

Module 404 practicals

- 1) Safety:** Please note the safety instructions on each form. These practicals pose low risk, providing sensible approaches are taken to your own jumping and handling of locusts.
- 2) Timetable:** Please attend each practical session once according to your **surname** as in this table:

	Computer simulation (A004)	Larval perception (A022)	Locust jump (A008)	Locust breathing (A032)
1. Tue am	A - E (20)	F - L (20)	R - Z (20)	M - Q (15)
2. Tue pm	F - L (20)	R - Z (20)	M - Q (15)	A - E (20)
3. Wed am	R - Z (20)	M - Q (15)	A - E (20)	F - L (20)
4. Thur am	M - Q (15)	A - E (20)	F - L (20)	R - Z (20)

- 3) Assessment:** The practicals are assessed by a written report with a deadline of **Monday 13 December 2010**. The report should not exceed 1000 words. It must be structured as a research paper with Introduction, Methods, Results, Discussion and Bibliography. The report must be word-processed and all graphs etc. drawn using graphical packages. Make sure that you address the questions posed in the schedule. If you are unwell or ill, and can only attend a proportion of the sessions you will have less choice in the write-up. If you are unwell for all the sessions, you need to arrange to complete a medical mitigating circumstances document.
- 4)** We hope you will enjoy the process of learning about nerve, muscle and movement from these practicals. Any comments welcomed (cje2@york.ac.uk)

C. J. H. Elliott October 14, 2010

Module 404

Practical 1

Computer Simulation of the Action Potential

Dr Gareth Evans

INTRODUCTION

In their seminal work of the 1940's using the squid giant axon, Hodgkin and Huxley discovered the ionic basis of the action potential. They developed a set of differential equations that accurately model action potentials observed *in vivo*. This practical will explore further the description of the action potential you received in Lecture 2 of the module. You will use an online computer program written by Dr Francisco Bezanilla (University of Chicago, USA) that simulates action potentials with Hodgkin and Huxley's differential equations. By varying the parameters of these equations you will be able to explore how ionic conductances, currents, and membrane voltage are related. In each exercise you will perform simulations to answer specific questions.

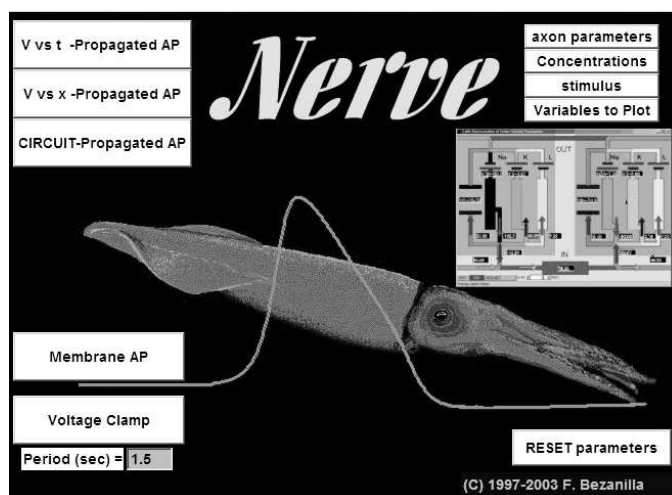
In this practical, all of the experiments are in current clamp mode. The experimenter applies current pulses and observes the voltage response. This is close to the physiological conditions of axon operation and experiments done under these conditions will give a close insight as to how the normal axon works.

PROGRAM STARTUP

Start the nerve program from: <http://nerve.bsd.uchicago.edu/nerve1.html>, which will give you a window as shown below. If at any time you restart the program it is possible some of the parameters may be different from the default values. Press **F10** to revert all parameters to their default values. Access to the different simulation modes is by way of the button on the left side of the main screen. For example, just click on the **Membrane AP** button. This will open a window that has a plotting area and several buttons to perform different operations. Some of these operations can be performed by using the shortcuts listed below:

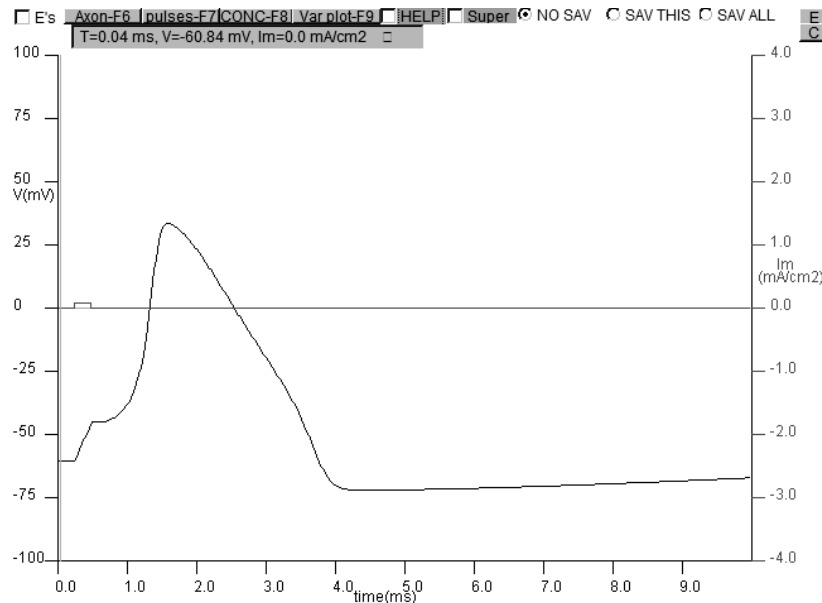
PROGRAM COMMANDS

- F6 Axon parameters
- F7 Pulses
- F8 Concentrations
- F9 Variables to Plot Menu
- F10 Reset parameters to default values
- ESC Close window in use.



EXPERIMENT 1 - Generating a simple action potential

To start, click on the **Membrane AP** button. You should see a plot of the **current pulse** used to stimulate the axon as a function of time (red trace) along with the plot of **membrane potential** ($V_{\text{inside}} - V_{\text{outside}}$, black trace), also as a function of time. What you have done is to simulate the injection of a current pulse (of 10 mA and 0.25 ms duration) to the axon and record the membrane potential. These are the default values and you will be able to change them in due course. Now save the picture - it should look like the figure below. In future you should save each new picture that you produce.



SAVING PICTURES

Copy your pictures to the clipboard using the PrintScreen key (top right on the keyboard) and then paste them into pages in a PowerPoint document. To avoid confusion, make sure you label each picture.

Now we can look at the flow of ions and the behaviour of the ion channels which determine the action potential. The program allows you to plot in the same screen the time course of many of the important variables that might be changing during the generation of the action potential. Press **F9** (Variables to plot) and experiment with checking or unchecking the various boxes to plot different variables. These are outlined below:

- INa sodium current - ie. how many sodium ions are flowing across the membrane
- gNa sodium conductance - ie. how permeable is the membrane to sodium = number of channels x probability of a channel opening = $N_{\text{Na}} \times P$
- m probability that Na channel subunit is active (3 active subunits are needed to open the channel)
- h $1-h$ = probability that the sodium channel inactivating ball is in position, ie. channel is inactivated
- IK potassium current
- gK potassium conductance

n n^4 = probability that the potassium channel is open
I_i total ionic current
I_m total membrane current

- Q1.** In which direction (into or out of the cell) are the sodium and potassium ion currents flowing during the action potential (the plots of I_{Na} and I_K might help)?
- Q2.** In this system, what are the values of the equilibrium constants for Na and K? (check the E's box in the top left corner of the graph screen to plot these values). What do these values represent?
- Q3.** Plot the conductances for sodium and potassium (g_{Na} and g_K) and comment on the relative rates of the activation and inactivation of sodium and potassium channels.

EXPERIMENT 2 - How does changing the ion concentrations affect the action potential?

Based upon Hodgkin and Huxley's formulae, when the external ion concentrations are changed the action potential waveform should change in predictable ways. Hodgkin and Katz did this experiment *in vivo* in the squid giant axon by exchanging the sea water for water with varying concentrations of sodium. We can test this directly by first changing the external sodium concentration.

Because we will be examining the effects of decreasing the external sodium concentration we need to increase the stimulus to insure that an action potential will occur each time. Click on **Pulses** (or **F7**) and set the stimulus strength to 20 microamps and increase the total time to 12 ms. Click on **Axon** (or **F6**) and reset the temperature to its default value (click on default in the temperature field).

To simplify interpretation we will run this simulation using *ideal* Na and K channel properties where only sodium or potassium ions go through their respective channels (ie. for an ideal Na channel, only Na can permeate). To do this, in the **Axon Parameters window** click on the **Ideal Channel Properties**. Click on **SAV THIS** to save the present simulation. Now press **F8** and reduce the external sodium concentration from its default value which is 440 mM. Check **Super** and you will see the new action potential superimposed on the reference action potential recorded in the default external Na concentration. Progressively reduce the external sodium concentration and observe the action potential. Before you run these simulations with reduced sodium concentrations try to predict what changes you will see.

- Q4.** When you have reduced the external sodium concentration to about 200 mM, notice that there is a small change in the resting potential and a delay in action potential firing. Explain the basis of these phenomena.

Now reset the Na concentration to default (440 mM) and then alter either the tetrodotoxin or pronase concentrations.

- Q5.** Based on the molecular action of these molecules on Na channels (see Lecture 2, p11 of handout), explain the effects on the action potential you observe.

Reset the variables to default and now increase the extracellular potassium progressively and examine its effect on the action potential. Be sure to examine g_K and g_{Na} . Try 20 mM extracellular K.

Q6.

- (i) Why do comparatively small changes in potassium have such a profound effect compared to the effect of sodium concentration changes?
- (ii) Why do large changes in potassium stop all activity? Examine the changes in membrane potential, and the equilibrium potentials.

We have seen that changes in external sodium affected many of the action potential properties. Let us now examine the *threshold*, which is a property that defines whether the action potential will or will not be generated. Restore the action potential parameters by clicking on **RESET** parameters or pressing **F10**. In the Pulses window change the amplitude to 10 μA and the total time to 10 ms. Now experimentally determine the amplitude of the current pulse that is required to just exceed threshold (that is, that generates an action potential).

To see the relationship between different stimulus intensities you can save each screen by clicking in **SAV ALL** and checking **Super** to superimpose the traces as you change the stimulus strength.

Q7. Why does the progressive reduction of the stimulus intensity cause the onset of the action potential to occur at progressively longer times after the cessation of the stimulus? [Hint: One suggestion as to how to approach answering this question is to examine the total ionic current (I_i) at high magnification, together with the Na and K currents. Observe the direction of the currents just when the action potential is taking off].

EXPERIMENT 3 - How do sodium ions contribute to the action potential?

We will now focus in more detail on the role of sodium ions in the action potential. Click on **RESET**, or press **F10** to restore the default settings. Select the plots for **sodium current** (I_{Na}), and **sodium conductance** (g_{Na}). By clicking several times on the right hand scale you can **display the legend** for I_m , g_{Na} and I_{Na} . You can also vary the **range** for the g_{Na} and I_{Na} plot by **expanding** or **compressing** the plotted scales using the **E** and **C** buttons.

Q8. There are several interesting and important features shown in the sodium current trace.

- (i) First, why is there a notch in the I_{Na} trace? What can you learn from this about the relationship between I_{Na} and g_{Na} ? [Hint: display E_{Na} and consider the relationship of E_{Na} to the membrane voltage V , particularly when $V=0$.]
- (ii) Notice that the peak I_{Na} curve occurs later than the peak of the g_{Na} curve, why?

Once you understand the reasons for the different time courses of I_{Na} , and g_{Na} , add the m and h curves to your plot. Note: these variables can best be understood if you

simplify your screen by removing the sodium current (deselect INa). Now you can compare the time course of the membrane voltage and the m and h curves during an action potential along with gNa (which is proportional to m^3h). Click on the right hand scale to display the legend for the m or h curves.

Q9. What is the significance of the fact that the m curve does not start at 0 and that the h curve does not start at 1?

Remember that m represents the probability that one of the subunits is in the active position and in the classical formulation three are required to open the channel (m^3) but at the same time, the inactivation particle has to be out of position for the channel to conduct. The probability that the inactivating ball is in position is given by 1-h. Therefore the probability that the Na channel is conducting will be: $P=m^3h$ and the conductance (g_{Na}) will be: $N_{Na} \times P = N_{Na} \times m^3h$. Thus, from these two curves you can get an appreciation of how the two gates of the sodium channel can act to regulate the sodium channel's permeability. To see how these interact, plot g_{Na} , m, and h together.

Q10.

- (i) Why does the sodium conductance begin to fall even though the sodium channel's m gate is still open?
- (ii) What would happen if you tried to stimulate the nerve with a second pulse timed to coincide with the peak of the sodium conductance?

EXPERIMENT 3 - How do potassium ions contribute to the action potential?

Clear the graph and this time display potassium conductance (g_K) and the potassium current (I_K). You should see plots of the **membrane voltage**, **g_K** , and **I_K** .

Q11. Notice the time courses for i_K and g_K are different. The I_K curve reaches its peak before g_K , why? [Hint: display the equilibrium potentials (by clicking on **E's** check button) and consider the relationship between E_K and the membrane voltage.]

Q12. Observe the falling phase of the potassium current. How can the potassium current (I_K) have fallen to nearly its resting level while g_K is still greater than 0? Is I_K equal to 0? You can check this more accurately by using a higher gain (higher magnification using the **E** button) for the I_K plot.

Once you understand the reasons for the different time courses of I_K and g_K , add the n curve to your plot. The n curve is a measure of the time course of the opening and closing of one of the subunits of the potassium channel (often referred to as the opening and closing of the n gate). Click on the right hand scale until you display the scale for the n curve. Remember that the probability of opening $P_o=n^4$ and that $g_K=NP_o$.

Q13. What is the significance of the fact that the n curve does not start at 0? Why is I_K nearly 0 at the end of the action potential trace even though at this same time n is even greater than its resting value of approximately 0.4?

Acknowledgement

We are grateful to F. Bezanilla for making this program and his manual available at <http://nerve.bsd.uchicago.edu/>

THE REPORT

The report should be structured as a research paper with Introduction, Methods, Results, Discussion and Bibliography. The report must be word-processed and all graphs etc. imported carefully. **Make sure that you address all 13 questions posed in this handout.**

The Introduction should give a background to the experiments and detail their aims. The Methods can be brief - only report aspects of the protocols that were not in the schedule or that were changed. The Results must be described in words and the main findings highlighted as well as being presented in suitable graphical or tabular form. Discussion - the findings should be discussed and placed in the context of the research literature. To save space, it is acceptable to combine the Results and Discussion sections. Bibliography - full reference must be made to information derived from other studies and these must be cited in the text.

Starting References

The ionic basis of the action potential is well described in several texts:

Purves et al. Neuroscience (Sinauer)

Aidley, DJ. The Physiology Of The Excitable Cell.

Hodgkin & Huxley's original papers are all online in the Journal of Physiology and include:

A. L. Hodgkin and A. F. Huxley (1945) **Resting and action potentials in single nerve fibres** J Physiol 104: 176-195. [[PDF](#)]

A. L. Hodgkin and B. Katz (1949) **The effect of sodium ions on the electrical activity of the giant axon of the squid** J Physiol 108: 37-77. [[PDF](#)]

Nerve Muscle and Movement Module 404

Circus Practical: Assessed Section, Dr Sean Sweeney

Drosophila larval responses to heat/pain and 'wasabi'.

This practical will expose and test the *Drosophila* larval responses to 1) pain/heat and 2) taste of a noxious chemical 'wasabi' (isothiocyanate).

Experiment 1: The 'pain/heat' assay:

When exposed (at the anterior end) to a noxious or painful stimulus, *Drosophila* larvae roll violently away from the stimulus with a highly stereotyped corkscrewing action. A touch stimulus applied to the same anatomical area on the other hand, will result in a retraction of the head and a changing of direction in crawling. We will take third instar crawling larvae and using a temperature regulated soldering iron we will apply a controlled heat stimulus to the larvae and record their responses. This will be carried out on larvae as they crawl across agar plates at room temperature. We will test two genotypes, a wild type and mutants of *pain1*.

Practical points.

- 1) Practice eliciting the corkscrew roll with wild type larvae so that your application of the temperature probe is consistent.
- 2) Before you test the experimental genotypes for pain sensation, touch each of the individuals in turn on the anterior end with your forceps and look for the retraction of the head, this will tell you that the larvae are competent for mechanosensation. If they do not respond, reject them for the experiment.

Experiment 2: The 'taste' assay.

Drosophila larvae normally avoid the taste of wasabi, presumably because it is noxious to them. We will test the taste avoidance ability of two genotypes of *Drosophila* third instar wandering larvae. Two blobs of yeast will be applied to

agar plates. One blob will contain isothiocyanate, the other will be plain yeast. One will be coded by the addition of red food dye, the other, blue food dye. Ten larvae of the wild type genotype will be placed on one plate, ten *pain1* larvae will be placed on another. They will then be tested on their choice for one blob of yeast or the other by leaving them to wander on the plate for 1 hour IN THE DARK. They will then be scored by examining the colour of the gut in each larvae.

Background Papers to read:

Tracey, W.D. Jr, Wilson, R.I., Laurent, G. and Benzer, S. (2003) *painless*, a *Drosophila* gene essential for nociception. *Cell*. 113: 261-273

Al-Anzi. B., Tracey, W.D. and Benzer, S. (2006) Response of *Drosophila* to wasabi is mediated by *painless*, the fly homolog of mammalian TRPA1/ANKTM1. *Current Biology*. 16: 1034-1040

Hwang, R.Y., Zhong, L., Xu, Y., Johnson, T., Zhang, F., Deisseroth, K. and Tracey, W.D. (2007) Nociceptive neurons protect *Drosophila* larvae from parasitoid wasps. *Current Biology*, 17: 2105-2106

The Assessed Component.

The Report:

Report only on the heat assay. We will not write up the taste assay.

The report should not exceed 1000 words. As much as possible, it should be structured as a research paper with Introduction, Methods, Results, Discussion and Bibliography. The report must be word-processed and all graphs etc. drawn using graphical packages. Make sure that you address the questions posed in the schedule.

The Introduction should give a background to the experiments and detail their aims. Methods can be short - only report aspects of the protocols that were not in the schedule or that were changed. Ideally the Methods section can be one sentence (it NEEDS to be minimal): "the experiment was carried out as per the practical handout". Results - the results must be described in words and the main findings highlighted as well as being presented in suitable graphical or tabular form. Look at the research papers for guidance on presentation of the figures. Discussion - the findings should be discussed and placed in the context of the research literature. Bibliography - full reference must be made to information derived from other studies and this must be cited in the text.

Your submission must carry your examination number and not your name.

LOCUST RESPIRATION

Safety: A very few people are allergic to locusts; and others may develop it over a long time (e.g. >1 year) handling locusts. If you are allergic to locusts, grass or other agents, you should minimise the risks by asking your colleagues to handle them. Always wash hands well after handling the locusts.

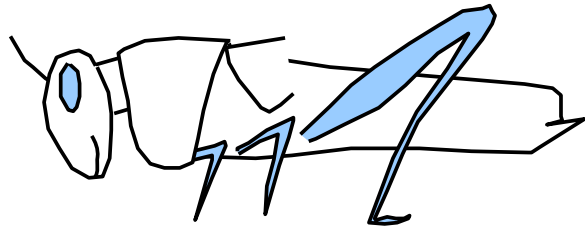
The problem in this practical is to study the mechanism of the insect ventilating system: the abdominal pumping movements and its associated spiracle control. The material will be locusts, *Schistocera gregaria*.

The practical is divided into 3 stages:

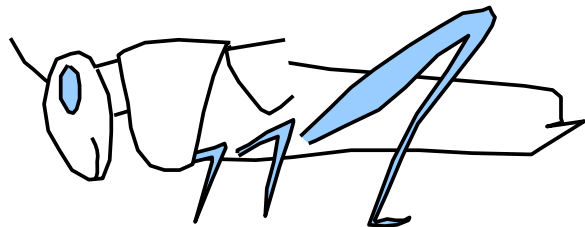
1. Observation on intact insects.
2. Examination of spiracle movements.
3. Analysis by electrical recording.

1. Observations on intact insects

- a. Strap a locust down so you can see its side using a piece of plasticine/ blutac. Look for movements of the abdominal sternites (the underneath plates on the abdomen. Draw the movements on the sketch to the right. See how they accelerate when the locust is gently touched or otherwise disturbed.



- b. Place the locust under the gassing (lunch) box and observe the effects of blowing CO₂ into the box through a clean (sterile) 1 ml pipette. Your expired air is about 4% CO₂. What happens to the amplitude and frequency of dorso-ventral pumping? Sketch any new forms of movement on the diagram.



- c. Now try a very small amount of CO₂ from the cylinder. *Safety Warning: Keep the cylinder standing upright, and do not put it where it can be kicked over.* Biological warning: too much CO₂ will kill the locust. Again sketch any new forms of movement on the diagram.



Q1. Does the inspiratory or expiratory movement change more as the locust breathes more?

2. Examination of spiracle synchronization

Spiracle valves open and close in a coordinated manner in such a way that there is a one-way airstream along the tracheal trunks during ventilation. (Why might this be more efficient than a tidal system?). Use Fig. 1 to locate the second spiracle, just above the middle leg. Use the binocular microscope to watch the opening/ closing of the spiracle and determine if it is open during inspiration, expiration or all the time.

Q2. What happens when the locust is placed in 4% CO₂ and in 100% CO₂.

3. Electrical recording

Technique: the neural and synaptic activity of the abdominal muscles will be recorded on the computer using a sensitive 1000x amplifier and input to the computer with the locust program (Fig. 2). Refer to Fig. 3 which shows the relation of the abdominal muscles to the exterior. Make a small pin hole near the end of M191. Place one of the fine copper wires just in each hole.

Can you record any electrical activity?? The most common artifact (Fig 4) is called hum (listen to your output) which is caused by pickup from the mains cables. If there is lots of 'hum' one (or both) of the electrodes is probably in a trachea or has not gone in far enough. Adjust the electrodes to reduce the hum: if it persists you may need to earth the locust with a fine pin and earth wire to the baseplate. Other artifacts may be caused by movement of the wires (eg if the locust can grab them, or if they move when you blow in CO₂). Record the signals on the computer – set it to record for 30s. You can save the output as emf or png format pictures which can then be inserted into your writeup.

Q3. What happens when you give CO₂?

Q4. Can you distinguish different size EJPS [see Commentary for an explanation of EJPS] ? Get the locust breathing rapidly and set the computer to record for 5s and see which ones come first?

Q5. How do the lengths of bursts and interburst intervals vary when the respiratory rate changes? Try a set of 30s recordings as the locust recovers from high CO₂.

Q6. How does M191 activity relate to the movement you see?

Observe the movements of spiracle 2 down a binocular microscope while listening to the output of the amplifier. .

Q7. How does the movement of the spiracle relate to the movements of the abdomen?

Figures

Figure 1: Location of spiracles on thorax and abdomen. Spiracle 1 is hidden under the pronotum, spiracle 2 has 2 halves, looking a bit like a bivalve mollusc, spiracles 3-10 are in small tubes. You should have no difficulty spotting spiracle 2, but keep the legs away. Watching spiracles 3 and 4 should be possible, but the more posterior ones are hard to see accurately.

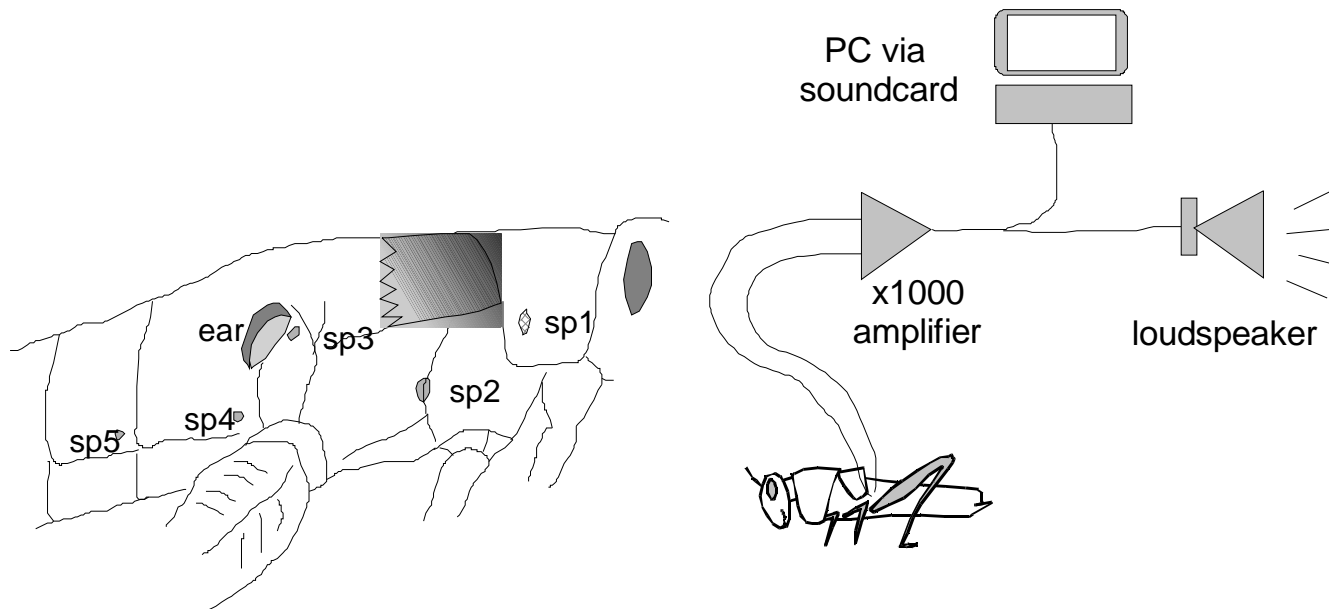


Figure 2 Circuit diagram of apparatus:

Figure 3: Map of the muscles in the abdomen

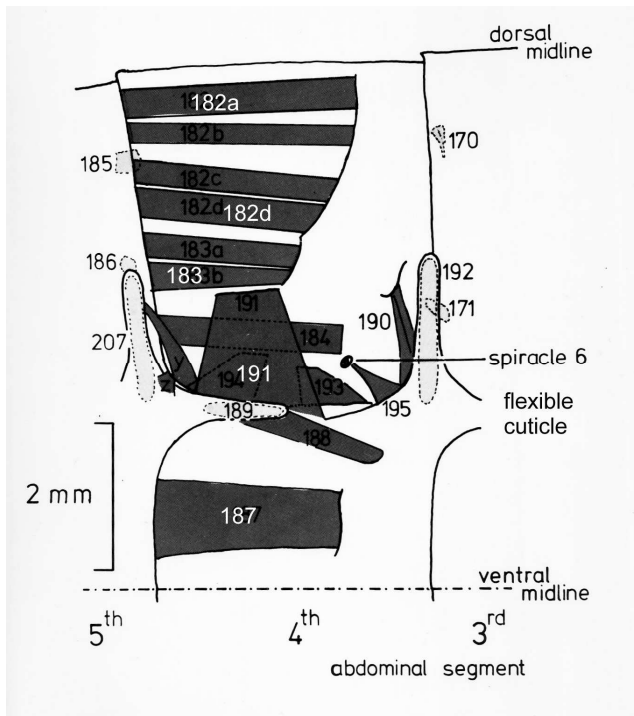
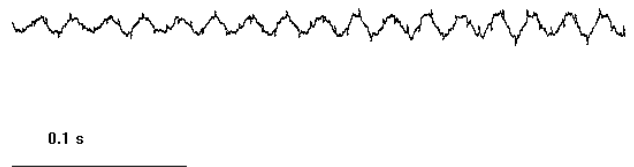
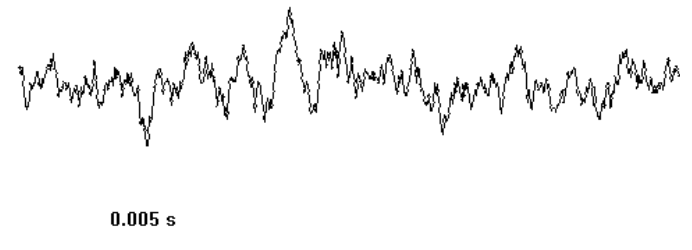


Figure 4: Artifacts of electrical recording

Hum: a regular wave with period 20ms. It hums if you play it over the headphones /speaker



Noise: nice unpredictable, random waves



Movement: note the shift in the baseline (e.g. under the n of baseline)



The Report:

The report should not exceed 1000 words. It must be structured as a research paper with Introduction, Methods, Results, Discussion and Bibliography. The report must be word-processed and all graphs etc. drawn using graphical packages. Make sure that you address the questions posed in the schedule.

The Introduction should give a background to the experiments and detail their aims. Methods can be short - only report aspects of the protocols that were not in the schedule or that were changed. Results - the results must be described in words and the main findings highlighted as well as being presented in suitable graphical or tabular form. The data must be presented in statistically valid form and appropriate tests carried out to support your conclusions. Discussion - the findings should be discussed and placed in the context of the research literature. Bibliography - full reference must be made to information derived from other studies and this must be cited in the text.

Your submission must carry your examination number and not your name.

Starting references

P. L. Miller Respiration in the Desert Locust: I. The Control of Ventilation
J. Exp. Biol. 1960 37: 224-
236. <http://jeb.biologists.org/cgi/content/abstract/37/2/224>

P. L. Miller Respiration in the Desert Locust: II. The Control of the Spiracles J.
Exp. Biol. 1960 37: 237-
263. <http://jeb.biologists.org/cgi/content/abstract/37/2/237>

P. L. Miller The Derivation of the Motor Command to the Spiracles of the
Locust J. Exp. Biol. 1967 46: 349-371
<http://jeb.biologists.org/cgi/content/abstract/46/2/349>

G. W. Lewis, P. L. Miller, and P. S. Mills Neuro-Muscular Mechanisms of
Abdominal Pumping in the Locust J. Exp. Biol. 1973 59: 149-168
<http://jeb.biologists.org/cgi/content/abstract/59/1/149>

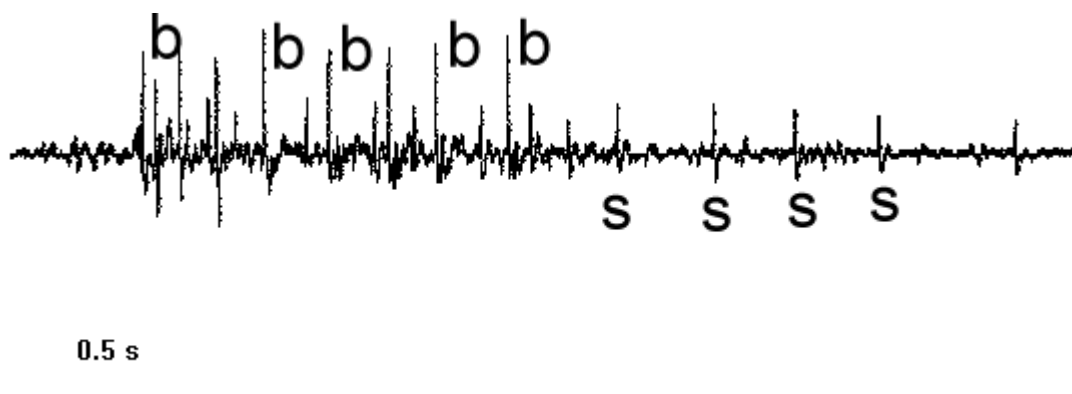
Greenlee, KJ & Harrison, JF (2004) Development of respiratory function in the
American locust *Schistocerca Americana* J exp biol 207, 497-508
<http://jeb.biologists.org/cgi/content/full/207/3/497>

Bustami HP, Harrison, JF & Hustert R (2002) Evidence for oxygen and carbon dioxide receptors in insect CNS influencing ventilation *Comp Biochem Physiol A* 133:595-604

[http://dx.doi.org/10.1016/S1095-6433\(02\)00155-1](http://dx.doi.org/10.1016/S1095-6433(02)00155-1)

Commentary:

Insect muscles are generally innervated by several neurons, and the total output is usually not big enough to generate an action potential in the muscle fibre. Therefore, the recordings you get are EJPs, (excitatory junction potentials, otherwise called synaptic potentials), and indicate the firing pattern of the neurons. Typically you get several EJPs which you can recognise by their different heights and widths: as shown by the big (b) and small (s) EJPs below.



Muscle Performance – Locust and Human

1. THE LOCUST JUMP

Allergies: *A very small fraction of people are allergic to locusts: You should all handle the locusts carefully wearing gloves. If anyone begins to feel unwell or discomfort, immediately stop handling the locust, move away from the animals and contact the demonstrator.*

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Locusts are well known for their ability to jump long distances, an ability that is important as an escape mechanism and for initiating flight. In these experiments you will be investigate the mechanisms and power output of the jump. There are two parts to your investigation:

1. The power output of the intact adult locust and juveniles
2. The functional organisation and mechanism of the jump

⇒ For all the tests, you should make several measurements. Record your data on the laptop so that, at the end of the week you can combine data from several groups to present the results in a statistically meaningful form, carrying out appropriate tests for significance.

The next section explains how we know the energetics of locust muscle and tendons from studies on a whole locust. This will be discussed in the first movement lecture.

Theoretical Background

The locust jump is powered by the contractile forces generated by the large *extensor tibiae* muscles of the metathoracic legs (the hindlegs). Jumping depends on the exertion of force by these legs against the ground; the active part of the jump, therefore, is only the brief period during which the legs remain in contact with the ground.

If we ignore the effects of air resistance, then the distance (d) in metres jumped by the locust depends on the velocity (v) (m s^{-1}) at take-off and the angle θ of the trajectory:

$$d = \frac{v^2 \sin 2\theta}{g}$$

where g is the acceleration due to gravity (9.81 m s^{-2}).

The distance travelled is maximal if the take-off angle is 45° . You can test if this is true by looking at the photographs in Figure 5 of a locust during jumping. If we assume the locust does take-off at 45° , then:

$$\sin 2\theta = \sin 90 = 1$$

eqn 1 therefore simplifies to:

$$d = \frac{v^2}{g} \quad \text{or} \quad v = \sqrt{gd} \quad 2$$

The velocity v depends on the energy E (in joules) produced by the animal which can be determined using the standard equation for kinetic energy:

$$E = \frac{mv^2}{2} \quad 3$$

where m is the mass of the animal in kg.

If we substitute for v from eqn 2:

$$E = \frac{mdg}{2} \quad 4$$

$$\Rightarrow d = \frac{2E}{mg} \quad 5$$

If the animal starts its jump from standing, then it has to accelerate from zero velocity to the take-off velocity. The acceleration period is restricted to the period of time the feet remain in contact with the ground and is therefore determined by the length of the legs and their degree of flexion at the start of the jump: when we make a standing jump, we first bend our knees and crouch, thereby maximising the time our feet retain ground contact while the extensor muscles are contracting and accelerating our body mass. This also improves the mechanical advantage and preloading of the extensor muscle, increasing the maximal power output.

For the locust jump, the legs are fully flexed prior to the jump and the extension occurs mainly as rotation of the femoro-tibial joint, see figure 1:

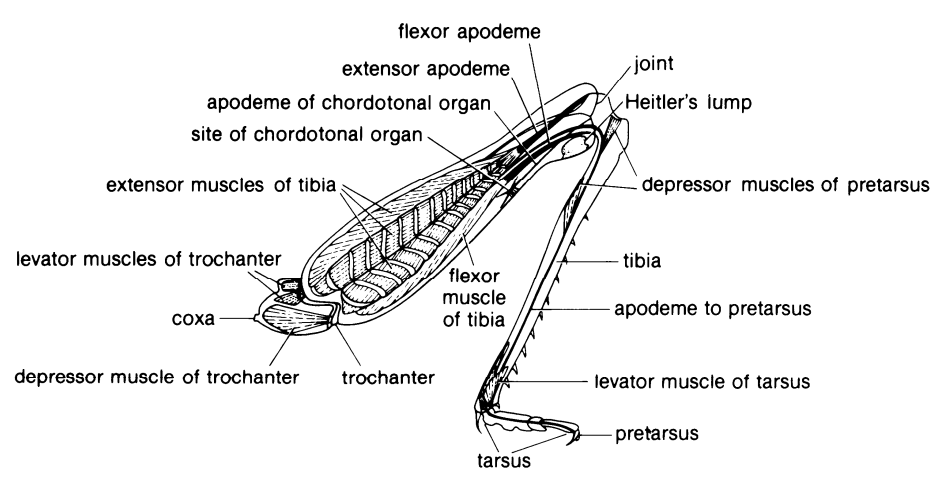
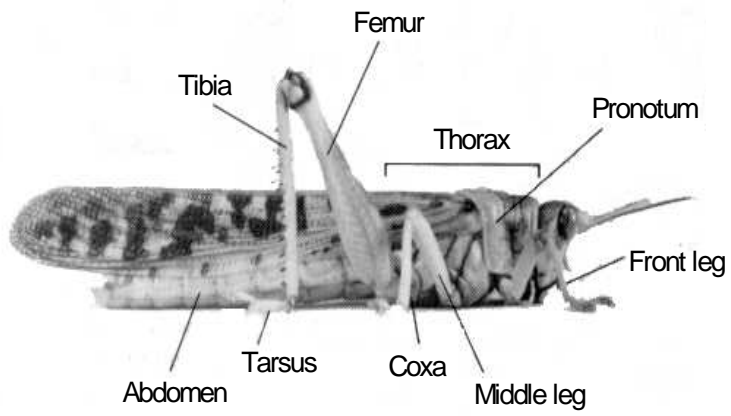


Figure 1 The adult locust and the internal organisation of the locust hindleg

The acceleration distance s is therefore determined by the length L of the femur and the angle of joint rotation. The leg starts fully flexed and rotates through an angle of 130° .

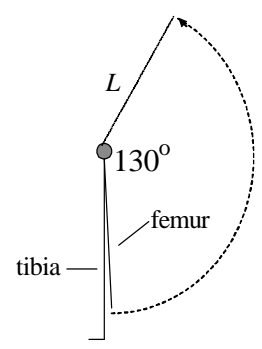


Figure 2 The rotation of the leg during jumping. The femur rotates around the joint with the tibia so that it describes the arc of a circle, the radius of which is the

We can calculate the acceleration distance (s), as the length of the arc of the circle, as follows:

$$s = 2\pi L \frac{130}{360}$$

□

$$s = 2.27 L \quad 6$$

The velocity at take-off is determined by the acceleration a and the distance s over which the muscles exert that acceleration. If we assume that the initial velocity is zero (a standing jump) then:

$$\frac{1}{2}mv^2 = m a s$$

$$v^2 = 2as \quad 7$$

To achieve this velocity the locust has to do work, over a period of time, to accelerate its mass. Work per unit time is expressed as Power, in Watts. The velocity is taken as $\frac{1}{2}v$ to approximate to the mean velocity during the acceleration phase:

$$P = \frac{1}{2} v a m \quad 8$$

Where m is the mass of the locust. The power output in Watts per kg of muscle is therefore: $P \div$ total extensor muscle mass. The theoretical maximum power output from living muscle is about 1 kW kg^{-1} of muscle, which is sustainable for only a fraction of a second.

The following data was recorded from an experiment to measure the jump length achieved by a human from a standing start:

	Human
Jump (m)	2.2
Mass (kg)	73
Femur length (m)	0.44
Extensor muscle mass (both legs) (kg)	8
Angle of joint rotation (deg)	70

Use the equations given above to complete the table:

	Human
Take-off velocity v (m s^{-1})	
Acceleration distance s (m)	
Acceleration a (m s^{-2})	
Total Power P (W)	
Power output per kg of muscle (W kg^{-1})	

⇒ How does the calculated power output compare with the maximum possible from skeletal muscle?

LOCUST JUMPING DISTANCE: experimental measurements

Measurements must be made in a warm environment (if possible 28-35°C)

- a) You should try to obtain records from at least four adult locusts (the wings of the adult locusts have been removed to prevent them from flying away) and 4th and 5th instar juveniles, as available. Each locust should be weighed and the length of the femur of the hindleg measured. Take care not to mix-up your locusts during the recordings: use ink-marks on the thorax/hindlegs for identification.. Place the locust on a rough surface such as a blue tissue roll placed on the floor - this gives the feet purchase so that they do not slip during the jump. Encourage the locusts to jump by blowing on them, pinching the abdomen etc.

You should record at least five jumps for each locust.

Using your best adult jumper, try the following experiments:

- b) Test the effects of body mass on jumping performance by sticking balls of plasticene to the pronotum: try 10, 20 and 50% of body mass (note that during egg bearing the mass of the female increases by up to 50%).
- c) Remove one hindleg. Keep the leg for measurements of muscle weight. Record some jumps made by the one legged locust. Take the leg removed from the locust and then weigh the leg. (How much does this overestimate the muscle mass?)
- d) Measure the jump distance achieved by 5th and 4th instars (“hoppers”)

Analysis of the Jumping Data.

Collate individual jumping data for the locusts tested by each group. For each locust plot the maximum jump distance against body mass and against femur length.

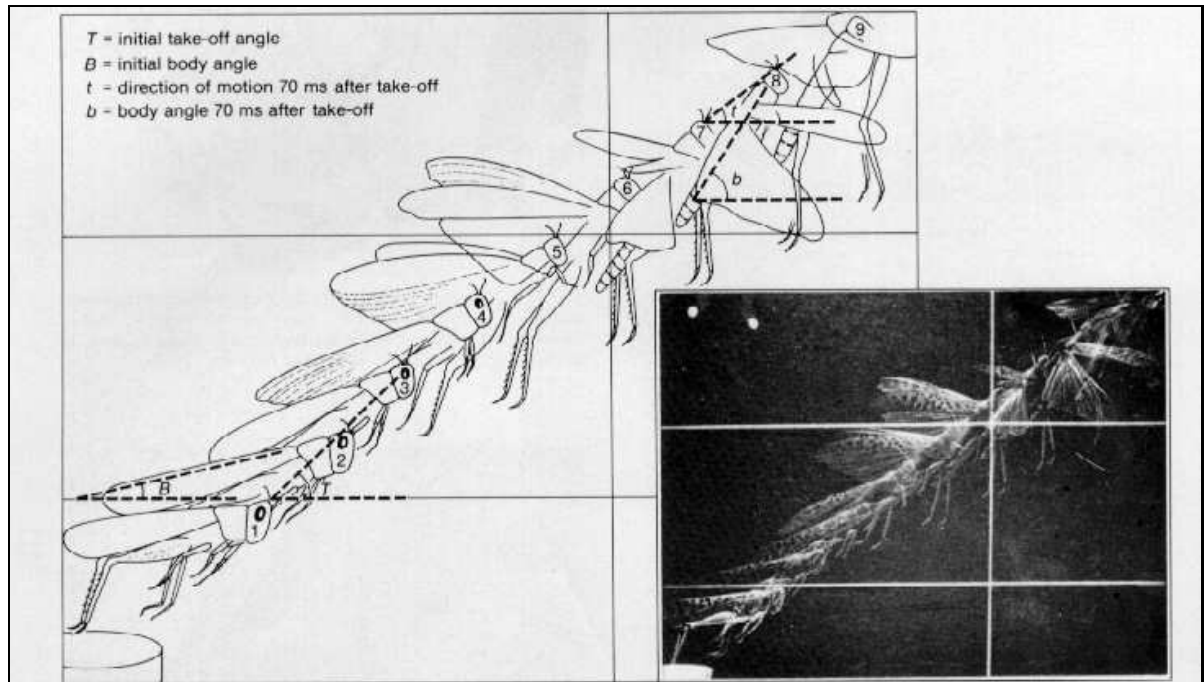
⇒ Is there a relationship between body mass, femur length and jump distance? (calculate the equation for any regression line you plot and the coefficient of correlation and determine its significance)

⇒ What are the effects of a) increasing the mass of the locust, b) removing one leg on jumping performance?

Draw up data tables similar to the sample human data and calculate the mean maximum power output per kg of muscle for the locust.

⇒ How does this compare with the physiological maximum output of striated muscle?

⇒ How does the performance of the instars compare with that of the adults? How does this relate to their behaviour in the wild?



|-----120 mm----->>|

Figure 3 Time sequence photographs of a locust during jumping for flight initiation.

Figure 3 shows strobe-synchronised photographs of a locust jumping from a platform to initiate flight. The photos were taken every 10 ms and the length of the large square is 120 mm.

⇒ Measure the angle of trajectory at take-off and compare this with the assumption we used for the calculations.

⇒ Calculate the velocity of the locust just after take-off and compare this with your calculated value from the jumping.

2. THE HUMAN JUMP

Human Jumping Performance Experiments

You are asked to investigate the effects of a) leg angle and b) adding weight, to human muscle performance using a standing jump.

You are expected to design your own experimental protocols as a group.

Before commencing please check your plans with the demonstrator.

Performance measurement:

The 'performance' will be a standing jump. The height of the jump will be assessed by the height you can place a 'post-it' note on the wall (Location of this activity will be defined by the Demonstrator).

You will need to decide what measurements (and how) you are going to make. For this you will get useful information from the information given in the locust jump experiment above.

Apparatus:

You will be provided with the following equipment:

- Weighing scales
- Metre rule
- Flexible tape measure
- Protractor
- A sub-aqua weight belt with weights

Recording:

Record your data in an appropriate way; please use the laptop provided to enter your data on an Excel spreadsheet.

Safety:

This is a very simple physical exercise but you should perform the exercise with care.

- *Identify one group member who will act as 'spotter' for each jumper (to ensure safety of the jumper in each jump). No one should be moving in proximity to the jumper during a jump.*
- *Only perform the activity in the place designated by the Demonstrator.*
- *You do not have to participate as an experimental subject if you do not wish.*
- *Remember that different people can be more or less comfortable with interpersonal physical contact and perform the experiments with due sensitivity.*
- *No student to jump with more than 32lbs of added weight.*
- *Weights must be properly and securely 'threaded' onto the belt. There are plastic 'keepers' that should be positioned between the weights and the ends of the belt so that if belt becomes undone the weights remain on the belt.*

- *The belt must be tightened sufficiently and securely to the subject so that there is no movement of the belt independently of the subject.*
- *When weight is being added to the belt it must be done so on a bench (to avoid dropping on toes etc.).*
- *The weighted belt must be carried to the subject in a two-handed manner by one student and the belt secured onto the subject by the subject – this will avoid the belt being dropped during application.*
- *The subject will then ascertain and report on whether the belt is tight enough.*
- *Removal of the belt will follow the above procedure in reverse.*

Note: If you have any concerns at all about the conduct of this experiment please discuss it with the Demonstrator.

Please note: Unlike the locust jump experiments we do not want you to compare one- and two-legged jumping performance by surgically or otherwise removing a leg of any participant.

The Report:

The report should be structured as a research paper with Introduction, Methods, Results, Discussion and Bibliography. The report must be word-processed and all graphs etc. drawn using graphical packages. Make sure that you address the questions posed in the schedule.

The Introduction should give a background to the experiments and detail their aims. Methods can be short - only report aspects of the protocols that were not in the schedule or that were changed. Results - the results must be described in words and the main findings highlighted as well as being presented in suitable graphical or tabular form. The data must be presented in statistically valid form and appropriate tests carried out to support your conclusions. Discussion - the findings should be discussed and placed in the context of the research literature. Bibliography - full reference must be made to information derived from other studies and this must be cited in the text.

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