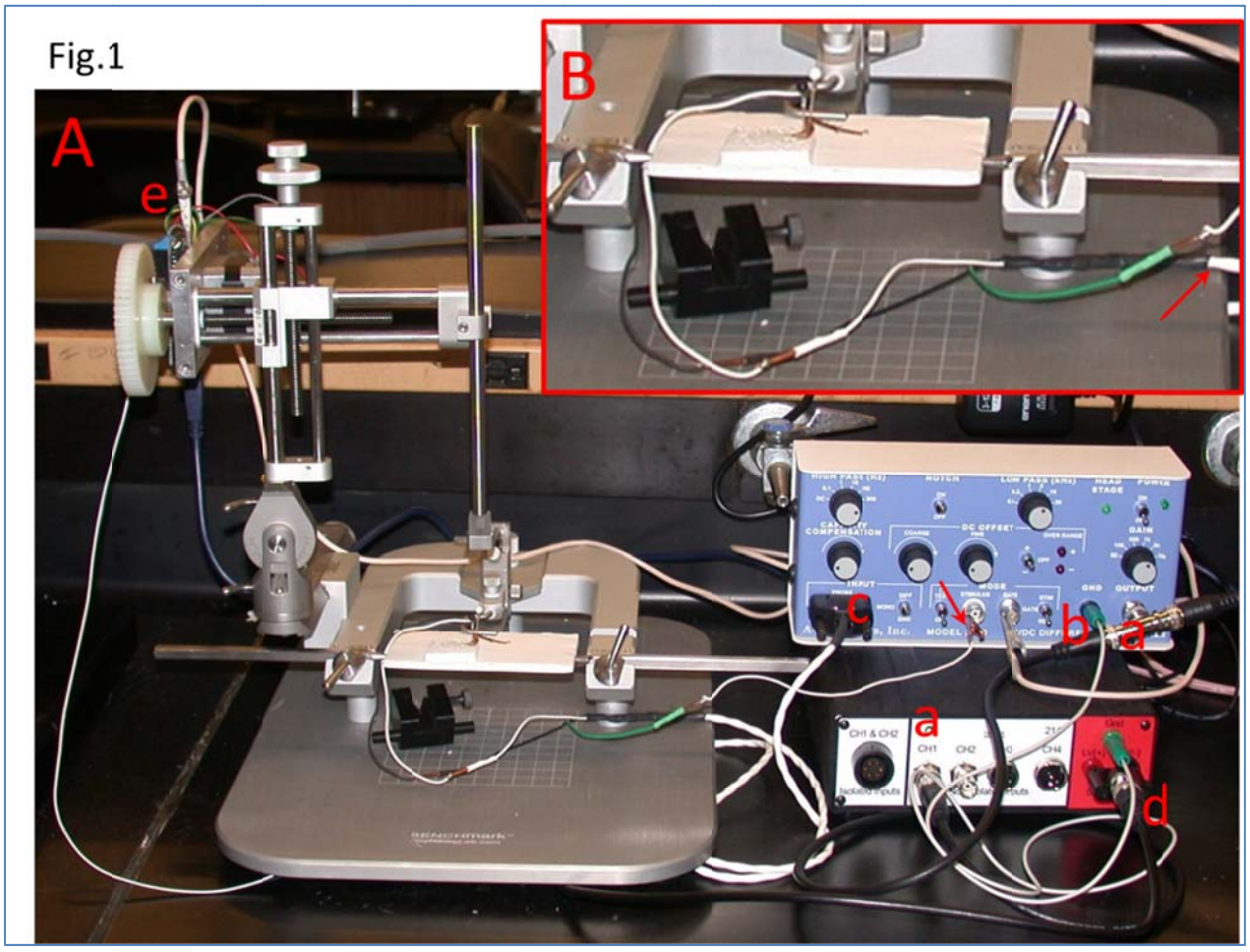
Cockroach lab

This laboratory exercise continues with extracellular recordings but with an emphasis on (i) identification of APs arising from different sensory axons and (ii) how these sensory neurons respond to mechanical stimulation. Here, we will do our recordings from sensory fibers in the cockroach leg and will observe changes in AP firing frequency in response to stimulation of sensory spines. Some of the experimental projects are adaptations inspired by a paper published by Ramos and colleagues (Ramos et al., 2007).

Instrumentation:

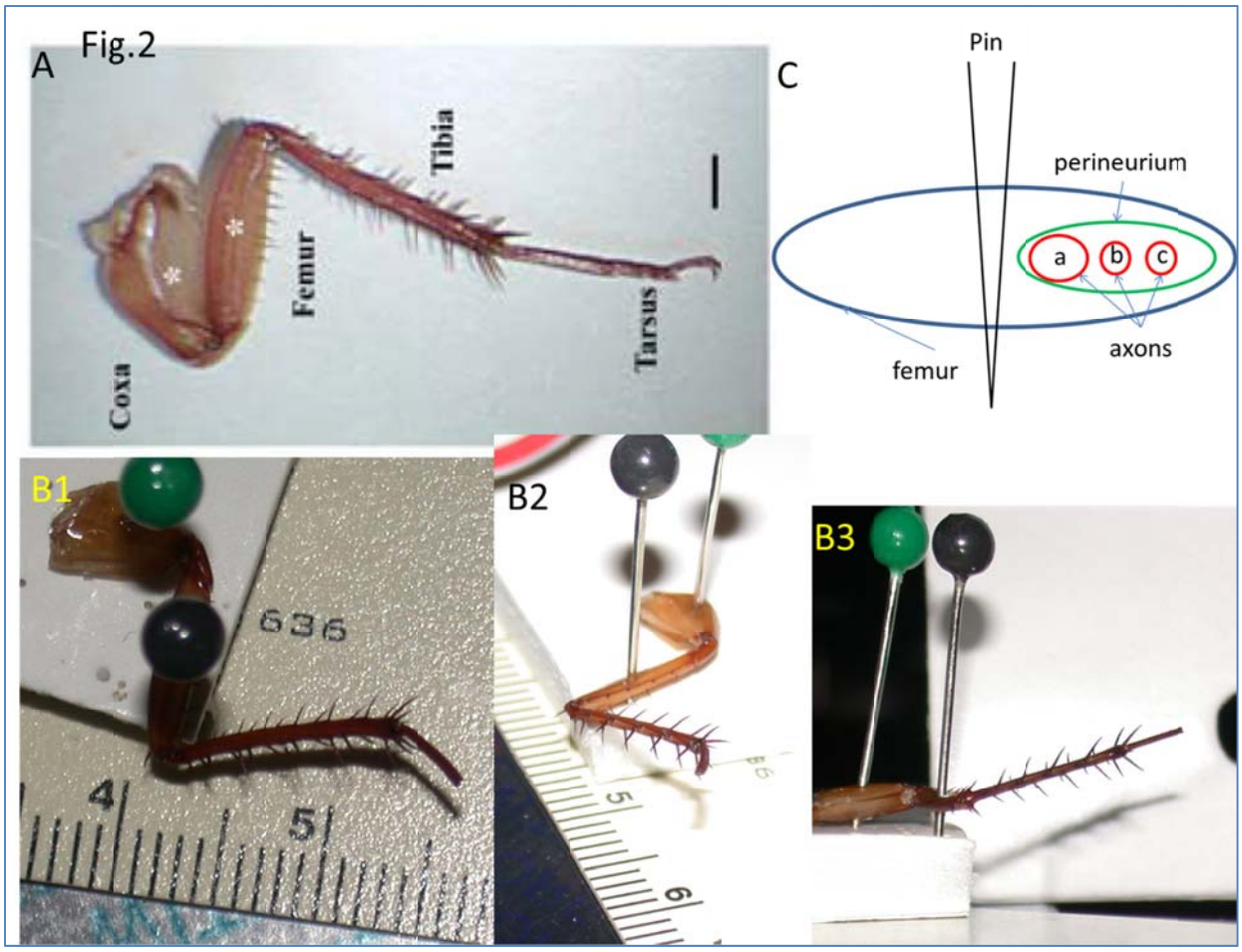


The equipment we will use is essentially the same as that of the earthworm lab. The cable connections between M3000 and IX214 are shown in Fig.1A. The output of M3000 feeds into Ch1 of IX214 through the black BNC (***Bayonet Neill-Concelman***) cable (Fig.1Aa). Both pieces of equipment share a common circuit ground by using the thin white wire with green plugs to connect GND posts together (Fig.1Ab). The pins for recording from the roach leg feed into the input of M3000, which is

the white cable ends in the black plug at the lower left of M3000 (Fig.1Ac). Fig.1B shows that the end of the white cable (arrow) from M3000 splits into three wires. The black and white wires are connected to the recording pins while the green one is connected to the amplifier ground by way of a small alligator clip (Fig.1A arrow). Finally, there is a black cable coming out of the stimulator section of IX214 (Fig.1Ad). This cable, which is tugged under the stereotaxic frame and emerges aa a white cable, is connected to the Arduino board (Fig.1Ae) mounted on the motor adaptor that controls the X-axis movement of the stereotaxic device. IX214 acts as an intermediate between iWORX in the Mac and Arduino board.

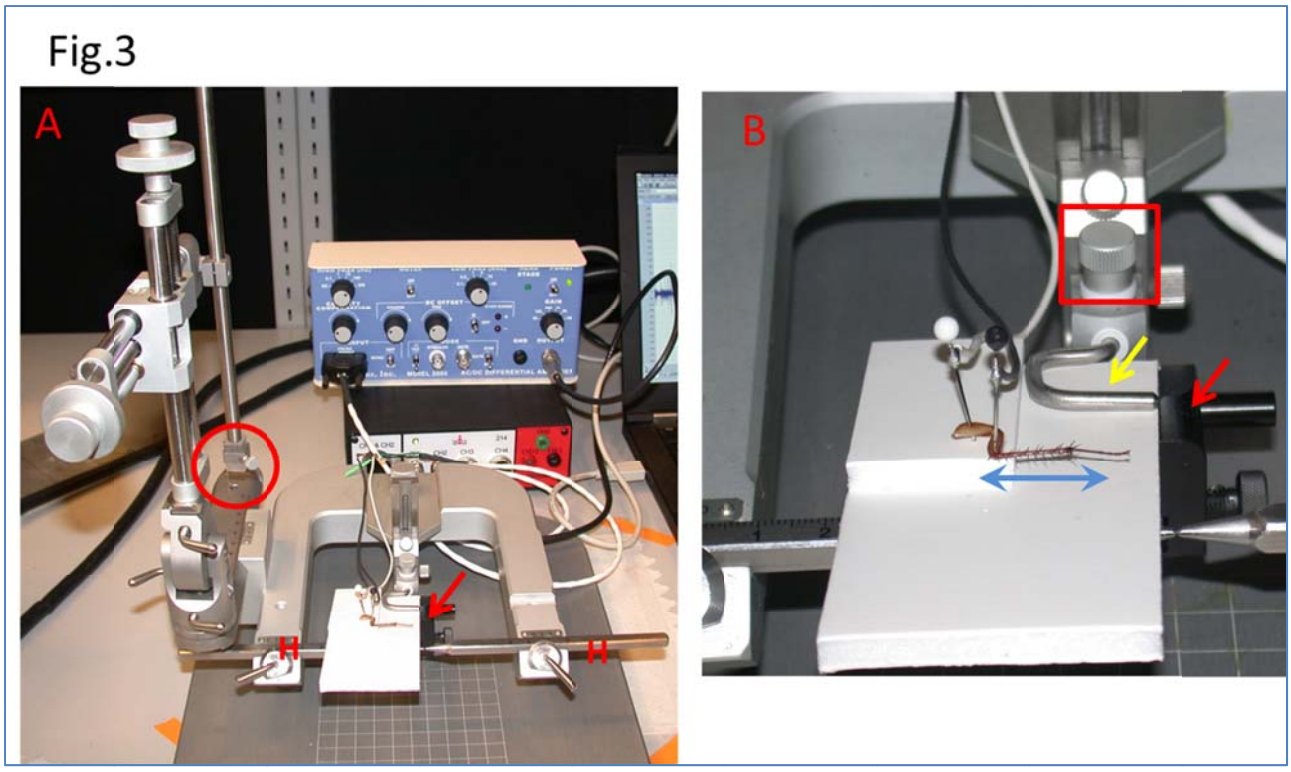
Software:

We use LabScribe. There will be only one panel for data recording for now. The recording period is much longer than before. In the earthworm lab, we recorded AP conduction after a stimulation pulse, where AP duration was 1ms or so, and conduction time was 3-5ms. The entire trace covered 30ms. Here, we will ask you to stimulate sensory spines manually with a manipulator. This hand controlled movement takes a second or so to complete. We will therefore collect data for a longer period, 5 seconds.
You should use the same note taking strategy as before, namely noting down the experimental conditions for each trace.
Biological preparation:
The preparation couldn't be simpler. The Roaches will have been immobilized by cooling in ice water and then killed by decapitation. All you need to do is to use fine scissors to remove the leg. Use a pair of iris scissors to cut one leg off and bring it to your bench. You should try to cut the leg so as to include coxa, femur, tibia and tarsus (Fig.2A). (It will also work if the coxa falls off.) You will find two pins connected to a white cable coming out of M3000. **The pins are very sharp, so be careful. Don’t pin the animal yet, finish reading this paragraph first.** You should pin the leg as shown in Fig.2B, with either one pin in the coxa and one in the femur (Fig.2B1,B2) or both pins in the femur. The white foam piece for you to pin the leg onto is ~3/16" thick. You should pin the leg near the edge/corner so that sensory spines on the tibia and tarsus are not in contact with anything (Fig.2B2,B3). The foam is quite tough, so you need to hold the pin firmly to push it through. Practice pushing the pin through the foam a few times before you actually pin the leg. Otherwise, the pin may slip as you push, and tear the leg. A good strategy is to pin the femur first then rotate the leg such that the tibia/tarsus are in the position described above (Fig.2 B1-B3) then put the 2nd pin in the coxa or femur. The two pins should not touch each other.



The next important task is to place the recording mat with wires and all onto the stereotaxic system. Before you start, pay attention to the vertical bar positioned to the back of the system (Fig.3A red circle). **Be careful, there is a very sharp pin at the end of the vertical, metal rod.** We have pushed the two horizontal, head clamping, pins of the stereotaxic system inward (Fig.3A H). The two pins together with the black head adapter (Fig.3A red arrow) should give you a platform on which to secure the recording mat, by wedging it into the space between the movable hook (Fig.3B yellow arrow) and the black base of the head adapter (red arrow). Move the recording mat carefully and make sure that the wires and the white cable move with it. The cable is a bit stiff, and you don't what it to pull or strain the pins. You can tighten the hook with the thumb screw (Fig.3B red square). The recording mat is firm but not hard, and you don't need to tighten the hook too much to keep it in place. One more thing before moving on: try to adjust/rotate your recording mat such that the tibia is oriented left to right (Fig.3B blue double headed arrow).

Fig.3

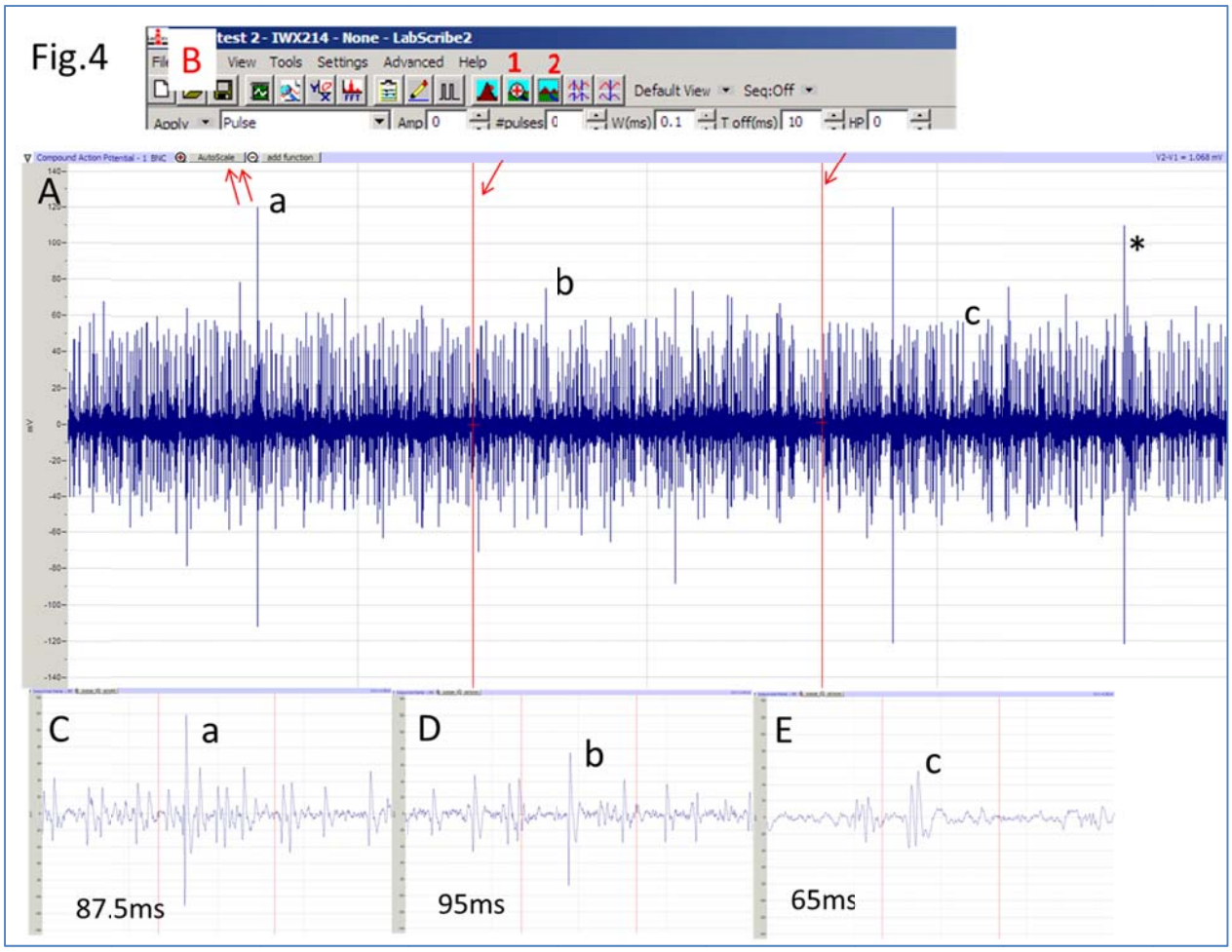


Once you have everything in place, you are ready to go. Turn on the M3000 and IX214. (The power switch of IX214 is in the back.) Launch Roach_setting.iwxset, click record, and you should see something similar to that in Fig.4A. To make sure that the display on the Y-axis is appropriate, click on autoscale, Fig.4A double arrows. How do you read a line like that? Every one of the vertical ticks is an AP. Sensory fibers of cockroaches have very high background activity. (The resting V_m in these fibers is likely to be very depolarized such that AP will fire continuously even without obvious mechanical stimulation.) The whole trace is 5 seconds long. We will need to expand it to see individual APs in detail. The AP labeled "a" (AP_a) is big, and we will look at this one first.

To expand the time scale and have a close look at the spikes you are interested in, move the vertical cursors (Fig.4 red arrows) to the left and right of the spike of interest, then click on the magnifying glass located on the top strip of buttons (Fig.4B 1). (Note that this button expands time, horizontal scale whereas the other magnifying glass, in the blue strip directly above the trace, expands the vertical scale.) Click on the top magnifying glass and AP_a should look like that in Fig.4C. In this example, the AP is biphasic, i.e. the trace goes down then up, not unlike the AP you recorded in the earthworm lab. Your AP may go up first before down if you put the white pin close to the tibia.

What about the medium AP labeled b (AP_b)? For this one, we first need to zoom out the expanded time scale to find AP_b, by clicking on the

button next to the magnifying glass (Fig.4B 2). Move the cursors to bracket AP_b and magnify it. This medium spike should look like that in Fig.4D. Finally, a small spike labeled c (AP_c) is shown in Fig.4E. What do these spikes, AP_a to AP_c, mean? Your pins act as extracellular electrodes. Fig.2C shows a cross section of the femur with your recording pin. Axons _a, _b and _c are encased in perineurium. Axon_a is large, close to the pin and expected to give rise to large signals. Axons _b and _c are similar in size but axon_b is closer to the pin than axon_c and is expected to generate APs of intermediate amplitude. Axon_c, being the farthest and not particularly large, should produce smallest AP. Thus, AP amplitudes are determined by axon diameters and their distances relative to the recording pin. Given the way recording pins penetrate the leg, the distance between your pins and axons is fairly stable. Therefore, every time we observe an AP with specific amplitude, we can confidently infer that the same axon had just fired again. Since each axon is connected to only one sensory spine of the roach leg, it is not surprising that, for example, AP_a doesn't change its firing until we touch a specific spine. At the same time, each sensory spine is innervated by multiple axons. It is very rare that you will detect no response when poking at a spine because one or more of the axons innervating that spine are likely to be close to the recording pin and give rise to detectable APs. In addition to specific anatomical connection between axons and spines, each axon is also likely to be unique in its physiological properties. For example, the axon that gave rise to AP_a have fired three times in the 5 seconds during the trace shown in Fig.4A, i.e. this axon has a firing frequency of 3/5 Hz. This firing frequency will likely stay the same for the entire lab period. AP_b probably fired 7-10 times in 5 seconds. You should collect 3-5 traces. Have a good look at these traces and see if the firing frequency of a given axon remains constant in the different traces. From now on, when we say AP_a, AP_b and AP_c, we implicitly suggest that they are from axon_a, _b and _c.



Major digression, optional reading:

In addition to record and display traces on computer, we also feed the recordings of spiking roach axons into audio, so that you could listen to APs.

Playing the audio of physiological recordings is common practice among electrophysiologists, because it allows us to continue monitoring APs, voltage gated currents, single channel currents or synaptic current/potentials as we turn away from the computer monitor or oscilloscope during an experiment. In fact, subtle changes in AP firing pattern are often not easily recognizable by looking at traces on oscilloscope screen, but can be instantly noticed during audio playback. There is no magic to this difference in sensitivity. After all, our visual system is optimized to detect features in two-dimensional images and is relatively slow at analyzing fast time-sequence signals. On the other hand, our auditory system is geared to analyze time sequences in real time, and is very good at picking out small changes in time sequences such as speech, tone and pitch. In general, subtle differences in firing pattern recognized only by the ears would require rigorous statistical analysis of spike trains to show the difference objectively. Experienced electrophysiologists have been known to be able to recognize the type of brain neuron—whether thalamic, cortical, cerebellar or hippocampal—simply by listening to recordings of intracellular AP firing.

Here we will be listening to the spontaneous activity of sensory axons in the cockroach. These responses, while exciting, are passive, i.e. the firing pattern only changes when we push the

sensory spines. For neuroscientists who do electrophysiological recordings from behaving animals, especially in monkeys, this kind of auditory monitoring takes on a different level of significance. Imagine a microelectrode implanted in a certain brain area of a monkey. As neuronal APs picked up by the electrode started to change their firing pattern while the animal was perceiving a sensory cue, planning or executing a motor output—you would be listening to the "thoughts" of an animal similar to us. On an abstract or metaphorical level, this kind of auditory monitoring brings us much closer, in an instinctive way, to the operation of our brain. Different neurophysiologists have been moved by this experience in different ways. Some claim that upon hearing this type of recording they feel a chill down their spine. Others have felt the concept of God hits them for the first time in their life. There are, of course, neuroscientists who think the sentiments described above are completely silly. Use your imagination and decide for yourselves.

Project 1: Identification of APs generated by different axons.

You should display a representative trace and pick out at least 3 AP/axons that are unambiguously different. Here, we will still follow the convention that notes window of slide 1 will be used for notes taking. Paste the traces in slide 2.

Here is how you should present your data: Right click on the trace panel. The second option on the right click menu is "Copy Graph". Launch PowerPoint and paste the copied graph on a slide. You can then adjust the size of the graph to fit the power point slide. A format similar to that in Fig.5 is good for this part of the report. In addition to the raw (5 second) trace where you should label your 3 identifiable APs, you should also display each AP on an expanded time scale, using the cursor/magnifying-glass trick followed by the right-click/copy-graph sequence. (**There is an important oversight in LabScribe, namely that the copied graph you paste into Powerpoint does not automatically include the time scale. The time scale is important in neurophysiology. One way to get around this is to move the cursors to the extreme left and right margins of the expanded trace, and T2-T1 will give you the length of the entire trace. Label the length of your expanded traces in a manner similar to that shown in Fig.4C,D and E.)**

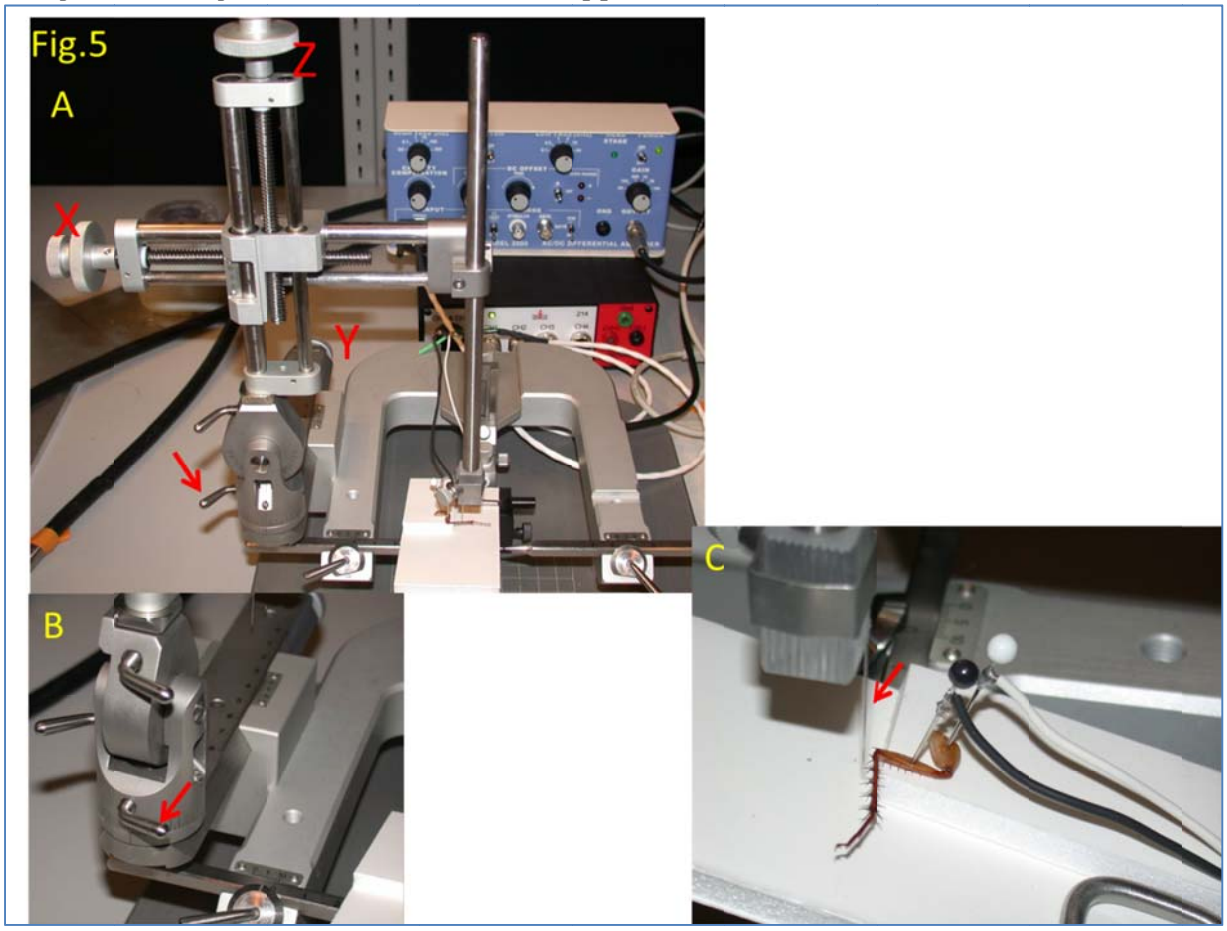
Write a figure legend that includes: (1) the trace IDs from which the APs were obtained, (2) the firing frequencies of the 3 axons. (To ensure that you have enough time for the projects below, you should avoid picking very small axons that seem to fire at very high frequency. (We don't want you to spend time counting hundreds of action potentials.)If you do decide to pick a small AP that fires a lot, in order not to spend too much time estimating firing frequency of the axon, you should count the number of AP within a 100-200ms time window and calculate the frequency (AP/sec) from the brief time window. Also, you should estimate the firing frequency now because you don’t have LabScribe to count APs on your personal PCs.)

What if you have only one or two clear, large APs? This may happen in some legs. The first thing you should do is to record a few more traces to make sure that you are not missing anything. (Also, click autoscale each time, to make sure that you are not missing APs by displaying the trace on the wrong scale.) You may want to discuss this

with your LI or LA, and then show only one or two clear APs in your report. As long as you show your raw (5 second) trace, it should be obvious whether or not you have three large/clear APs. Don't panic if you don't see large APs; they may just be dormant, i.e. not have any background firing activity, and may show up when you poke the right sensory spines.
To get a feel for the sensitivity of the mechano-recptors on the roach legs, you can click record then blow gently on the leg. The recording should register your blow, in terms of one or two puffs etc. Play with this paradigm a few times, and also try a light and a hard blow during the same recording trace to see if the trace registers the difference. Everyone should have a go. No need to report this part, but I hope that you are impressed by the sensitivity and robustness of the sensory response.

Project 2: Map the "receptor field" of a sensory spine.
(Receptor field is also call receptive field.)
First we need to set up the manipulator and microscope to make it easier to: (1) finely control the stimulation of sensory spine by uaing the manipulators of the stereotaxic device and (2) to visualize the mechanical stimulation we deliver to the sensory spines. You should first swing the tower of the stereotaxic system into place such that the vertical rod with a sharp pin is right above the leg (Fig.5A). You accomplish this by unlocking and relocking the L-shaped key at the base of the tower (red arrows in Fig.5A and B). (There are three L-shaped keys at the base of the tower; use the lowest one.) Fig.5C shows roughly where the small insect pin (red arrow) should be with respect to the leg. Next, you need to swing the dissection scope into a comfortable position. The whole set up should look roughly like that shown in Fig.6A & B. **The most important thing to keep in mind is that manipulation of the insect pin, with the X-Y-Z knobs (Fig.5A), should not result in any contact between the microscope and the stereotaxic system. It is easy to push the microscope too far such that it crashes into the tower of the stereotaxic system and causes damage! At least at the beginning, while one person is working the knobs and watching the pin under microscope, another member of your team should watch and make sure that there is a safe distance, >5 cm, between the microscope and the stereotaxic tower.**
There is one more fine adjustment to make. You may have to rotate the tower slightly, so that the left-right movement of the insect pin, as you turn the X-knob, is aligned with the long axis of the tibia. (I asked you to align the leg earlier, but the femur-tibia joint may have relaxed over time and need realignment. Reposition the recording mat for large-scale adjustment, and rotate the tower for fine adjustment.) This is important because our projects require consistent stimulation of sensory spines. Consistent movement is best delivered by turning

only the X-knob, Fig.5A. (Note that the X-axis knob in this lab is the large white gear mated to the stepper motor.)



Now you can look down the microscope and maneuver the insect pin to push a single spine first in the direction of 0°, then 180° (Fig.7). Most spines will give you a detectable response. Check and play around with spines in any of the six sections, namely inner/outer prox/mid/dist. Since we want to know if our test spine has any directional preference, we need to first ensure that we can provide similar stimulations in both 0° and 180° directions. Doing this by hand is not trivial, but we must do our best. There are three important factors we need to consider for this project. **First**, we mustn't move the femoral-tibia (F-T) joint as we push/pull a sensory spine. Bending the joint will trigger mechano-sensitive receptors there and result in impressive firing. To know how the F-T joint response looks/sounds like, You should try bending the joint a few times, by moving the pin toward or away from you with the Y-knob in the back, Fig.5A. If we want to stimulate a spine selectively, we need to displace the spine carefully—i.e. not pushing too far such that you bend the F-T joint but the small push you deliver should trigger clear response. This is easier said than done. You will need to search around until you find the "best spine": defined as sensory spine that will give clear response from relatively small spine displacement that does not involve F-T joint bending. Generally, I find spines on the outer side better. However, everyone pins the leg down differently. Check the spines on inner and outer sides to find your "best spine". To

encourage participation by all, you should compare two candidate spines for the "best spine" and the selection should be confirmed by at least one other member of your team. The **second** important consideration is that if we want to compare whether a spine prefers 0° or 180° stimulation, we must try to displace the spine by the same distance in both directions. There is no easy way of doing this because the knobs on the stereotaxic system do not have a digital readout, and the vernier reading on the stereotaxic system is not fine enough. The best practice is to have the same person look under microscope and turn the knob at the same time, so that he/she knows the distance the insect pin has moved using both visual cues and the degree of X-knob rotation. Neither cue is quantitative, but if you pay attention and practice a little, you can achieve consistent stimulation. Furthermore, this is an occasion for an independent second opinion. Have a second member of your team to do the same experiment, on the same spine, and confirm that the preferred orientation determined by the first person is correct. If it is not confirmed, you should discuss it and consider getting 3^{rd} and even 4^{th} opinions. Finally, we need to keep a **third** factor constant, namely the rhythm of our stimulation. Here is our standard pattern: Start by placing the pin as close as possible to the spine but not touching it. Then click record. Wait one second. Then push the pin and stimulate for one second. Then pull back the pin. It is sometimes helpful to have a separate member of your team call out the timing. If you think of the timing in terms of a musical beat, you should be able to get a consistent rhythm going easily.

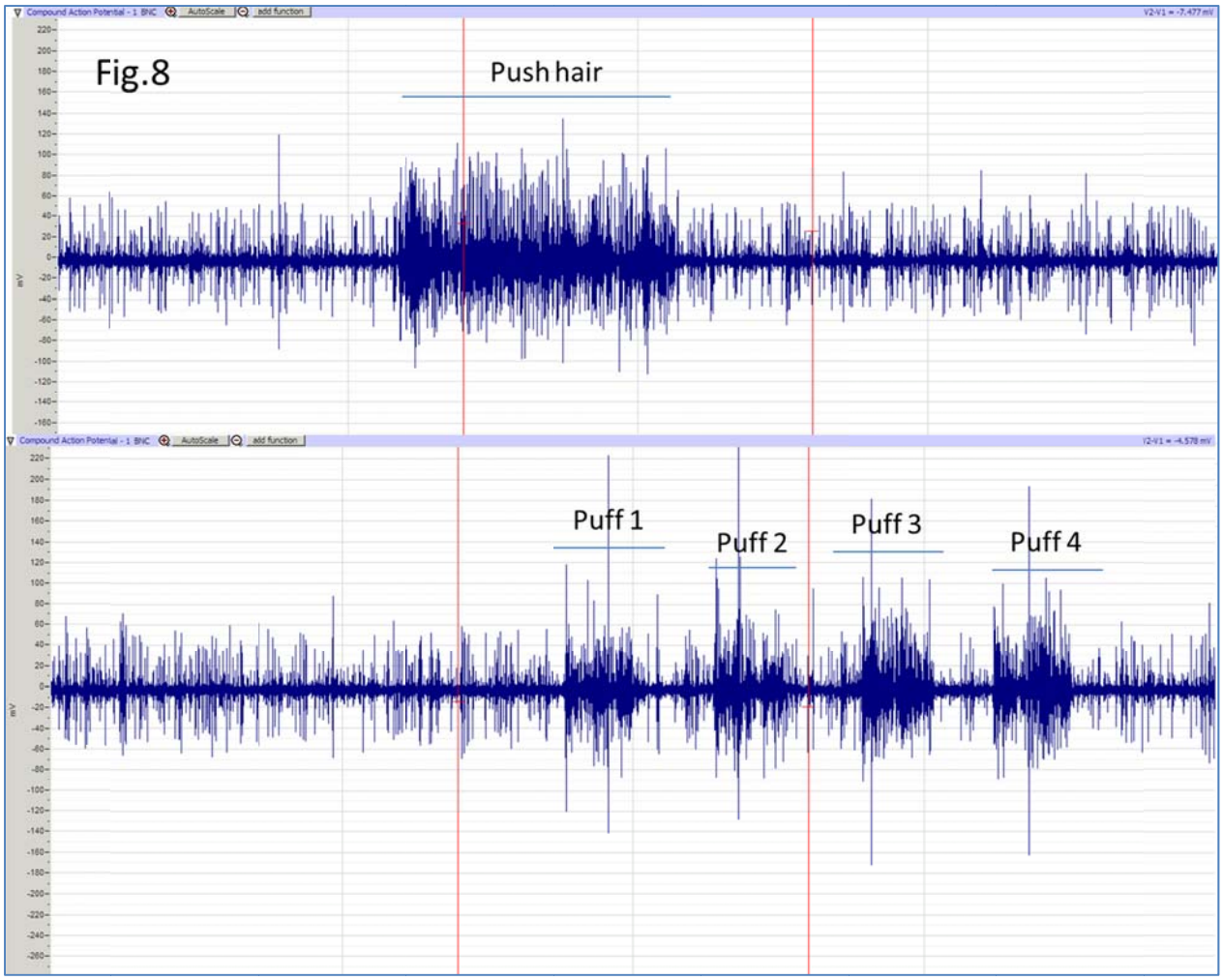
This project seems to have a lot of details that need attending to. The truth is that when we do science, we do have to control parameters important for the question we are trying to answer.

In slide 3, you should first show two traces from the two "candidate" spines you have screened and compared, by displaying both traces on the same slide, as top and bottom panels. Also, mark on the trace the time when the stimulation is delivered—see Fig.8 for an example. In the figure legend, describe your traces.

Write the figure legend then comment on the reasons you chose the spine giving rise to the recording shown in the top (or bottom) panel for further study. Trace IDs should also be included in the legend.

In a separate PowerPoint slide (4), display the responses activated by 0° and 180° stimulation of the spine you have chosen for detailed analysis. Show both traces on the same slide, as top and bottom panels, so that it will be easy to see whether they are different or not. Label the traces in terms of direction and time of stimulation etc. Write figure legend that describes the traces as instructed before and, in a separate paragraph, draw a conclusion, by referring to the traces, as to which direction the spine prefers, i.e. is more sensitive.

In sensory systems, the concept of receptor field is important. The receptor field defines the space within which a sensory neuron will respond. The definition of space is obvious for visual or somatosensory receptors. For example, a retina ganglion cell will only respond to a bright spot located at one particular point on a screen in front of an experimental animal. The location of the bright spot is the receptive field of that ganglion cell. In the case of mechanosensitive spines, the receptor field is defined by its directional sensitivity. In other words, the spines are tuned to be more sensitive to stimuli, e.g. air flow, from some directions compared to others. So, we have just finished mapping the receptor field of a sensory spine in a roach leg. However, be aware that there are two additional dimensions that we did not test. The pin is too sharp to provide a consistent downward push and there is no hook to move the spine upward.



Project 3: Rapidly vs slowly adapting properties.

A common theme in sensory systems is that some sensory neurons will fire action potentials continuously as long as a stimulus is present, while other sensory neurons will only respond to the beginning and termination of a stimulus. These responses are classified respectively as slowly and rapidly adapting. (Fig.9.4 of your textbook also address this topic.) We now are going to determine whether sensory spines at the tibia-tarsal (T-T) joint (Fig.7) are of the rapidly or slowly adapting type. These spines look thicker than those on the tibia, so you should have no difficulty identifying them.

First, follow the procedure you used in Project 2. Select a sensory spine from which it is easy to get clear response and that is easy to stimulate selectively, namely easy to access without bumping into other spines nearby and without causing the tibia to move. We will focus on only one direction in this project. (Don't get too obsessed and spend a disproportionate amount of time in search of the perfect spine. Keep an eye on the clock.) In the process of choosing your

target spine, you should also establish a consistent stimulation as before, i.e. use visual cues under the microscope along with manual cues as you turn the knob.
You should now practice a few times using the three stimulation paradigms described below. In the first case, you use the same strategy as that in Project 2: Press "Record", wait one second, push the spine for one second and pull back the pin. This will be your brief/rapid stimulation. In the second case, press "record", wait one second, push the spine **but don't pull back the insect pin.** Leave the insect pin in place, where it is still displacing the spine. This will be your continuous stimulation. **Don't release this stimulation.** For the third paradigm, click record again, wait for 2 seconds, then release the stimulation. In this case, you will be releasing an existing, continuous stimulation. You may want to repeat these three types of stimulation a few times, preferably allowing a second person to try if time allows, to convince yourselves that you are getting consistent results.
The question is: does the increase in firing persist unabated during continuous stimulation?
You should display one representative recording from each type of stimulation on your slide. Label your traces and describe them in your Powerpoint notes. Write figure legend. After the figure legend, in a separate paragraph, you should explain why you think the spine in question is rapidly or slowly adapting, by pointing to various labeled features of the recordings—such as whether firing drops off or not, for example. The best way to do this is to put labels on your traces—arrows, a, b, c, d marks etc—and refer to these labels as you discuss changes, or the lack of, firing frequency.

Project 4: Investigate the relationship between sensory axon firing and bending of the femur-tibia joint

An important question common to the study of sensory systems is the quantitative relationship between intensity of sensory stimuli and responses of sensory neurons. The understanding of this relationship is essential for us to gain insights to the range and sensitivity of our sensory organs. As the last project of this lab, we are going to investigate the relationship between sensory axon firing activities and the bending of the femur-tibia (FT) joint. There are sensory fibers innervating the FT joint to inform the roach brain about the state of their legs. These sensory fibers will change their firing frequency as the joint is bent or straightened. The goal in this project is to determine the relationship between the angle of the joint and the rate of neuronal firing. The experiment design is simple, we will use the stepper motor to displace the tibia and bend the FT joint while recording axonal firing. Although one could do the

same experiment with manually turning the knob, it is not ideal for this type of studies. The main reason is that manually turning the knob could be variable between trials and between individual experimenters. These variabilities make it inappropriate to carry out signal averaging—because the pin movement in each trial may not be the exact replica of the previous one—or pooling data between groups—because your knob turning may be different from that of the person in the next bench. Using computer controlled devices avoid these reproducibility issues and makes it possible for scientists from different laboratories to compare or pool data.

Set Up

The stepper motor activity is controlled by the voltage output from the Stimulator section of IX214. The Arduino board reads the voltage and controls the degree of motor turning which, by way of gears, moves the pin. This sounds simple but we have a few problems to deal with. First, the turning of the stepper motor causes vibration of the stereotaxic frame. The vibration is subtle but you can feel it by placing your hand in the frame, so could the roach leg. Thus, the turning of the motor itself could cause some APs, which has nothing to do with the joint bending. There are many professional ways to deal with this issue but we just want our data and will take the quick and dirty approach.

You need to remove the foam board with the leg and pins in place from the stereotaxic frame and attach the whole assembly to a piece of long foam board on your bench. Here are the steps:

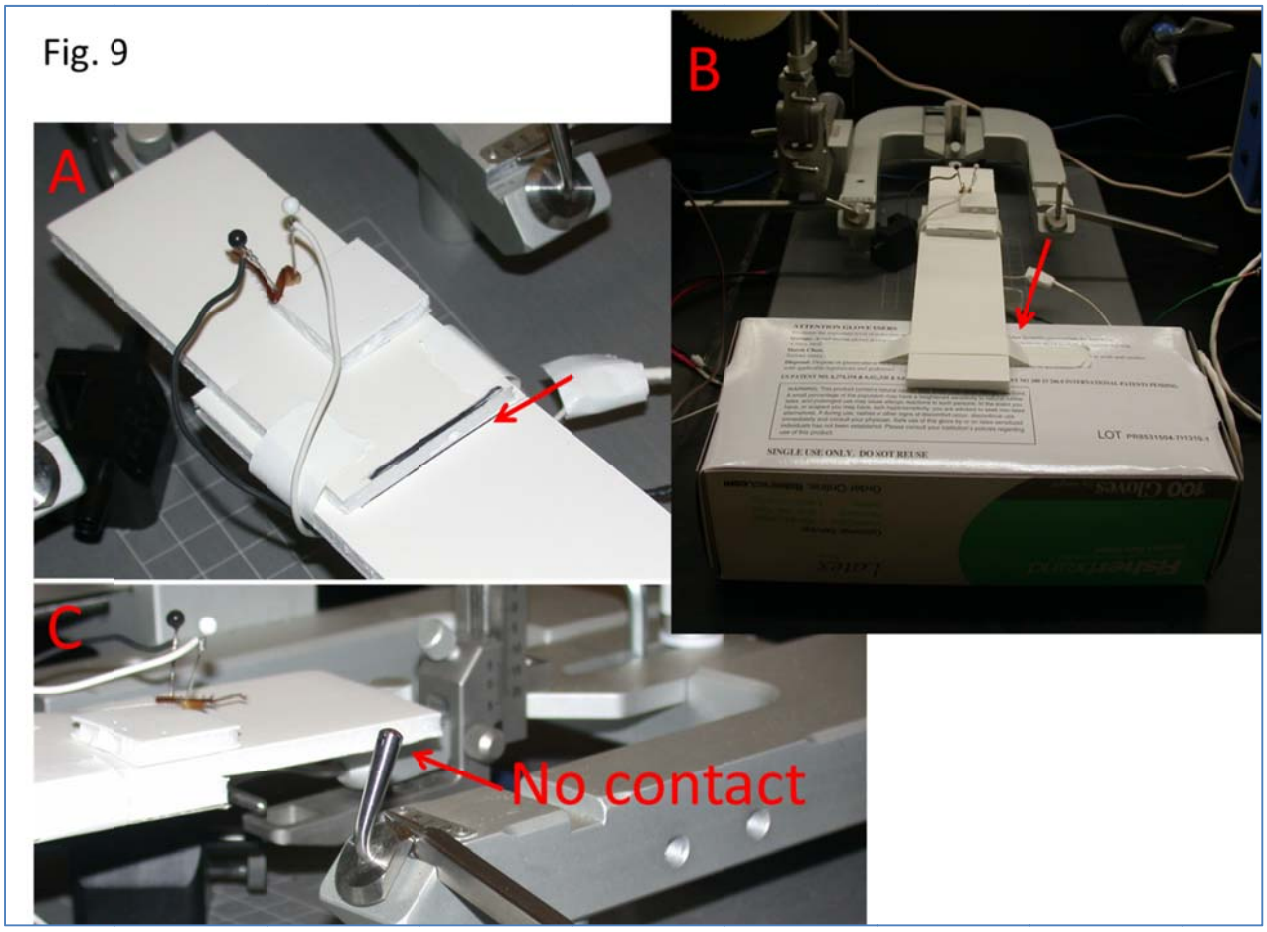
1. Turn the M3000 amplifier off.

2. Remove the foam board with the leg and pins in place.

3. Tape the foam board onto the long board with the tibia pointing to the end of the long board (Fig.9A). (You should be able to find a black line on one side of the small foam board, the one with the leg on. Align this line with the black line on the long foam board (Fig.9A arrow).) The other end of the long board will be taped to a glove box (Fig.9B arrow). You can now position the glove box such that the tibia is pointing to the back of the stereotaxic frame (Fig.9B). This way, the movement of the pin along X-axis will be able to bend the joint effectively.

Note that the glove box should not make contact with the stereotaxic frame. Also, the end of the foam board should not touch any component of the stereotaxic frame (Fig.9C). (You may need to put a pen under the glove box to tilt it so that the foam board point upward and is lifted a little.) Otherwise, the vibration of the motor will be transmitted to the leg.

4. Turn the M3000 back on.



Experiment

Save and close Labscribe with data from your previous projects.

To open iWORX set for the stepper motor controlled movement:

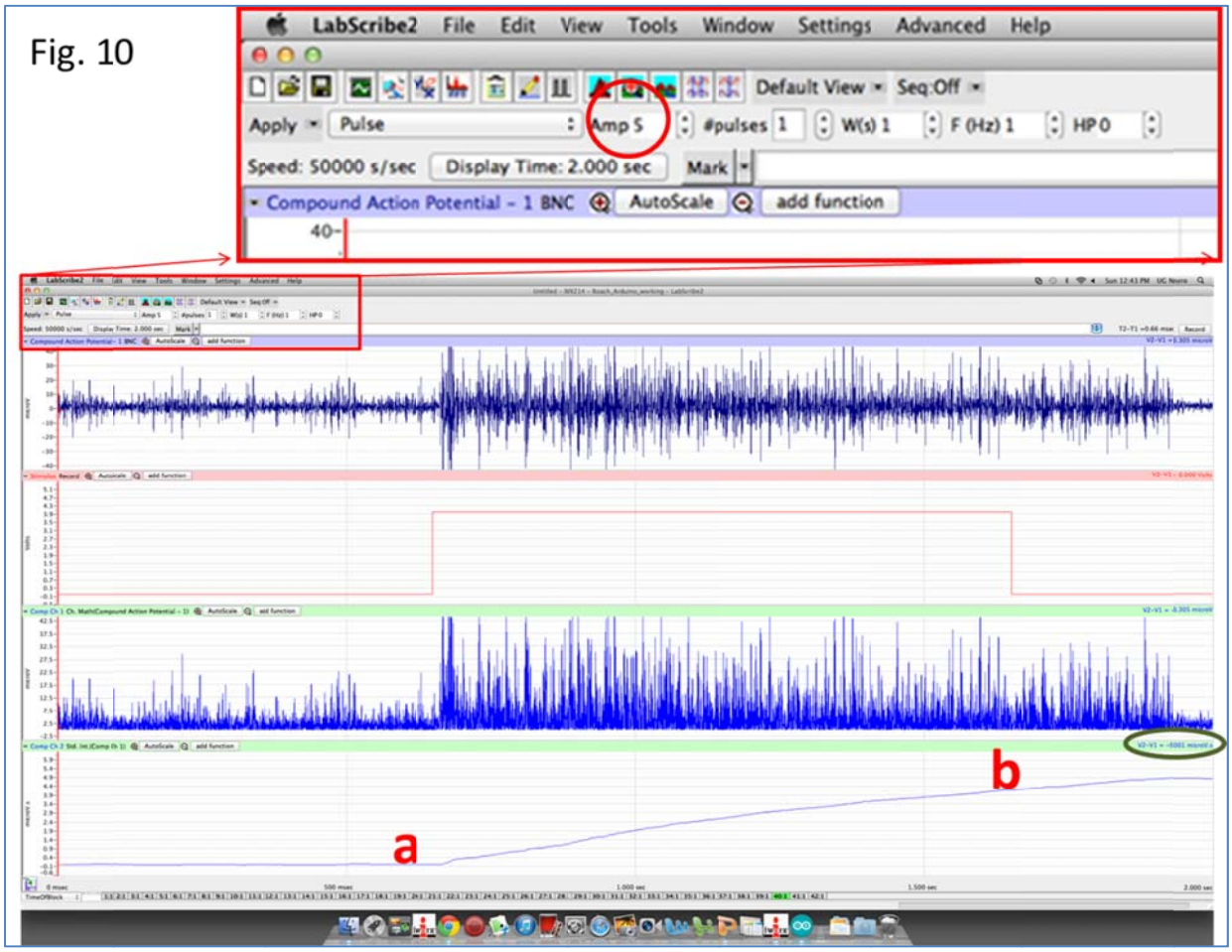
File➔open➔Roach_project4_arduino_setting.iwxset. You should see four panels (Fig.10).

Top Panel: Your recording will be displayed here.

Second Panel: the timing and amplitude of your pin movement command will be displayed here.

Third panel: We rectify the recorded trace shown in the top panel, namely all the negative part of the traces are flipped positive and added to the part of the trace that are already positive. This is the first step to calculate the sum total of all the neuronal activities.

Bottom panel: This is an integration of the trace in the third panel. The overall neuronal activities could be measured as the difference between the base line (Fig.10a) and the amplitude near the end of the pin displacement (Fig.10b). This measurement, between point a and b, should be proportional to overall neuronal activities. (Similar data processing steps are used to analyze electromyograms by clinicians and research scientists.)



Why do we massage the beautiful trace, which has all the crisp APs,

into a mushy and feature-less trace in the bottom panel? Ideally, we should do this analysis by identifying the best axon that fires big APs. We could then monitor changes in AP firing frequency as we bend the joint. However, this is not practical. First, we push our recording pins into the femur and coxa randomly. The axons innervating the joint may not be near the pins and give us clear, big APs. Second, iWORX doesn't have spike/frequency counting features. Third, there ae many axons that innervate the joint and we would like to include firing activities of all the axons. This rectification-integration approach incorporates contribution from those axons that give rise to small APs.

Experimental Protocol:

1. Pin positioning.

We would like to move the tibia consistently during repeated trials. The best way to do this is to wedge the pin near the base of a spine such that the pin is able to push and pull the leg forward and backward (Fig.11 circle and blue arrows). However, this arrangement is not completely "fool proof", the pin may slip off on large movements. You need to keep an eye on the prep and don't use the traces that movement was not complete because the pin has slipped for later analysis.

Another important trick in positioning the pin, before running the iWORX protocol, is that while you turn the large gear to move the pin along X-axis, always finalize your position with a movement of you pin in the direction you intend to move, i.e. don't back into your final position move forward into your final position. This way, the pin will start to move once the gear starts to turn and we avoid backlash issue of the mechanism.

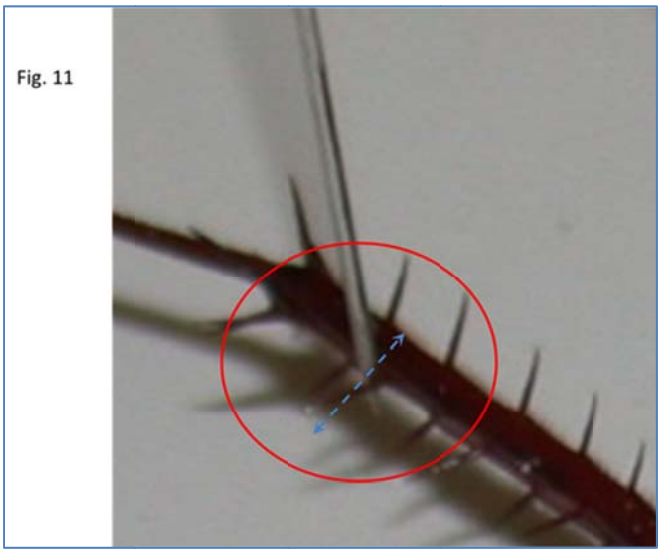


Fig. 11

2. Parameters to control:

First, find the toggle switch on a line attached to the Arduino board and make sure that the switch is in F position. "F" for forward and application of positive voltage will move the pin to the right.

We will change the value of amplitude (Fig.2 red circle in inset) from 1 to 5 with increment of 1. After that, flip the toggle switch to B, for backward. Move the pin to the opposite side of the tibia and run through amplitude 1 to 5 again. (Don't forget to wedge the pin near the base of a spine.)

Note that each time you press "Record", the iWORX will make four repeats of the same movement and averages the four trials. Be patient, iWORX will show you the progress as each trial is added to the averaging process. Do not touch the set up or press any buttons as the four repeated movements are being carried out.

2. Data presentation.

For each voltage command, you should have a measurement of the integrated trace at the bottom panel. You can place the cursors are positions "a" and "b" and read the difference at the upper right corner of the bottom panel (Fig.2 green circle). (Right click the upper right corner and choose V2-V1 if the display shows something else.)

By the end of this experiment, you should have:

Movement amplitude	1	2	3	4	5	-1 (- for backward)	-2	-3	-4	-5
V2-V1										

Double click the IGOR template, "roach_project4_plot.pxt" to generate your plot. Is the relationship between the axon firing activities linearly related to the movement of the joint, or the relationship is complicated? We should keep in mind that we are not plotting the distance or the angel of the FT joint. However, the voltage amplitude should be proportional to the distance of the pin movement.

You can then try a linear fit by: Analysis➔Quick fit➔line. Linear fit may not be appropriate, you can play with other curve fits and decide which mathematical function describes your data best.

(If there are data points you decided not to use, such as the case when the pin did not move the tibia correctly, you should type in "nan", for "not a number", in both voltage and response cells of the table.)

Data from this series of experiment should be displayed in two slides.

The first slide should show AP traces—the trace in the top panel—from 4 voltage levels. (You decide which traces to show.) Voltages used for each trace should be clearly labeled on the slide. For the figure legend, you should briefly compare features in these traces. Examples of features to comment on included: firing frequency, duration of responses or the density of axonal firing. Feel free to come up with more comparisons of your own. Keep it brief.

The second slide should show the plot you made in IGOR. As before, provide a brief description of the plot, trend and linearity etc.

Reference

Utility and Versatility of Extracellar Recordings from the Cockroach for Neurophysiological Instruction and Demonstration. R. L. Ramos, A. Moiseff and J. C. Brumberg The Journal of Undergraduate Neuroscience Education (JUNE), 2007, 5:28-34

**We want to thank Johnathan Perry of the Biology Department Machine Shop for his thoughtful inputs in the design stage of this project, for his skills in making all the hardware components and for his patience and efforts that make it possible to finish this project in a timely manner.