1. Neuroscience using the nematode worm *Caenorhabditis elegans*

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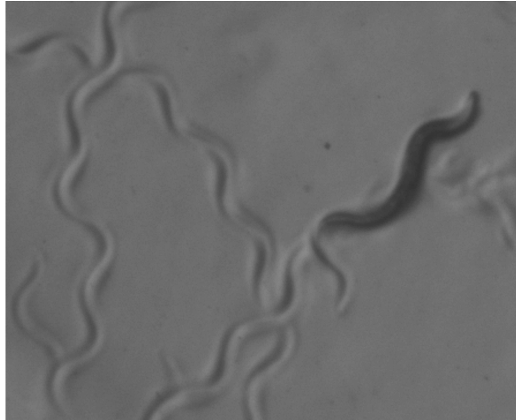


1.1 General Background for the experimental model:

Caenorhabditis elegans (*C. elegans*) is a nematode worm whose natural habitat is the soil and rotting vegetation. It was adopted by cell biologists in the 1960s under the leadership of Sydney Brenner. Sydney Brenner had the superb vision to realise that what biological research really needed was a simple animal model that might provide answers to fundamental questions about how an animal develops. He was particularly interested in '*finding a simple experimental system which might tell me how brains were constructed*' (Brenner; Nobel prize autobiography (http://www.nobelprize.org/nobel_prizes/medicine/laureates/2002/brenner-autobio.html).

C. elegans has just 302 brain cells (neurons) and in 1976 the 'wiring diagram' of this simple nervous system was published following painstaking electron microscopy analysis by John White and his colleagues. Now all this information is managed through an on-line Wormatlas (http://www.wormatlas.org/). Combined with this neuroanatomical map there is very detailed information on the animal's genome (www.wormbase.org) and an unsurpassed resource of mutants and molecular genetic tools (see www.wormbook.org to learn all about the worm!)
This makes *C. elegans* a very attractive model system for neuroscientists who are interested in understanding the relationship between brain function and behaviour. Despite the fact that this worm's

brain is anatomically simple, it stores information and signals in a very similar manner to human brain. The worm will seek out food, avoid food which is toxic, respond to drugs such as alcohol, and adapt its behaviour over time in response to environmental challenges. It also provides a great model for looking at the biology of ageing as it only lives for 2 to 3 weeks and its genetic tractability enables a precise investigation of genes which are involved in either shortening or extending lifespan. Our longest living worm survived for 102 days, the equivalent of a 400 year old human!

1.2 Suggestions for Research Questions:

You can get some ideas by looking at one or two worm papers before you decide on your project. These are available online from the Public Library of Science (PLoS: www.plos.org):

A Modular Library of Small Molecule Signals Regulates Social Behaviors in *Caenorhabditis elegans* Srinivasan J, von Reuss SH, Bose N, Zaslaver A, Mahanti P, et al. (2012) A Modular Library of Small Molecule Signals Regulates Social Behaviors in *Caenorhabditis elegans*. PLoS Biol 10(1): e1001237. doi:10.1371/journal.pbio.1001237

Genetics of Aging in *Caenorhabditis elegans* Antebi A (2007) Genetics of Aging in *Caenorhabditis elegans*. PLoS Genet 3(9): e129. doi:10.1371/journal.pgen.0030129

A Role for Autophagy in the Extension of Lifespan by Dietary Restriction in *C. elegans* Hansen M, Chandra A, Mitic LL, Onken B, Driscoll M, et al. (2008) A Role for Autophagy in the Extension of Lifespan by Dietary Restriction in *C. elegans*. PLoS Genet 4(2): e24. doi:10.1371/journal.pgen.0040024

***Caenorhabditis elegans* Battling Starvation Stress: Low Levels of Ethanol Prolong Lifespan in L1 Larvae** Castro PV, Khare S, Young BD, Clarke SG (2012) *Caenorhabditis elegans* Battling Starvation Stress: Low Levels of Ethanol Prolong Lifespan in L1 Larvae. PLoS ONE 7(1): e29984. doi:10.1371/journal.pone.0029984

Cocaine Modulates Locomotion Behavior in *C. elegans* Ward A, Walker VJ, Feng Z, Xu XZS (2009) Cocaine Modulates Locomotion Behavior in *C. elegans*. PLoS ONE 4(6): e5946. doi:10.1371/journal.pone.0005946

Having read these you may think of some questions that you could investigate using *C. elegans*. For example, not much is known about the effect of ethanol on *C. elegans* development, particularly if exposure of the parent affects development of the offspring. There are also some very simple assays that can be designed to look at food choice and navigational behaviour. To get you started here is an experiment you can try:

1.3 Testing the effect of alcohol on sensorimotor coordination in *C. elegans*

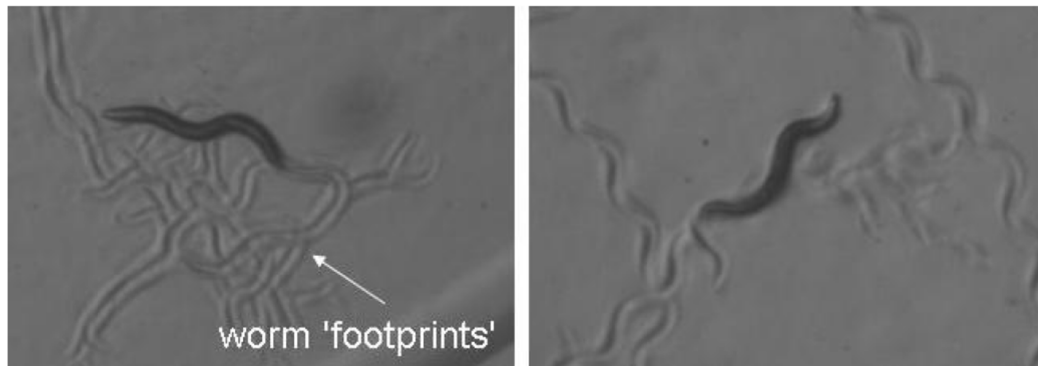
Objective: To quantify the concentration-dependent effects of ethanol on the navigational performance of *C. elegans (*or '*Can a drunk worm find food?*')

Time required: Preparation, approximately 1 hour; running the experiment approximately 2 hours.

The research question: Alcohol is a drug with a profound effect on the brain. The effects are concentration-dependent and increase in severity with increased amount of alcohol consumed. After one or two drinks the blood alcohol concentration (BAC) increases to about 0.5mg/ml (0.1% v/v or 20mM). At this concentration mild intoxication is induced (0.8mg/ml is the UK drink drive limit), the person becomes more relaxed and talkative. After more drinking the effect of alcohol on behaviour becomes more severe graduating from impaired judgement, slowed reaction time and motor function to marked ataxia (staggering and slurring of speech) and blackouts (periods of time that cannot be recalled). At BAC above 3.0mg/ml (0.38% v/v or 65mM) sedation is induced and the person will be approaching a coma. A BAC of 4.0mg/ml is the lethal dose for 50% of people.

How does alcohol bring about these diverse effects on human behaviour? The answer to this question is still far from complete. We know that alcohol acts on specific neurotransmitter signalling pathways in the brain. However, unlike other drugs which typically interact with just one or two signalling pathways, alcohol interacts with many neurotransmitter systems. Understanding the role of each of these in the overall effect of alcohol on behaviour is thus a complex question.

The nematode *C. elegans* employs the same neurotransmitter signalling pathways as the human brain. It therefore provides a simple and genetically tractable experimental model in which to start to unravel the effect of alcohol on distinct pathways and determine how this alters an animal's behaviour.



The effect of alcohol on *C. elegans*. The left hand picture shows a worm that has been exposed to alcohol whilst the right-hand picture shows a 'sober' worm.

How can you measure alcohol intoxication in a worm? *C. elegans* eat bacteria and are accomplished at finding this in their environment. An adult worm placed on an agar plate diametrically opposite a spot of bacteria will quickly detect the food and move towards it. This requires precise and coordinated signalling in the worm's brain. Chemosensory neurones (the worm's 'nose') detect the food. This information is signalled to the central nervous system where it is interpreted in the context of the current state of the worm i.e. how hungry is it? The central nervous system then signals to the motor nervous system which controls the movement of the worm and directs a pattern of activation of the musculature that enables the worm to move towards the food.

Experimental protocol: You will conduct 'food race' assays in which the ability of worms to navigate towards a point source of food, in presence and absence of ethanol (50mM, 150mM, 250mM and 500mM) will be measured.

Equipment and resources:

Access to a binocular microscope with approximately x10 mag

One plate of ~ 50 one day old adult *C. elegans*

One 9cm 'food race' agar plate

One eppendorf tube

5ml M9 buffer

BSA

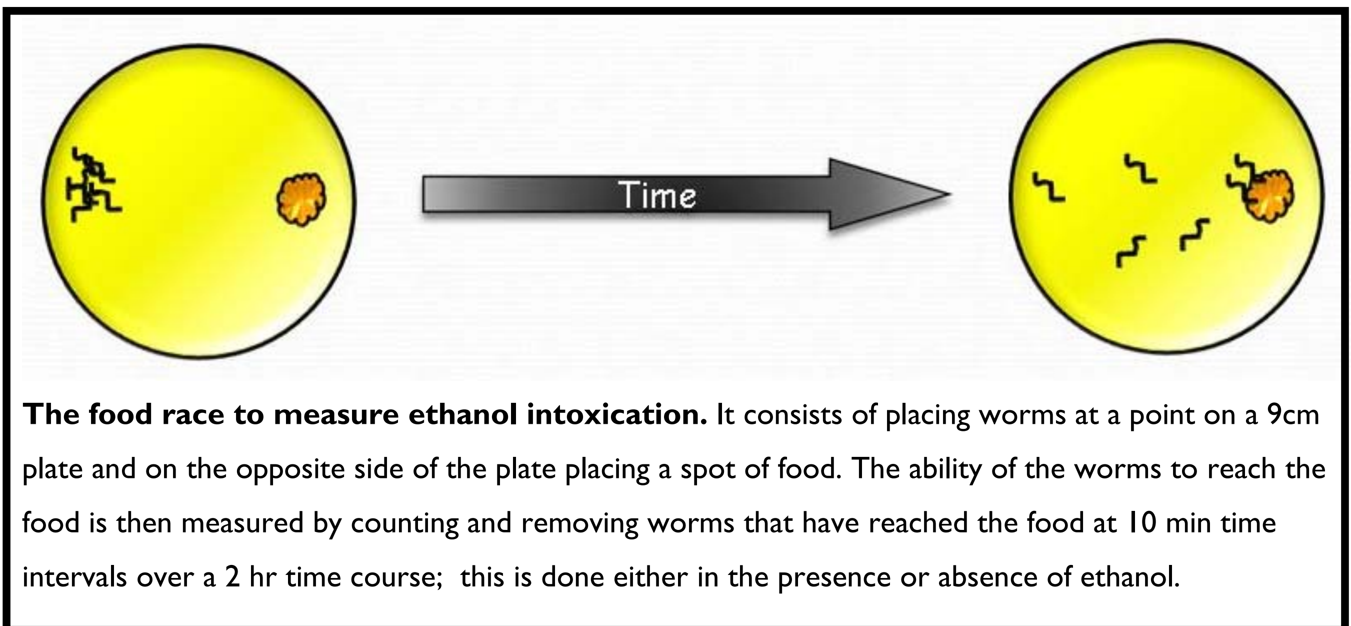
One glass Pasteur pipette and bulb or a 1ml Gilsen pipette with tips

One small beaker full of ice

A small piece of fine tissue paper

One worm pick

Method:



The food race to measure ethanol intoxication. It consists of placing worms at a point on a 9cm plate and on the opposite side of the plate placing a spot of food. The ability of the worms to reach the food is then measured by counting and removing worms that have reached the food at 10 min time intervals over a 2 hr time course; this is done either in the presence or absence of ethanol.

1. Unseal the agar plate of young adult worms.
2. Carefully pipette about 1ml of M9 buffer onto the plate and swirl the liquid around the plate to collect up the worms.
3. Tilt the plate slightly on its side so that the liquid collects at the edge and aspirate the worms and liquid and decant into the eppendorf tube.
4. Close the eppendorf tube and rest it gently in the ice beaker. Leave for about 2 to 5 minutes. (This allows the worms to settle to the bottom).
5. Very carefully remove the top layer of liquid from the eppendorf tube, taking care not to disturb the worms at the bottom. Then add another 1ml of M9 to the tube. Allow to settle for another 2 minutes and then remove the top layer of liquid again. This procedure is designed to wash the bacteria off the worms before they are tested in the food race and may be repeated a couple of times.

6. Open the food race plate. Suck the worms up into the pipette and place on the agar at the point marked X. If there is a lot of liquid with the worms still try to gently soak it up with a fine piece of tissue paper.
7. Put the lid back on the food race plate. Make a note of the time. This is the 'start' of the food race.
8. For the rest of the experimental period look at the food race plate every 10 to 15 min. At each time point, count how many worms have got to the food spot. (If you have a very steady hand you might like to try to remove the worms that have already got to the food with the worm pick.) At the end of the experiment count how many worms are left on the plate i.e. haven't made it to the food spot.
9. Make a table of your results like this:

Time from start of food race (min)	Number of worms that reached the food	Cumulative number of worms that reached the food
0	0	0
etc...		

and draw a graph to represent your data. Work out the % of the total number of worms that had reached the food after 90min (Depending on the time available this may be altered on the day).

1.4 Suggestions for further study:

This assay could be modified to study the effects of other parameters on sensorimotor coordination; other drugs, drug withdrawal, age, food deprivation (e.g. do starved worms find food faster?). You could modify it to look at food preference (i.e. use different strains of bacteria or live versus dead bacteria). You could also test different genetic mutants to investigate which neurotransmitter pathways are required for the behaviour. All of these assays would provide insight into how a simple nervous system can respond to changes in the environment to regulate animal behaviour.

1.5 Experimental Protocols

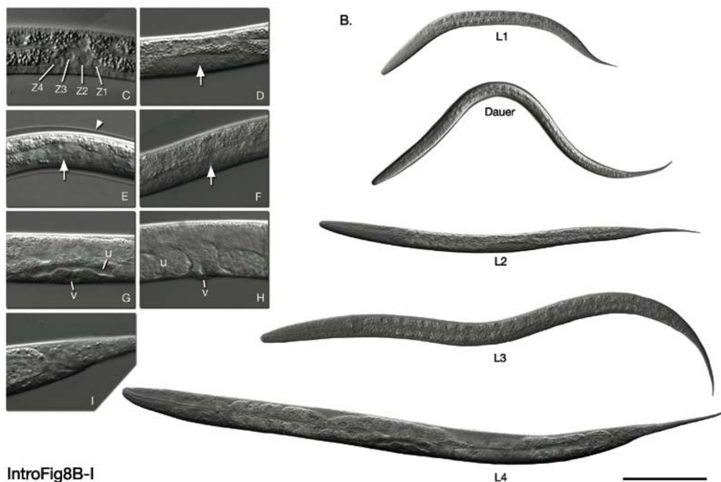
There are a few techniques you will need to acquire if you are going to work with *C. elegans* as an experimental model. You will need to learn how to i) culture the worms from egg to adult ii) recognise the different developmental stages iii) score their behaviour e.g. motility iv) for some assays you will need to be able to pick individual worms.

1.5.1 Culturing worms

Wild type *C. elegans* (N2) can be grown on NGM plates seeded with *E. coli* OP50. This is done by 'chunking' a piece of agar from a plate with worms to a new plate seeded with bacteria. Use a scalpel (dipped in ethanol and flamed to sterilise first) to cut a small chunk from an old culture of worms and transfer to a new plate seeded with OP50. Seal the plate with parafilm.

1.5.2 Recognising different developmental stages:

Worms progress from egg to adult in 3 days and have 4 larval stages prior to adulthood. There is also a developmentally arrested dauer stage which the worms enter if food is scarce or they are overcrowded. see http://openwetware.org/wiki/BISC_219/F10:_Worm_Info



1.5.3 Scoring worm behaviour (phenotyping worms). One of the attractions of studying *C. elegans* is that they exhibit a repertoire of behaviours which can be easily quantified. In combination with the power of molecular genetics this have provided an extremely successful model for defining the genetic basis of behaviour and has led to the discovery of key mechanisms that control signalling in the brain. The following is just a brief example of some of the behaviours you could study. However, you should bear in mind that just like human, worm behaviour is age-dependent! Try to synchronise the age of the worms that you are working on if you want to make a careful comparison between the effects of different treatments.

1.5.4 Motility

On solid medium the worms move in a sinusoidal fashion making body bends. You can count the frequency of these. The worm moves at different speeds on and off food, and when it is avoiding a noxious stimulus. Worms in liquid move rhythmically in a motion called 'thrashing' in which they flex around a midpoint. This is quite fast but very consistent and provides a good assay for looking at drug effects. Worms also make reversals and deep body bends and turns and all of these can be quantified to define the pattern of behaviour.

1.6 Resources required

Chemicals:

NGM (Nematode Growth Medium)

M9 Buffer

BSA (bovine serum albumin)

Absolute alcohol

Plastics:

3, 5 and 9 cm petri dishes

Autoclave bags

Equipment:

Counter

Worm pick

Binocular microscope

Gilsen pipette (20μL, 100μL and 1000μL)

Gilsen tips

Other:

C. elegans cultures

Mutants available on request

1.7 Risk assessments

Risk assessments have been completed and submitted to the college for all the experimental procedures.

There are no exceptional hazards associated with the procedures.

Additional risk assessments may be required if specific drugs or chemicals are used.

The projects may use mutants that have been generated by chemical mutagenesis as these do not require GMO (Genetically Modified Organism) approval.