Design and Production of Behavioral Assays on *C. elegans*

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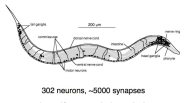
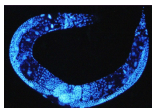
Background and Motivation

Caenorhabditis elegans (*C. elegans*) is a transparent nematode whose 302-neuron nervous system has been fully mapped. *C. elegans* started being used as a model organism about 50 years ago.


Obtained from scientificamerican.com



Obtained from courses.biology.utah.edu

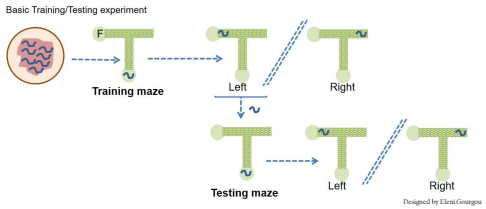

Obtained from macroevolution.net

We use *C. elegans* in our project in T-shaped mazes to test decision making and spatial learning. The long-term goal of this project is to discover the specifics of if and how aging affects spatial learning and consequent decisions.


Courtesy of Eleni Gourgou


Courtesy of Eleni Gourgou

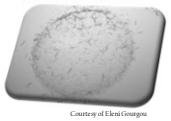
To determining if worms are capable of spatial learning, a test as depicted below was ran. Food was placed in the left end of the Training Maze, the worms who found it were placed in the Testing Maze immediately after.



The high homology between the genes of *C. elegans* and humans allow our results to reveal a substantial amount of information about aging and learning in higher organisms.

Methods

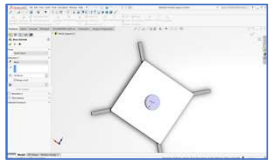
60mm diameter petri dishes to test and culture worms


Courtesy of Eleni Gourgou

Olympus SZ61 microscope


Obtained from Nomarksi.com

I use SolidWorks, a Dassault Systemes Product, to design the maze molds and we print them in a Form Labs 1+ desktop 3D printer




Obtained from 3Dprint.com

Abstract

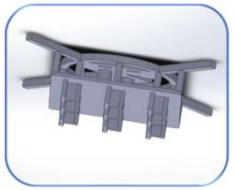
We use a hydrogel called agar to culture and test out worms. It is transparent and easily manipulated. Currently we are using a T-shaped maze with a left-right decision, changes in elevation of the worm while in the maze are disregarded. I am tasked with improving the design to increase efficiency. I increased the number of mazes per dish from five to nine and eliminated post solidification prep time (trimming). Apart from the T-mazes, I designed several other types of mazes, including radial and sequential, along with a method for a removable food source. After printing all of my parts I inspect the molds themselves and the mazes they create under the microscope for defects.

The other part of my project is to develop a method to prepare 3-D mazes. This is something that has never been done before in relation to our model organism, *C. elegans*. There is a myriad of reasons why this type of maze is worth exploring. The seemingly most obvious explanation is that a 3-D test space more closely resembles the wild-type environment. In nature these worms are not limited to 2-D decisions. Classical molding techniques where agar is inserted into a mold, allowed to solidify and the mold parts are then removed have shown promising results.

MazEnnea


Top View


Side View


Angled Side View


Bottom View

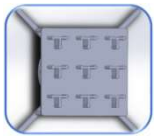
Results

Two–Dimensional Design

Maze + Ennea (Greek for Nine)


The Maze Mold as it was when I entered the Hsu Lab in Fall 2017


The first version with the trim plate

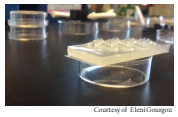

Mazennea - Bottom View

Adding the trim plate, as we call it, removed any need for trimming. Adding mazes, decreased material waste.

Mazelia
Maze + Elios (Greek for Sun)

Meant to test the worms preference to different substances

Mazethea
Maze + Ethea (Greek for Air)


Courtesy of Eleni Gourgou

For use on an inverted microscope

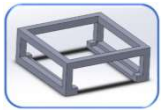
Mazeira
Maze + Seira (Greek for order, line)

Original Sequential Maze


Courtesy of Eleni Gourgou

We needed a way to remove the food from the maze after the worm found it and before it tasted it.

So I designed these parts to allow the food to hang from a glass cover on top of the maze instead of on the floor.


Holds the glad cover in place on the mazes

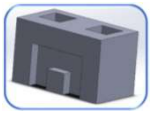

Shows where to put food on the glass cover

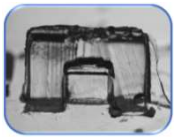
The food on the glass cover is aligned with the left end of the maze.
We plan to design a similar apparatus for a sequential testing platform.

Three-Dimensional Design

The Archway
We print two of each of these parts, hold the mold halves together with the clips and inject liquid agar into the mold from underneath


Clip


Mold


The resulting arena can be seen here from the side

Conclusions

Adding a planar platform on the molds where the surface of the agar meets the mold removed the need for trimming, this technology has been implemented on all the maze designs.

Designing maze molds in this fashion has proven to work very well. The maze designs are as diverse as the questions they can answer. This method is easy to implement in any *C. elegans* lab.

We have formulated a new way to put food in the mazes. The worm is able to find it without being able to eat it.

Agar is rigid enough to support itself, despite being a relatively soft and wet hydrogel. This characteristic will be essential to the creation of 3D arenas.

Acknowledgements
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References
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Corsi A.K., Wightman B., and Chalfie M. (18 June 2015). A Transparent window into biology: A primer on Caenorhabditis elegans. In *WormBook* (Introduction: 8)

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