Guided Projects CSSI 2011
Examination of mutants with defective male-specific neurons

BACKGROUND

Sexual Dimorphism in *C. elegans*

C. elegans nematodes exist in nature as two sexes, the self-fertile XX hermaphrodite and the XO male. Nearly every tissue in *C. elegans* exhibits sex-specific characteristics, or sexual dimorphism (Figure 1). Some sexually dimorphic characteristics are immediately obvious by just looking at *C. elegans* in the dissecting or compound scope. For example, the adult hermaphrodite is much bigger than the adult male, lays "eggs" (fertilized embryos, actually) through a central vulva, and has a whip-shaped tail. The adult male lacks a vulva, and his tail is a spade-shaped structure that is highly specialized for mating, containing neuronal projections called V rays and T rays (Figure 2). Other sexually dimorphic traits are only obvious when we examine gene expression in specific cell types. One way to do this is to compare expression of green fluorescent protein reporter transgenes (see below) in males and hermaphrodites. Not surprisingly, with all of its sexually dimorphic tissues, *C. elegans* behavior also differs between the sexes. As you watch your worms crawl elegantly around their plates, try to catch a couple in the act of mating, and notice the differences in male and hermaphrodite behavior.

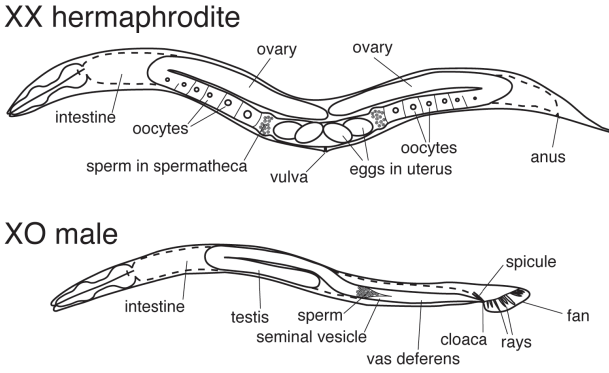

Figure 1. Sexual dimorphism in *C. elegans*

Sexual dimorphism in the nervous system

Sexually dimorphic neurons, which are distinct in males and females (or hermaphrodites), control unique behaviors and are essential to reproductive success in most animals, including humans. Learning how sex-determining genes work with regulators of cell identity, division, and patterning to generate unique neurons in each sex is critical for understanding normal development, and may also provide clues about developmental disorders with higher incidence in one sex. As a result, the relatively simple neuronal development of *C. elegans* has become an essential model for understanding sex-specific neuron specialization. Because many *C. elegans* genes are conserved in other organisms, there is a possibility that what we learn in *C. elegans* might tell us something about vertebrates, including ourselves.

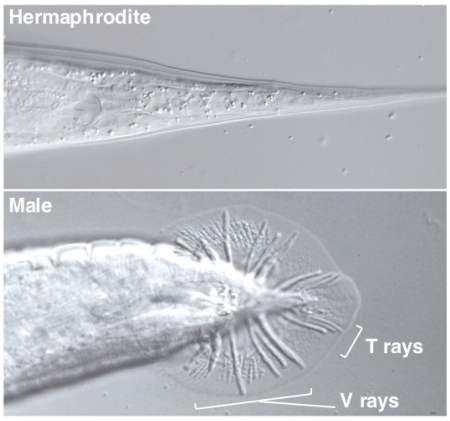

Figure 2 The male tail is sexually

The nervous systems of *C. elegans* hermaphrodites and males have much in common: they share a core nervous system of 294 neurons. However, males and hermaphrodites play very different roles in reproduction, and their nervous systems are sexually specialized to control their unique reproductive behaviors (Figure 3).

The hermaphrodite-specific nervous system controls egg-laying, and consists of eight neurons, the VC motor neurons and the HSNs (hermaphrodite-specific neurons). The male nervous system is more complex; 89 male-specific neurons are used to execute intricate mating behaviors. Four sensory neurons in the head, the CEMS, detect hermaphrodites. 19 male-specific CA and CP neurons are located in the ventral nerve cord. Most CAs and CPs are motor neurons that help control the body movements of mating. The remaining male-specific neurons are concentrated in the elaborate tail. The most prominent tail structures are the V rays and T rays. Each ray is composed of two sensory neurons and a structural cell, and is unique in its morphology, position on the body axis, and the orientation of its sensory opening. Ray development has provided a model for studying the coordinated regulation of cell fate specification, developmental patterning, and nervous system development.

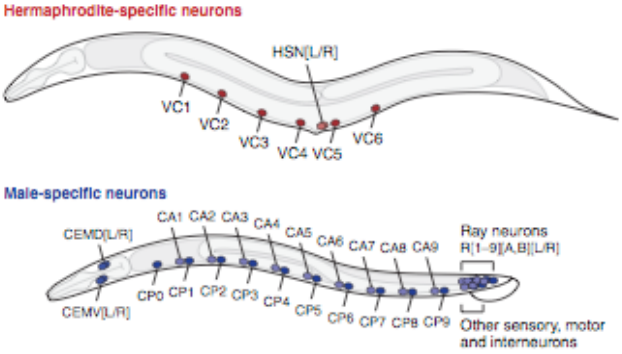

Figure 3. Sexual dimorphism in the *C. elegans* nervous system. The adult hermaphrodite (above) has eight sex-specific neurons :six VC neurons in the ventral cord and two HSN motor neurons. The adult male (below) has 89 sex-specific neurons. Portman (2007

Mutants missing male-specific serotonin neurons

tph-1::gfp is a reporter transgene that is expressed in all serotonergic neurons in *C. elegans* (Fig. 4). (Serotonergic neurons are neurons that use serotonin as their neurotransmitter.) The sex-specific serotonergic neurons include two hermaphrodite-specific neurons (HSNs), which control egg laying and six male CP neurons, which are located in the ventral nerve cord and control male ventral curling during mating. Both males and hermaphrodites also have serotonergic neurons in the head.

My students, postdoctoral associate, and I have isolated mutant worm strains that have reduced expression of *tph-1::gfp* in the male-specific CP neurons in the ventral nerve cord. Our lab is interested in using these mutants to identify genes involved in the development of male-specific neurons. We are most interested in further studying mutant strains with defects in only these male-specific neurons, and not in all neurons in the nervous system. We are thus interested in examining neuronal function in these worm strains to determine which ones are most interesting for further study.

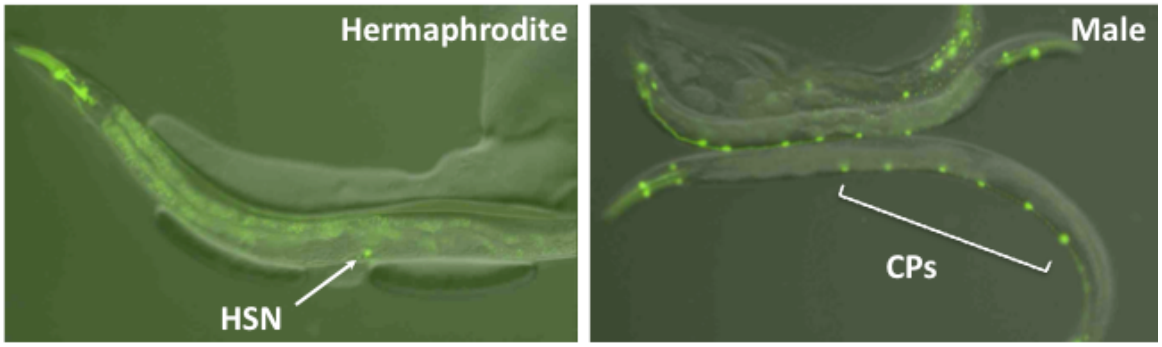

Figure 4. Expression of *tph-1::gfp* is sexually dimorphic. Photo courtesy of team KDJ, CSSI 2011

GUIDED RESEARCH PROJECT GOALS

1) Quantify the extent of loss of *tph-1::gfp* in males and hermaphrodites in these worm strains
2) Use techniques and tools that you have learned this week to assess neuronal function in these worm strains
3) Create an excellent poster summarizing your findings

TOOLS AVAILABLE TO YOU

- Worms
  - A wild-type strain containing the *tph-1::gfp* reporter transgene
  - A mutant strain containing the *tph-1::gfp* reporter transgene.
    *All strains contain a *him* (or *high incidence of males*) mutation that causes males to arise at a high frequency.
- DiI
- Chemotaxis plates and supplies (each group may have 15 plates/per experiment; see appendix for more info on chemotaxis reagents)
- Any worm handling tools that you've used so far (picks, plates, slides, etc.)
- Dissecting microscopes
- Compound fluorescence microscopes
- Other tools may be available upon request—ask, and if it's reasonable, we'll see what we can do.

LOOKING FOR BACKGROUND INFORMATION?

- Wormbook.org is a great source of information about *C. elegans* biology, including review articles on the nervous system and research methods
- Wormbase.org is a great source of information about specific *C. elegans* genes
- Pubmed (http://www.ncbi.nlm.nih.gov/sites/entrez?db=pubmed) is a great source of biomedical science related journal articles

- The Carleton Gould library biology research guide page (http://gouldguides.carleton.edu/content.php?pid=69510&sid=514322) has links to useful biology-related literature databases

TIMELINE

Friday 7/22
Getting to know your mutant strain, including initial examination of GFP expression. By the end of today, you should be prepared to present your experimental plans to your classmates and instructors. We will give you feedback so you will be ready to start your experiments on Monday. A draft of your experimental plan (protocol) is due by the end of lab on Friday. Please keep in mind that previous lab handouts are good sources of background information and information about experimental protocols.

Monday, 7/25-Tuesday 7/26
Your final experimental protocol is due at the beginning of class. I have set aside Monday to set up a chemotaxis experiment, and Tuesday to collect and interpret your data. Depending on your plans, you might also do additional analyses on these days.

Wednesday 7/27
Minnesota Zoo trip

Thursday 7/28-Friday 7/29
Further experiments, which could include repeating chemotaxis, DiI staining, and GFP analysis

Monday 8/1-Wednesday 8/3
Poster making; Final poster DUE Wednesday 4 PM
Observations of zebrafish development (we hope)

Thursday 8/4
Lab Olympics
More zebrafish observations

Friday 8/5
Poster presentations at CSSI Research Symposium

Research planning form

Group Name_____

Mutant Strain _____

1. For your first experiment, I have suggested that you do a chemotaxis assay. What odorants, dilutions, and strains will you use? Fill in as many lines as you need. Be sure to include controls. You may have up to 15 chemotaxis plates per experiment.

Odorant	Dilution	Strain	Number of Plates

2. What strains have you chosen to use in your chemotaxis experiment? Why have you chosen these strains?

3. What odorant(s) have you chosen to use in your chemotaxis experiment? Why have you chosen these odorants? Include any background information about odorants (such as chemical structure) that you think would be helpful.

4. What additional research questions would you like to answer about your mutant strain?

5. What techniques and equipment will you use to address these questions?

6. On a separate sheet, write or type the steps of your experimental methods. We encourage you to use diagrams when appropriate. Please include a timeline for how you plan to use your time on Monday, Tuesday, Thursday, and Friday afternoons.

Appendix: Chemotaxis of WT *C. elegans* to volatile compounds

From Bargmann et al. Odorant-selective genes and neurons mediate olfaction in C. elegans. Cell (1993) vol. 74 (3) pp. 515-27

Table 1. Volatile Odorants Tested for Chemotaxis

Attractants	
Alcohols	1-Butanol, 1-pentanol, 1-hexanol, 2-pentanol, 2-hexanol, 3-hexanol, 4-heptanol, isobutanol, isoamyl alcohol, sec-butanol, β-mercaptoethanol, 4-penten-1-ol
Ketones	Acetone, 2-butanone, 2-pentanone, 2-hexanone, 2-heptanone, 2-octanone, 3-pentanone, 3-octanone, diacetyl (2,3-butanedione)
Esters	Ethyl acetate, propyl acetate, n-butyl acetate, n-pentyl acetate, isoamyl acetate, ethyl propionate, ethyl butyrate, ethyl isobutyrate
Pyrazines	Pyrazine, 2-methyl pyrazine, 2,3-dimethyl pyrazine, 2,5-dimethyl pyrazine, 2,6-dimethyl pyrazine, 2-methoxypyrazine
Thiazoles	2-Ethoxythiazole, 2-isobutylthiazole, 2,4-dimethylthiazole, 2,4,5-trimethylthiazole
Aromatic compounds	Benzaldehyde, p-tolualdehyde, furfural, nitrobenzene, benzonitrile, methyl salicylate, aniline
Aldehydes	Valeraldehyde, capronaldehyde, heptaldehyde
Diethyl ether	
Weak attractants (attractive undiluted only)	
Citronellol, 2-phenylethanol, geraniol, methyl isobutyrate, benzene, cyclohexane, benzyl alcohol, benzylamine, pentylamine, heptylamine, 1-propanol	
Neutral or repellent compounds	
Alcohols	Ethanol, 2-heptanol, 3-heptanol, 2-octanol, 3-octanol, 1-nonanol, isopropanol, linalool, 1-heptanol,* 1-octanol,* 1-nonanol*
Ketones	2-Nonanone,* 3-nonanone,* 5-nonanone*
Esters	Ethyl valerate, ethyl hexanoate, propyl propionate, propyl butyrate, pentyl valerate, hexyl acetate, ethyl heptanoate,* butyl butyrate*
Pyrazines	2-Methoxyl-3-methylpyrazine, 2-methoxy-3-ethylpyrazine, 2-methoxy-3-isopropylpyrazine, 2-methoxy-3-isobutylpyrazine, 2-ethylpyrazine, 2-ethyl-3-methylpyrazine, 2-ethoxy-3-ethylpyrazine, 2-acetylpyrazine
Thiazoles	Thiazole, 4-methylthiazole, 2-acetylthiazole, 2,4,5-trimethylthiazole*
Aromatic compounds	Xylenes, chlorobenzene, toluene, benzyl benzoate, m,o-anisidine, m,p-anisaldehyde, trans-anethole, eugenol, 2-methylquinoxyline, 5-methylquinoxaline, 6-methylquinoxaline, vanillin, benzaldehyde*
Aldehydes	Butyraldehyde, caproylaldehyde, isobutyraldehyde
Amines	Diethylamine, triethylamine, butylamine, hexylamine, octylamine
Other	Limonene, +/−camphor, butyric acid, isovaleric acid, a-ionone +/−pinene, menthol

Repellents are listed with an asterisk. Some molecules (e.g., benzaldehyde) are attractive at low concentrations and repellent at high concentrations. In general, a 10^{-2} dilution of odorant was tested; in some cases, when no response was observed, the odorant was also tested undiluted.

Available Odorants

Acetone
Benzaldedhyde
R-(-)-carvone (spearmint)
S-(+)-carvone (caraway, rye bread)
Citral (citrus, lemon-lime)
Cineole (eucalyptus oil)
Trans-cinnamaldehyde
Cinnamon Oil
Citronellal (citronella)
Diacetyl (buttered popcorn)
Eugenol (clove oil)
Geraniol (rose)
Isoamyl acetate (banana oil)
Linalool (spicy flower)
Methyl Salicylate (wintergreen oil)
Nerol
Octanol
Pyrazine (red wine-ish)
Turpineol (pine, pine-sol)
Vanillin
Triethylamine
Galaxolide (musky)

Maddy Jack M Allison	Kira Ducky Steven
Kelly Dakota Shraddha	Charles Jack W Rey

FRONT OF LAB (WHITE BOARD)