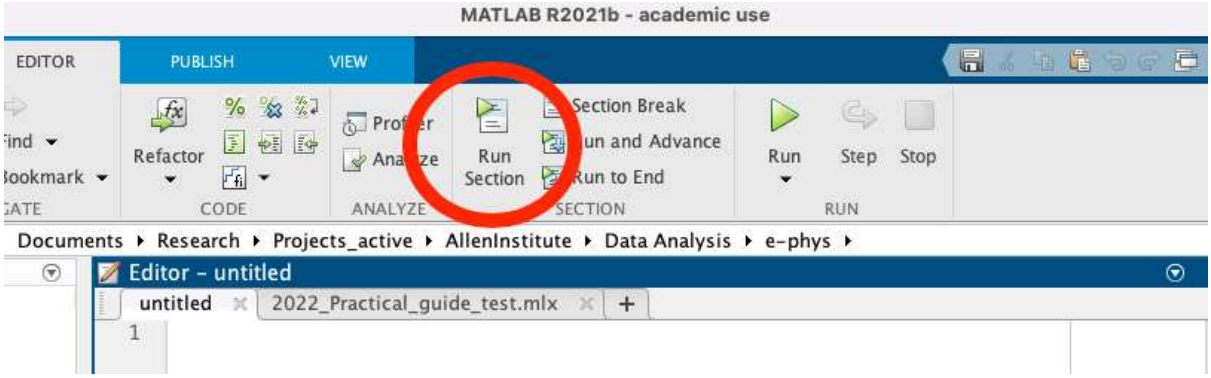
Before you begin...

This is a live script document that you can open and run in Matlab. This document contains both explanation text for the practical as well as the Matlab code for the data analysis. In this way can run the analysis as you go and analyze your files during the practical. Here are some important things you have to know before you start:

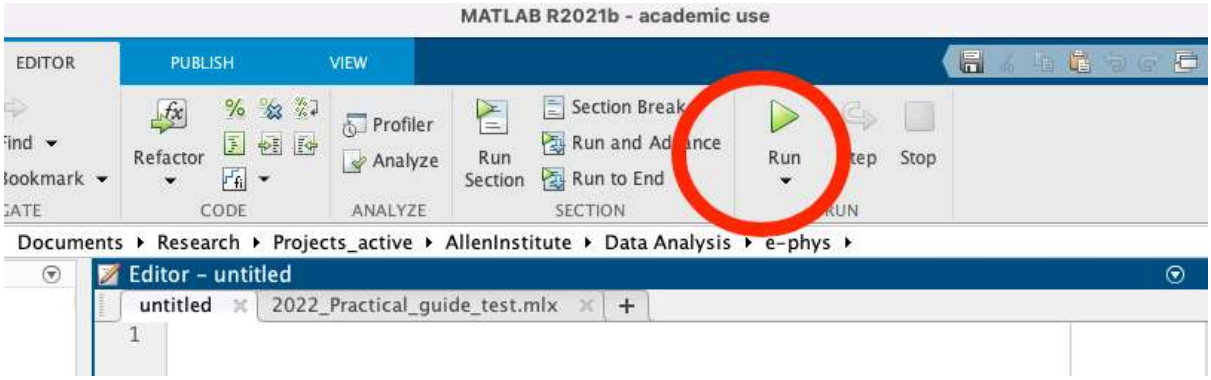
- The code is written in small sections, so that you can easily understand what you do and run it section by section. You can recognize the section limits by blue lines around the section. If you click on the code within the section you are in!



- Now you can run the code in the section by pressing the button "Run Section".



- Do not press "Run", it will execute all the code in the scirpt, take a lot of time and generate a lot errors because of still missing data. If this still happens by accident, go back to the section of the code you were working on and proceed from there (as you go further you will rerun the code with the correct variables and the errors will disappear).



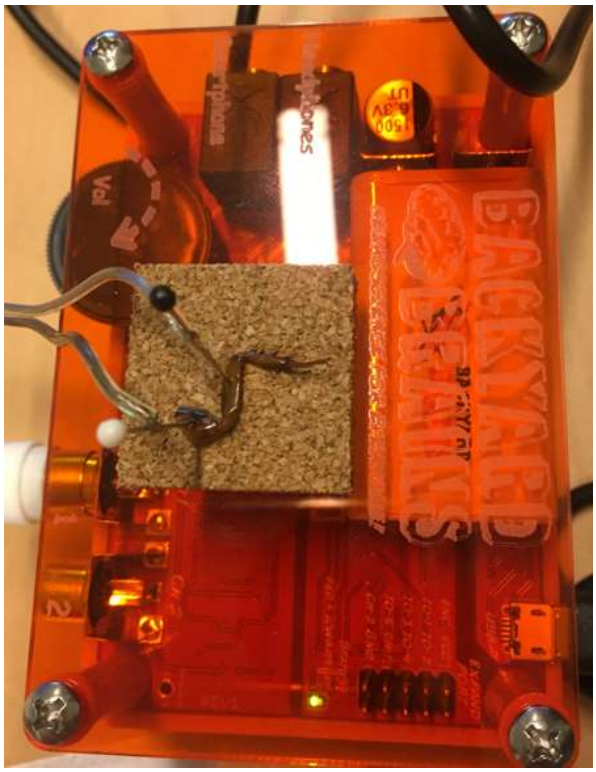
- Be careful to change the names of the files you analyze and the figures you are going to save for the report.
- There are questions that you will have to answer during the practical. Don't forget to save your answers to the questions, you will need them for your report. You can also just type your answers in the text below the quesitons in this file and save this file as .mlx file and .docx file at the end of the practical. In this way you will be able to see later what you have done during the practical and rerun the script if you want.
- At the end of the practical don't forget to copy the folder with your analysis, figures and recordings to your own memory device or upload on your cloud drive.

You are set to go!

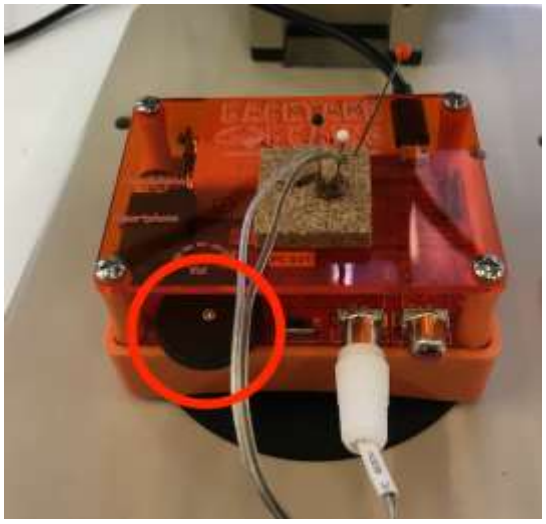
Part 1: Recording Spontaneous Action Potentials

Preparation

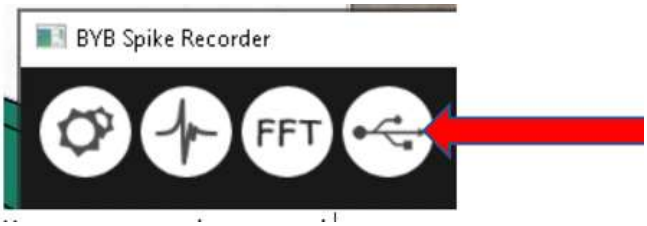
- Get the cockroach leg from pracitcal assistant and mount it on the amplifier (Backyard Brains Neuron Spikerbox pro). Clean your electrodes. Insert the ground electrode (black pin) into the femur, piercing it into the underlying corkboard. Insert the recording electrode (white pin) into the coxa.



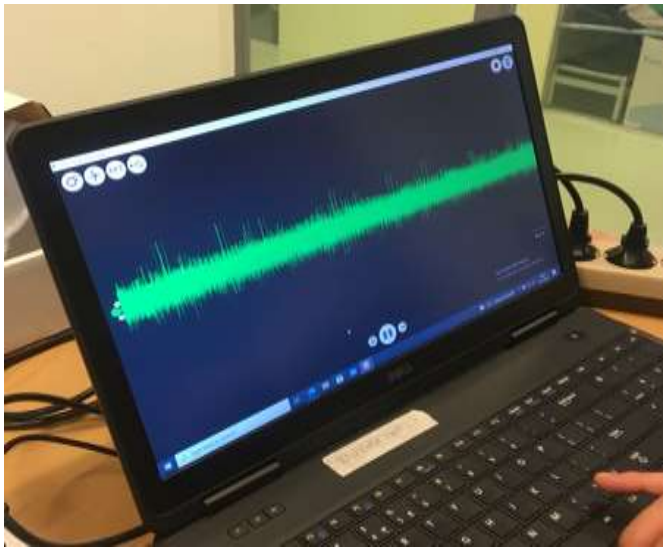
- Plug in the USB connector to laptop and turn on the amplifier by turning the black volume knob:



- Open the Spike Recorder software.
- Click the top left usb icon to connect to amplifier



- You are now ready to record s**pontaneous activity** of axonal fibers in cockroach's leg
- In a high-quality recording you will clearly hear spontaneous action potentials through the speaker. It should sound like raindrops hitting a window over a quiet background noise. You will also be able to see spikes on the Spike Recorder oscilloscope window.



- If you cannot see and hear the spikes, try placing your electrodes at different locations in the coxa and femur.
- If you still cannot see and hear the spikes after a few electrode placements, ask the TA for another leg.
- Record action potentials for about 10s using the top right record button.
- Rename the file "spont" *(.wav is the extension of the file, it is added to the file automatically).*

Step 1. Import data and plot the spontaneous signal

Close all figures and clean the variables from your workspace and start with an empty workspace:

```
clear all

close all
```

Define here the path to the folder in which you are working on the computer. If you are going to rerun the analysis on your own computer, don't forget to put all the files in one folder and specify here the path to this folder:

```
folder='C:\....';

addpath(genpath(folder));
```

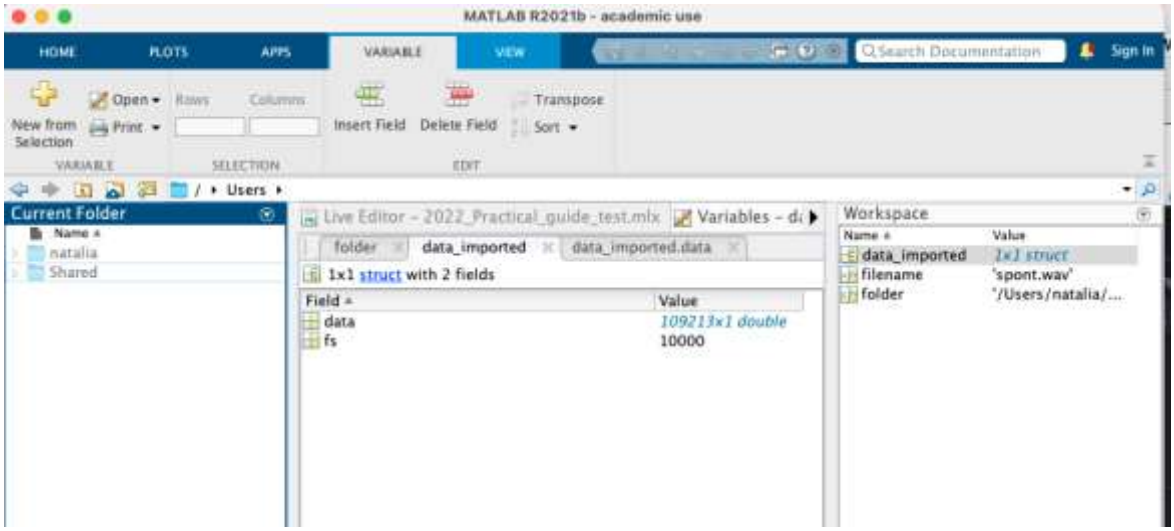
Input the filename you recorded (make sure the name of the recorded file is *exactly the same* as in the code).

```
filename='spont.wav';
```

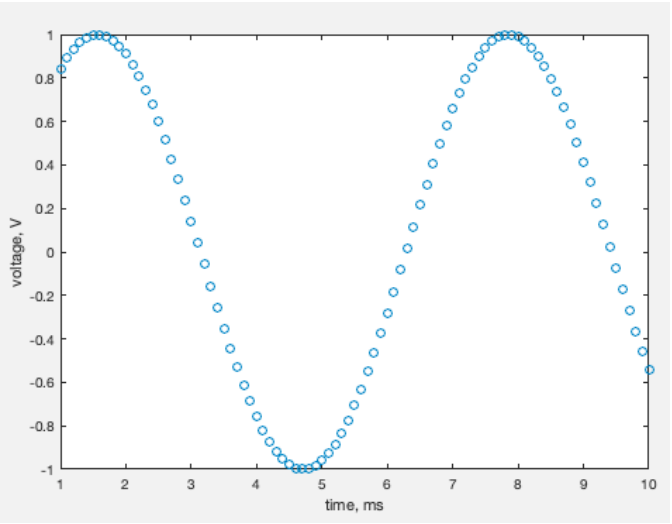
import your data from the file and save it in variable "data_imported". If you get an error here, it is most probably because the folder name to the files or the name of the file do not match with the real names. Check folder and file name again.

```
data_imported=importdata(fullfile(folder,filename));
```

To your right, in Workspace panel, you can now see the created variables, if you click on the variable it will appear in a "Variables" tab and you can examine it. "data_imported" has 2 fields. One is “data” it contains your recorded data in time, where each row represents the recording data point (sample) in time. In the example below, there is 1 column, 1 recorded trace with 109213 samples.



The recording was sampled with a certain frequency - a number of samples per second. The field “fs” contains the sampling frequency in your file. To be able to analyze and convert the data into seconds, you need to know the sampling rate of your data. In the example below you can see the recording in samples (shown as blue circles). In this example, there are 100 samples in a 10 ms long recording, so the sampling frequency is 10 000 samples in a second, or 10 000 Hz.



The file you just imported contains this information in the field "fs". Run this to check the sampling frequency:

```
data_imported.fs
```

You can see that the sampling rate is 10000 Hz or 10 kHz. Save it as a variable "srate":

```
srate=data_imported.fs;
```

Save your data as a variable "data":

```
data=data_imported.data;

% we will not need data_imported anymore, so we clear it

clear data_imported
```

How many rows does your data variable have? how long was the recording?
We will cut the data file to the nearest full second, e.g if your file is 10.564 s long, we will cut it to exactly 10 s long. This will help us when we will devide the file in 1s bins to calculate spike frequency.

```
data=data(1:floor(size(data,1)/srate)*srate,:);
```

The data variable contans 1 or several columns, we will only analyze the signal from your recording electrode in the first column. Let's name it *trace* and reverse its polarity (all the negative numbers become positive):

```
trace=-data(:,1); % reverse polarity if needed
```

Create a new variable “x” that contains the data corresponding to the time of each sample in seconds, in this way we will be able to plot the data in seconds.

```
x=(1:size(data,1))/srate;
```

Now we will create a figure and plot the trace:

```
h=figure;

plot(x,trace);
```

Add y and x axis labels and the title.

```
hold on;

ylabel('Voltage,mV');

xlabel('Time, s')

title(filename);
```

Step 2. Analyze the trace: extract the spikes

Now that we have imported the trace, we can start the analysis and extract spikes (action potentials). First find the peaks of action potentials by using the function "findpeaks". If you get a warning like this:

```
Warning: Invalid MinPeakHeight. There are no data points greater than MinPeakHeight.
```

It means that your threshold for defining peaks is too high and no spike were detected. The reason for this is that your recording most probably does not contain spikes or the threshold value is too high. Try to make a new recording of sponaneous activity.

```
clear locs % in case locs already exist clear it

amp_threshold=0.05;

[~,locs]=findpeaks(trace,'MinPeakHeight',amp_threshold,'MinPeakDistance',3);
```

If in your signal the spikes are negative you can reverse the signal of the trace by running this code in the command line below:

trace=-trace;

Plot the trace and mark all action potentials (peaks) with a red circle:

```
plot(x, trace,'b');

hold on

plot(x(locs),trace(locs),'ro');

hold off
```

You can play with the settings and zoom in a part of the trace to better see the spikes. We will do it by defining the interval of the trace to plot.

```
%define here the start and end of the interval to plot (in samples)

start_interval=5000;

end_interval=7000;



interval=[start_interval:end_interval];
% plot the interval

plot(x(interval),trace(interval),'b');

hold on;

ylabel('Voltage,mV');

xlabel('Time,s');

title('Trace with action potentials');



% select only the peak indices that fit within the interval
newlocs=locs(locs>start_interval & locs<end_interval);



% plot the spike peaks with red circle
plot(x(newlocs),trace(newlocs),'ro');

hold off
```

Now we can tune our analysis. Since the threshold for detecting the spikes is different for each recording conditions, you can play with the amplitude settings to define the minimum peaks. In your analysis you want to avoid mistaking noise for spikes on the one hand, but also missing the small spikes. The default amplitude is 0.05. Change the threshold value. What happens with the detection of peaks if you increase or decrease this threshold value?

```
clear locs % in case locs already exist clear it

amp_threshold=0.03; %change amplitude threshold here!

[~,locs]=findpeaks(trace,'MinPeakHeight',amp_threshold,'MinPeakDistance',3);

plot(x, trace,'b');

hold on

plot(x(locs),trace(locs),'ro');

hold off
```

Choose the best interval to plot:

```
%define here the start and end of the interval to plot (in samples)
```

```
start_interval=2000;

end_interval=3000;

interval=[start_interval:end_interval];

% plot the interval

plot(x(interval),trace(interval),'b');

hold on;

ylabel('Voltage,mV');

xlabel('Time,s');

title('Trace with action potentials');

% select only the peak indices that fit within the interval

newlocs=locs(locs>start_interval & locs<end_interval);


% plot the spike peaks with red circle
plot(x(newlocs),trace(newlocs),'ro');

hold off
```

When you found the optimal threshold and time window, save the figure. Enter the name for the saved figure in control field, replace xxx with your own name:

```
% if you don't have a folder, make a folder here called 'Figures' for saving
your figures

if not(isfolder(fullfile(folder,'Figures')))

    mkdir(fullfile(folder,'Figures'))

end

saveas(h,fullfile(folder,'Figures','Fig1_xxx.jpeg')); % Now your figure is in
the folder Figures
```

Check if the figure was saved.

Q1: What ions cause the phases of the action potential?

Step 3. Frequency of firing

Let's find out what the average frequency of firing is in your trace. To do this you need to divide the number of spikes in the trace by the duration of the trace. Try to estimate by eye the frequency in your recording, is it similar to the calculated mean_freq?

```
mean_freq=size(locs,1)/max(x); % this will give you a number of spikes per
second
```

Q2: What is the average firing frequency in your recording?

Plot firing frequency in your recording as a function of time:

```
h2=figure;
histogram(x(locs),size(trace,1)/(1*srate));
ylabel('spike freq, Hz');
xlabel('time, s');
saveas(h2,fullfile(folder,'Figures','Fig2_xxx.jpeg')); % save your figure here
```

Q3: What might cause changes in frequency in your plot?

Q4 What is the number of spikes in the first second? Is it similar to what you calculated previously with mean_freq?

Step 4. Spike amplitude

Now let's have a look at an average spike (waveform). We will extract the waveforms for all detected spikes and calculate the average waveform. We will plot the traces in the window of 20 samples, the traces for individual spikes are black, the average waveform is plotted in blue, standard deviation in shown in red lines.

```
wave=[];
window=20; % how many samples on either side of spike
for i=1:size(locs,1)
    if locs(i)>window
    wave(i,:)=trace([locs(i)-window:locs(i)+window]);
    end
end
wave(wave==0)=NaN;
wave_ave=mean(wave,1);
wave_std=std(wave,1);
h3=figure;
plot(wave(:,:)','k');
hold on
plot(wave_ave,'Color','b','LineWidth',3);
errorbar(wave_ave,wave_std, 'Color','r');
xlabel('Time, samples');
ylabel('Voltage,mV');
title('Average spike');
```

```
hold off
```

You can change the window to plot more or less samples around the peak and plot only the average trace:

```
wave=[];
window=20; % change the value of window here
for i=1:size(locs,1)
    if locs(i)>window
    wave(i,:)=trace([locs(i)-window:locs(i)+window]);
    end
end
wave(wave==0)=NaN;
wave_ave=[];
wave_std=[];
wave_ave=mean(wave,1,'omitnan');
wave_std=std(wave,1,'omitnan');
plot(wave_ave,'LineWidth',3);
hold on
errorbar(wave_ave,wave_std); % plot the sandard deviation of the average spike
hold off
xlabel('Time, samples');
ylabel('Voltage,mV');
title('Average spike');
```

Q6: What is the interval between two samples? What is the half width at half height of the average spike?

Save the figure for your report:

```
saveas(h3,fullfile(folder,'Figures','Fig3_xxx.jpeg'));
```

Now we find out what is the distribution of all amplitudes (maximum point of each waveform) in the recording and plot this distribution as histogram:

```
amp=max(wave,[],2);
h4=figure;
histogram(amp,20);
```

```
xlabel('Spike amplitude, mV');
ylabel('Count');
title('Amplitude histogram');
```

Q7: What is the most frequent amplitude of the waveform? Do you have multiple peaks in your distribution? What does it mean?

Save the figure for your report:

```
saveas(h4,fullfile(folder,'Figures','Fig4_xxx.jpeg'));
```

Step 5. Isolate different axons based on amplitude

If there are multiple amplitudes, we can try to isolate the separate axons with different characteristics based on the amplitude. Rerun the code for analyzing your trace and extracting spikes, but now change the threshold to only extract information on one axonal fiber with the biggest amplitudes.

```
clear locs % in case locs already exists, clear it and recalculate
amp_threshold= 0.03; %change amplitude threshold here!
[~,locs]=findpeaks(trace,'MinPeakHeight',amp_threshold,'MinPeakDistance',3);


mean_freq=size(locs,1)/max(x); % this will give you a number of spikes per
second
h5=figure;
plot(x,trace);
hold on
ylabel('Voltage,mV');
xlabel('Time,s');
title('Trace with action potentials');
plot(x(locs),trace(locs),'ro');
```

Save the figure:

```
saveas(h5,fullfile(folder,'Figures','Fig5_xxx.jpeg'));
```

Plot now the spike shape and amplitude distribution for this new analysis:

```
wave=[];
window=20; % change the value of window here
for i=1:size(locs,1)
    if locs(i)>window
```

```
    wave(i,:)=trace([locs(i)-window:locs(i)+window]);
    end
end
wave(wave==0)=NaN;
wave_ave=mean(wave,1);
wave_std=std(wave,1);
% plot waverform
h6=figure;
plot(wave_ave,'LineWidth',3);
hold on
errorbar(wave_ave,wave_std); % plot the sandard deviation of the average spike
hold off
xlabel('Time, samples');
ylabel('Voltage,mV');
title('Average spike');
```

Note: if you have no detected spikes in the recording, you will not be able to plot the spike shape. In that case, try to rerun the steps above with a lower threshold.

Q8: Is the half width now different from previous analysis?

Save the figure:

```
saveas(h6,fullfile(folder,'Figures','Fig6_xxx.jpeg'));
```

Now plot the amplitude distribution for this new analysis. Is it different?

```
amp=max(wave,[],2);
h7=figure;
histogram(amp,20);
xlabel('Spike amplitude, mV');
ylabel('Count');
title('Amplitude histogram');
```

Save the figure:

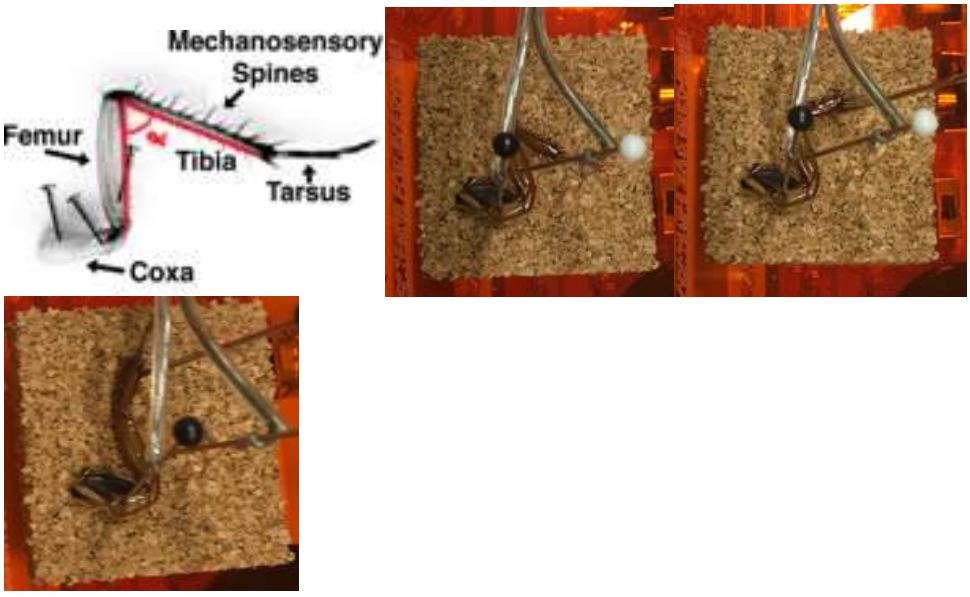
```
saveas(h7,fullfile(folder,'Figures','Fig7_1xxx.jpeg')); % save your figures
here
```

Part 2: Sensory stimulation

Step 1. Sensory encoding of leg flexion

In this part we will activate the proprioceptors in the leg by changing the angle of the joint. We will analyze the differences in action potential firing at different positions of the leg.

- Use pins to set the femur-tibia joint at different angles. Make a short recording at each angle after you changed the position of the leg.



- Each recording should last about 5s
- After recording, rename the files according to the angle of deflection, e.g. A_00.wav, A_45.wav and A_90.wav – you can go up to 180 degrees.

Lets first clear all variables, and close all figures.

```
clear amp amp_threshold ans data end_interval filename locs interval mean_freq newlocs ...
    start_interval trace wave wave_ave wave_std window x i h h2 h3 h4 h5 h6 h7
close all
```

Which files did you record? Enter the file names and number of the recordings in the script below.
You can add or remove the files by adding or removing the control field boxes. Alternatively, you can insert a comma after the last box and type the name of extra files in quotation marks.

```
filenames={'A_00.wav','A_90.wav','A_180.wav'};
```

```
n_recordings=3; % input the number of recordings(files to open) here
```

Import the data by looping through the recordings one by one (we use "for loop"). Many of the steps are very similar to importing data from the recording in the first part of the practical, so you should recognize this code by now.

```
h8=figure;


for i=1:n_recordings % loop through all the recordings
    filename=fullfile(folder,filenames{i});
    % import data for the files one by one
    data_jointangle=importdata(filename);
    data=data_jointangle.data; %import the data
    srate=data_jointangle.fs; %import the sampling rate
    clear data_jointangle; % clear the data you are not using anymore


    data=data(1:floor(size(data,1)/srate)*srate,:); %cut to nearest full
second
    trace=-data(:,1);%reverse polarity



    % Plot the traces
    x=(1:size(data,1))/srate;
    ax(1)=subplot(2,n_recordings,i);
    plot(x,trace);
    hold on;
    ylabel('Voltage,mV');
    title(filenames{i});


    % Find peaks:
    clear locs
    amp_threshold=0.05; %change amplitude threshold here!
    locs=[]; %start with an empty locs variable for each iteration through
files

[~,locs]=findpeaks(trace,'MinPeakHeight',amp_threshold,'MinPeakDistance',3); %
change amplitude threshold here
```

```
    plot(x(locs),trace(locs),'ro');
    ax(2)=subplot(2,n_recordings,i+n_recordings);
    histogram(x(locs),size(trace,1)/(1*srate));
    ylabel('Spike freq, Hz');
    xlabel('Time, s');
    linkaxes(ax,'x');
    mean_freq(i)=size(locs,1)/max(x)
end % this is where the "for" loop ends. you are now through plotting all your recordings
```

Now let's find how the firing rate encodes different angles:

```
h9=figure;
angles=[0,90,180]; %input the joint angles you recorded here in degrees
plot(angles,mean_freq,'ko-','LineWidth',3);
xlabel('Joint angle, degrees');
ylabel('Spike freq, Hz');
title('joint angle encoding');
```

Save your figures:

```
saveas(h8,fullfile(folder,'Figures','Fig8_xxx.jpeg')); % change the name of the figure in the control field
saveas(h9,fullfile(folder,'Figures','Fig9_xxx.jpeg'));
```

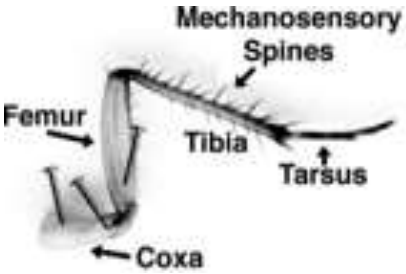
Q9: How is joint angle encoded by the sensory spikes you recorded?
Q10: What is rate coding?

Q11: For which behavioral situations might the roach need joint angle information?

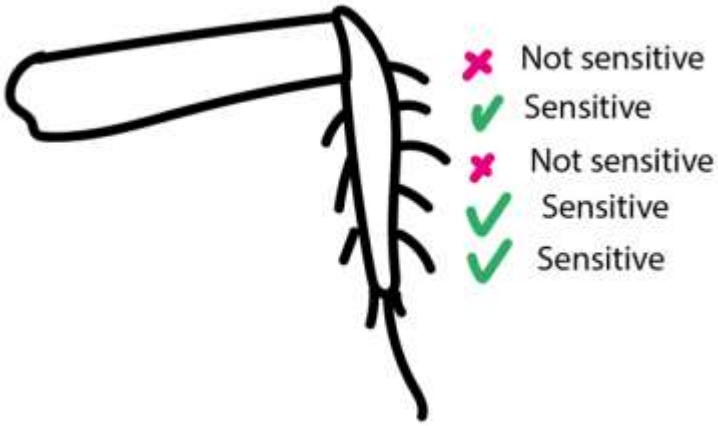
Part 3: Sensory stimulation – tactile spines

Step 1. Sensory encoding of spine deflection direction

- While keeping your recording and stabilizing the femur-tibia joint with pins, use a tooth pick to stimulate different tactile spines:

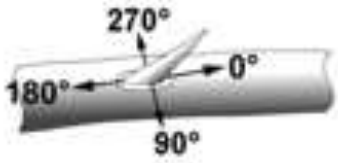


- Go spine mapping: Draw a sketch of the leg and label the responsive spines on it.



Q12: Are some spines more responsive than others? Is there a logic to their positions?

Choose one sensitive spine and record firing for different orientations. Use a toothpick to deflect the spine in a different direction in each recording. Name the files according to the angle of deflection, e.g.: D_00.wav, D_90.wav, D_180.wav and D_270.wav. Record 4 different deflection angles.



.

```
clear amp_threshold angles ax data filename filenames h8 h9 i locs
mean_freq...
    n_recordings trace x

close all
```

Let's input the filenames of the files you have just recorded and renamed:

Input the filenames you recorded here.

```
filenames={"D_00.wav","D_90.wav","D_180.wav","D_270.wav"};

n_recordings=4;%input the number of recordings here
```

Make a figure and plot the results of the frequency analysis:

```
h10=figure;



for i=1:n_recordings
    filename=fullfile(folder,filenames{i});

    data_orientation=importdata(filename);

    data=data_orientation.data;

    srate=data_orientation.fs;

    clear data_orientation;

    data=data(1:floor(size(data,1)/srate)*srate,:); %cut to nearest full
second



    trace=-data(:,1);%reverse polarity


    % plot

    x=(1:size(data,1))/srate;

    ax(1)=subplot(2,n_recordings,i);

    plot(x,trace);

    hold on;

    ylabel('Voltage,mV');

    xlabel('Time, s')


    title(filenames{i});


    %find peaks

    amp_threshold=0.05; %change amplitude threshold here!

    locs=[]; %start with an empty locs variable for each iteration through
files


[~,locs]=findpeaks(trace,'MinPeakHeight',amp_threshold,'MinPeakDistance',3); %
change amplitude threshold here

    plot(x(locs),trace(locs),'ro');
```

```
    ax(2)=subplot(2,n_recordings,i+n_recordings);
    histogram(x(locs),size(trace,1)/(1*srate));
    ylabel('Spike freq, Hz');
    xlabel('Time, s');
    linkaxes(ax,'x');
    mean_freq(i)=size(locs,1)/max(x)
end
```

Q13: Look at the figures, do you see any difference between the subplots for different orientations?

Q14: What do the differences mean? What are the mean frequencies for the different stimulations?

Save the figure:

```
saveas(h10,fullfile(folder,'Figures','Fig10_xxx.jpeg')); % change the name of the figure in the control field
```

Now let's plot the sensitivity of the roach's leg to different orientaitons of the leg spines.

```
%Deflection angle / firing rate plot
clear x
h11=figure;
x=deg2rad([0,90,180,270]); % input the deflection angles you chose here
polarplot(x([1:end 1]),mean_freq([1:end 1]),'ko-','LineWidth',3);
hold on;
title('spine angle tuning');
rmax = max(mean_freq);
text(rmax/2, 0, 'spike freq, Hz', 'horiz', 'center', 'vert', 'top',
'rotation', 80);
text(pi/4, rmax*1.2, 'deflection angle, degrees', 'horiz', 'center',
'rotation', -45);
hold off
```

Q15: is there evidence for sensitivity of the spine to a specific stimulus? What is the difference in spike frequency between the most and least sensitive angles?

Save the figure:

```
saveas(h11,fullfile(folder,'Figures','Fig11_xxx.jpeg')); % change the name of
the figure in the control field
```

Step 2. Sensory encoding of spine deflection intensity

You can also use a toothpick to deflect the spine in one direction only, but with different force in each recording. Try this yourself! Look at the code below and change relevant lines. Name files according to the recorded files.

```
clear amp_threshold ax data filename filenames h10 h11 i locs mean_freq...
    n_recordings trace x
close all


% Change the names of files here to import the data from your recordings:
filenames={'F_light.wav','F_middle.wav','F_hard.wav'};
h12=figure;
% Change the number of files here:
n_recordings=3;


for i=1:n_recordings
    filename=fullfile(folder,filenames{i});
    data_orientation=importdata(filename);
    data=data_orientation.data;
    srate=data_orientation.fs;
    clear  data_orientation;
    data=data(1:floor(size(data,1)/srate)*srate,:); %cut to nearest full
second


    trace=-data(:,1);%reverse polarity


    % plot
    x=(1:size(data,1))/srate;
    ax(1)=subplot(2,n_recordings,i);
    plot(x,trace);
    hold on;
    ylabel('Voltage,mV');
    xlabel('Time, s')
```

```
    title(filenames{i});


    %find peaks


    % Change amplitude detection threshold here:
    amp_threshold=0.05;
    locs=[]; %start with an empty locs variable for each iteration through
files

[~,locs]=findpeaks(trace,'MinPeakHeight',amp_threshold,'MinPeakDistance',3); %
change amplitude threshold here
    plot(x(locs),trace(locs),'ro');
    hold off
    ax(2)=subplot(2,n_recordings,i+n_recordings);
    histogram(x(locs),size(trace,1)/(1*srate));
    ylabel('Spike freq, Hz');
    xlabel('Time, s');
    linkaxes(ax,'x');


    mean_freq(i)=size(locs,1)/max(x);
end
mean_freq(i)=size(locs,1)/max(x);
saveas(h12,fullfile(folder,'Figures','Fig12_xxx.jpeg')); % change the name of
the figure in the control field


%Deflection intensity vs firing rate plot
clear x


h13=figure;


% input different intensities you used:
x=[1, 2, 3];
plot(x,mean_freq,'ro-');
title('spine intensity tuning');
ylabel('Spike frequency, Hz');
```

```
xlabel('Stim intensity');



saveas(h13,fullfile(folder,'Figures','Fig13_xxx.jpeg')); % change the name of
the figure in the control field
```

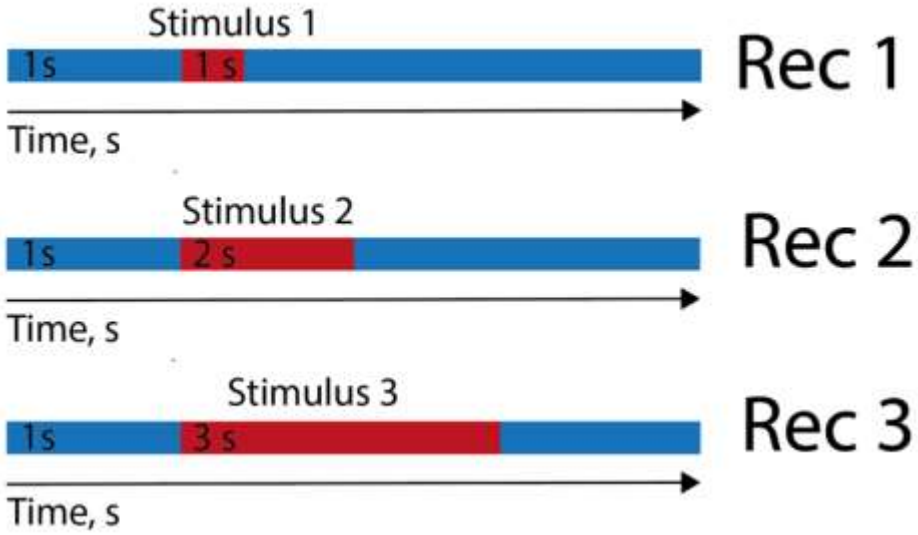
Q16: Is the intensity of sensory stimulation coded by spike frequency?

Q17: What is a receptive field? How can complex receptive fields of CNS neurons arise from the simple receptive fields of sensory receptor neurons?

Part 4: Sensory stimulation – response habituation

Here we will study how the duration of constant stimulus is encoded.

- Choose one tactile spine and stimulate it with similar short deflections of variable time durations. For example, start your recording, then stimulate for 1s, record without stimulation, finish the recording. Start another recording, record for 3s, record without stimulation, finish the recording. Make several recordings of ~15 s with different durations of stimulation in each file.



- Name the files according to the duration of deflection, e.g., short_.wav, medium.wav, long__.wav.

```
clear amp_threshold ax data filename filenames h12 h13 i locs mean_freq...
    n_recordings rmax trace x
close all
```

Input the filenames you recorded here:

```
filenames={"short_.wav","medium_.wav","long_.wav"};
```

Make a figure and plot the results of your frequency analysis:

```
h14=figure;

n_recordings=3;%input the number of recordings here



for i=1:n_recordings
    filename=fullfile(folder,filenames{i});

    data_habituation=importdata(filename);

    data=data_habituation.data;

    srate=data_habituation.fs;

    clear data_habituation;

    data=data(1:floor(size(data,1)/srate)*srate,:); %cut to nearest full
second



    trace=-data(:,1);%reverse polarity

    % plot

    x=(1:size(data,1))/srate;

    ax(1)=subplot(2,n_recordings,i);

    plot(x,trace);

    hold on;

    ylabel('Voltage,mV');

    xlabel('Time, s')


    title(filenames{i});


    %find peaks

    amp_threshold=0.05; %change amplitude threshold here!

    locs=[]; %start with an empty locs variable for each iteration through
files


[~,locs]=findpeaks(trace,'MinPeakHeight',amp_threshold,'MinPeakDistance',3); %
change amplitude threshold here

    plot(x(locs),trace(locs),'ro');

    ax(2)=subplot(2,n_recordings,i+n_recordings);

    histogram(x(locs),size(trace,1)/(1*srate));
```

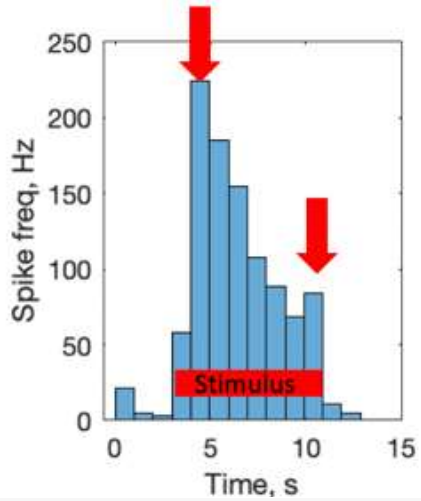
```
    ylabel('Spike freq, Hz');
    xlabel('Time, s');
    linkaxes(ax,'x');
    rate(i).h=histcounts(x(locs),[1:1:size(trace,1)/(1*srate)]);
end


saveas(h14,fullfile(folder,'Figures','Fig14_xxx.jpeg')); % change the name of
the figure in the control field
```

Q18: Study the plots. Do you see the evidence of habituation (diminishing response while stimulus is still present)? What is this evidence? Which stimulus duration gives the highest habituation?

From your plots, how many seconds **after** the peak did the stimulus last? Input the duration in seconds for each recording below:

```
clear x trace
x=[1,1,6];
```

Now we will calculate the habituation index, how much habituation there is in the reponse (Habituation is the decrease in the response to a repeated or prolonged presentation of that stimulus). This is the ratio of spike number at the peak of stimulation to the number of spikes at the last second of the stimulation. The higher this index, the more habituation there is. response to a ecreases after repeated or prolonged presentations of that stimulus




```
h15=figure;
for i=1:size(rate,2)
    trace=[];
    trace=rate(i).h;
```

```
    habi(i)=max(trace)/trace(find(trace==max(trace))+floor(x(i)));
%habituation index

end

plot(x,habi,'ko-','LineWidth',3);hold on;

title('spine habituation');

ylabel('habituation index');

xlabel('duration of stimulus, s');

saveas(h15,fullfile(folder,'Figures','Fig15_xxx.jpeg'));
```

Note: if you get an error message similar to one shown below, it means that the duration of the stimulus after the peak is too long, change it to a lower value.

```
Index exceeds the number of array elements. Index must not exceed 14.
```

Q19: What is habituation index during the longest stimulation?

Q20: What is the physiological importance of response habituation?

Congratulations! You came to the end of the practical!
Do not forget to save this file - as .mlx to rerun the analysis if needed and as .docx or .pdf file to save answers to all the questions and analysis output that will be saved with the file. Also **copy all your analysis, figures and recordings** to your own memory device! You will need the figures and analysis for the report.

3.3 Report template

General guidelines:
Your report should follow the IMRaD style in this order: Introduction (including research questions), Methods, Results (including figures) and Discussion. It should be brief and focused - do not use jargon when you do not need it and edit out useless words from your sentences. E.g., instead of "Moreover, we fabricated patch pipettes from borosilicate glass capillary by means of a puller.", it is preferable to write: "We pulled glass patch pipettes." The methods in particular should be clear and concise so that anyone can replicate the experiment. For the report you can select 2-3 main research questions that you addressed during the practical (do not write about everything you did). On the last pages include your answers to the questions from the practical guide.
The report (including all text and figures, excluding your answers to the questions from the practical guide) should not exceed 3 pages.

Title:

Student names and student numbers:

Introduction:

Materials and methods:

Results (should include the research questions, the figures showing results, your findings):

Discussion (what are your conclusions, what do your findings mean, what could you do to improve the method):

References (the list of literature references if used):

Questions and answers (answer the questions from the practical guide, provide data figures from the practical where necessary):

Q1: What ions cause the phases of the action potential?

A:

Q2: What is the average firing frequency in your recording?

A:

Q3: What might cause changes in frequency in your plot?

A:

Q5: What is the number of spikes in the first second? Is it similar to what you calculated previously with mean_freq?

A:

Q6: What is the interval between two samples? What is the half width at half height of the average spike?

A:

Q7: What is the most frequent amplitude of the waveform? Do you have multiple peaks in your distribution? What does it mean?

A:

Q8: Is the half width at half height now different from previous analysis?

A:

Q9: How is joint angle encoded by the sensory spikes you recorded?

A:

Q10: What is rate coding?

A:

Q11: For which behavioral situations might the roach need joint angle information?

A:

Q12: Are some spines more responsive than others? Is there a logic to their positions?

A:

Q13: Look at the figures, do you see any difference between the subplots for different orientations?

A:

Q14: What do the differences mean? What are the mean frequencies for the different stimulations?

A:

Q15: is there evidence for sensitivity of the spine to a specific stimulus? What is the difference in spike frequency between the most and least sensitive angles?

A:

Q16: Is the intensity of sensory stimulation coded by spike frequency (show figure to support your point)?

A:

Q17: What is a receptive field? How can complex receptive fields of CNS neurons arise from the simple receptive fields of sensory receptor neurons?

A:

Q18: Study the plots. Do you see the evidence of habituation (diminishing response while stimulus is still present)? What is this evidence? Which stimulus duration gives the highest habituation?

A:

Q19: What is habituation index during the longest stimulation?

A:

Q20: What is the physiological importance of response habituation?

A:

4. Assessment and feedback

4.1 Practical report assessment rubric

1. Introduction (3 points)

- Overall background information logically required for posing the research questions (1 point)
- A convincing, logical reasoning is presented for why the research questions were posed (1 point)
- 2-3 Research questions are clearly stated (1 point)

2. Methods (3 points)

- Used methods (how the recordings were performed) are succinctly described (1 point), yet comprehensively enough that another researcher can replicate the work (1 point).
- Used materials are clearly described such that another researcher can replicate the work (1 points).

3. Results (4-6½ points)

- For each research question it is clear which experiment was performed (½ point per RQ)
- Figures and legends are present (1 point) and Figures illustrate clearly the results and address the questions (½ point for each RQ).
- Extra analysis (not included in the practical guide) was performed (bonus 1 point)
- Conclusions/main findings are clearly stated for each RQ (½ point for each RQ)

4. Discussion (2 points)

- Discussion of the findings is logical (1 point)
- Findings are discussed in the context of literature and adds insight beyond the results section (1 point)

5. Questions (5 points)

- ¼ point for each answer which is not clearly wrong (5 points)

6. Report overall (3 points)

- Contains introduction, methods, results, discussion (1 point)
- Relevant literature is cited appropriately (1 point)
- The report is well written (quality of the language) and fits in 3 pages (1 point)

Points to grade conversion:
>20 → 10
18-19→ 9
16-17 → 8
14-15 → 7
11-13→ 6
<11 → fail