# *C. elegans* learning and decision making in T-shaped mazes

MECHANICAL ENGINEERING
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## Background & Introduction

*C. elegans* demonstrate non-associative **learning** (habituation) and they are capable of associative learning and imprinting.

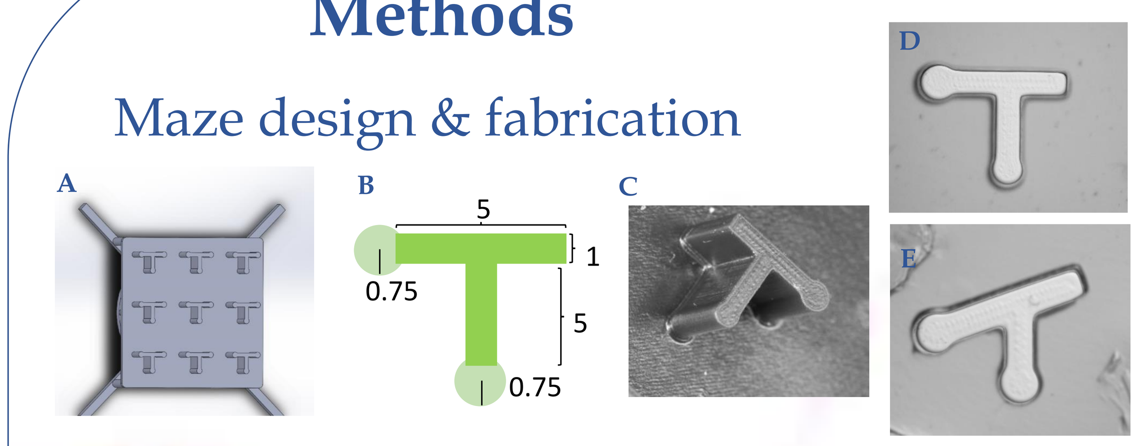
**Mazes** have been broadly used as a tool to investigate the ability of organisms to **learn navigation-related tasks** and to test their **decision making** process.

We introduce a **custom-made T-shaped maze platform**, the **Worm-Maze**, to explore *C. elegans*' **navigation, learning and decision making**. We characterize this new behavior in relation to diverse environmental cues, and we explore the role of chemosensation, mechanosensation and propriosensation.

We show that **aging has a differential effect on maze-related behaviors**, as middle-aged worms can still locate food in the maze, but their learning ability is severely declined.

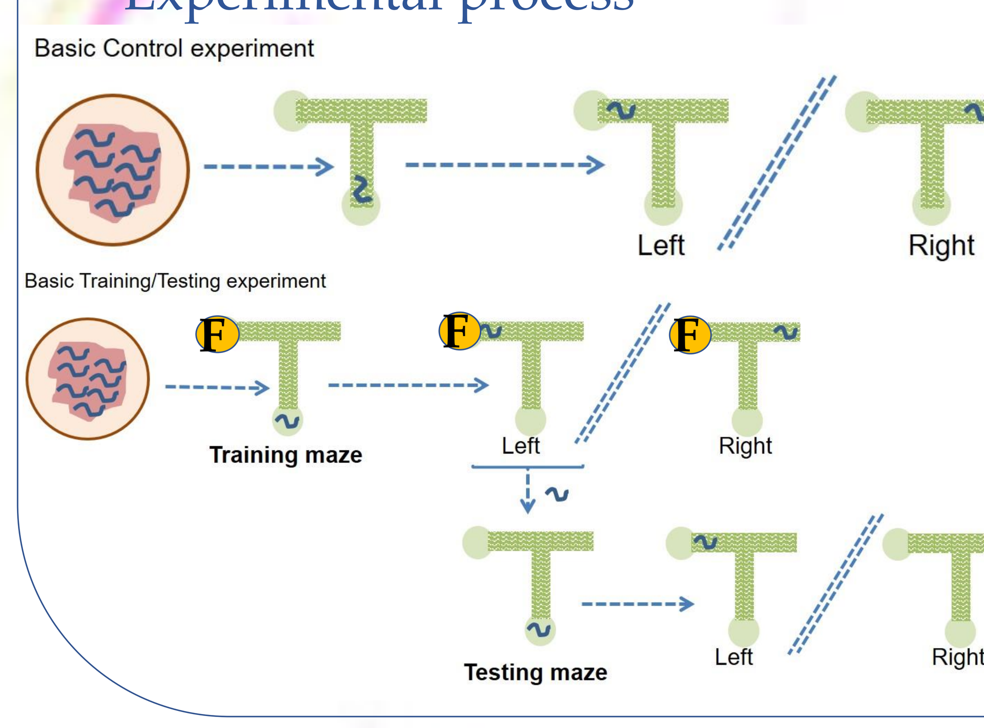
## Methods

### Maze design & fabrication




A. 3D-printed maze mold;
B. Maze dimensions in mm;
C. Individual mold closeup;
D. Simple T-maze (90°/90° );
E. Angled T-maze (60°/120°)

### Experimental process

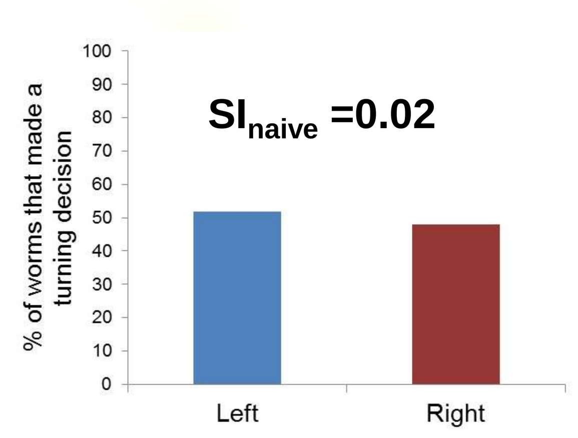



**Control experiment (empty maze):** process followed for N2 and mutant strains, as a reference case.

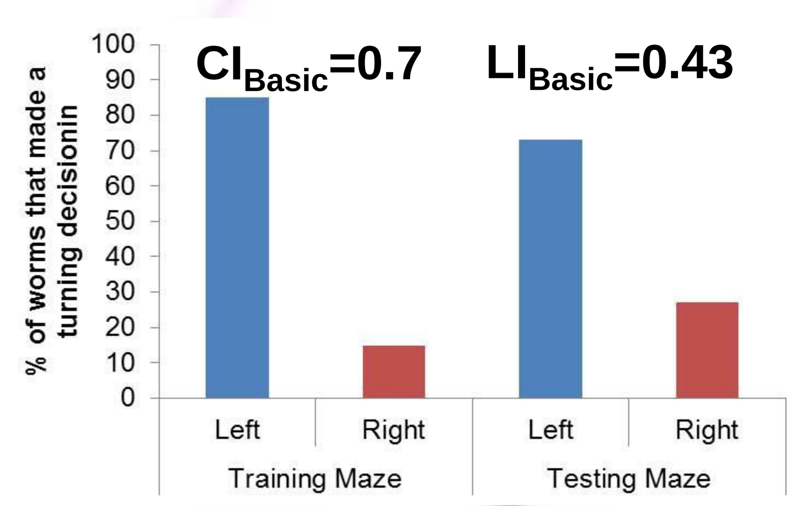
**Training (food)/Testing (no food) experiment:** process followed for N2 and mutant strains with T-shaped mazes, and for N2 with cantered mazes.

## Results

Control Experiment: no inherent preference



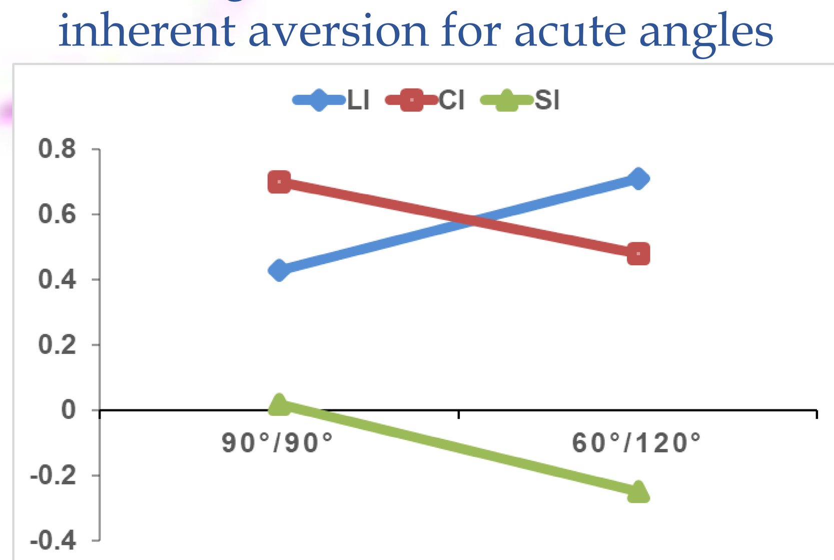

Training/Testing Experiment: worms locate food and learn where to turn




**Solving Index**, **SI= $(n_L-n_R)$/Total worms-$n_{censored}$**
**Chemotaxis Index**, **CI= $SI_{Train}$-$SI_{Naive}$** **(comparing performance in Training maze)**
**Learning Index**, **LI= $SI_{Test}$-$SI_{Naive}$** **(comparing performance in Testing maze)**

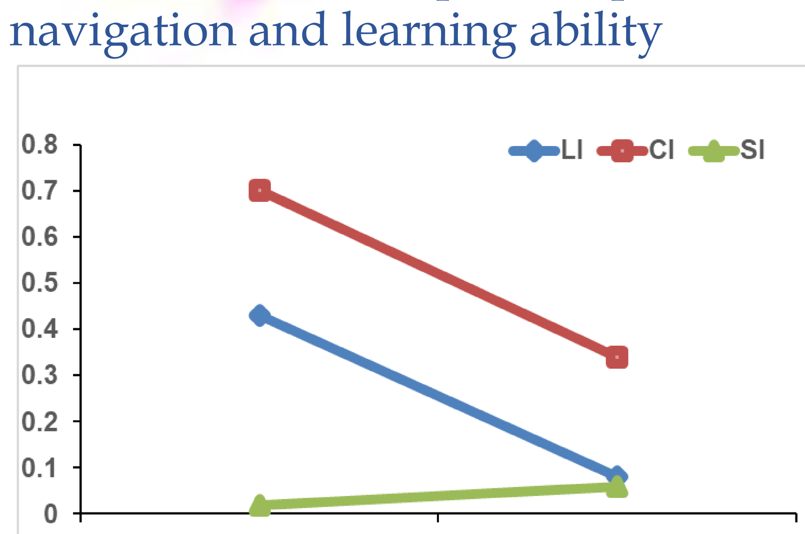
$n_L$: worms that turned Left /towards food, $n_R$: worms that turned Right /not towards food
$n_{censored}$: worms censored (immobile, inconclusive, lost—given as % in Tables below)
$SI_{Naive}$= $(n_L-n_R)$/Total worms-$n_{censored}$ (naïve worms, maze **empty-Control**)
$SI_{Train}$= $(n_L-n_R)$/Total worms-$n_{censored}$ (worms in **Training maze**),
$SI_{Test}$= $(n_L-n_R)$/Total worms-$n_{censored}$ (worms in **Testing maze**)

Learning is sufficient to overcome inherent aversion for acute angles

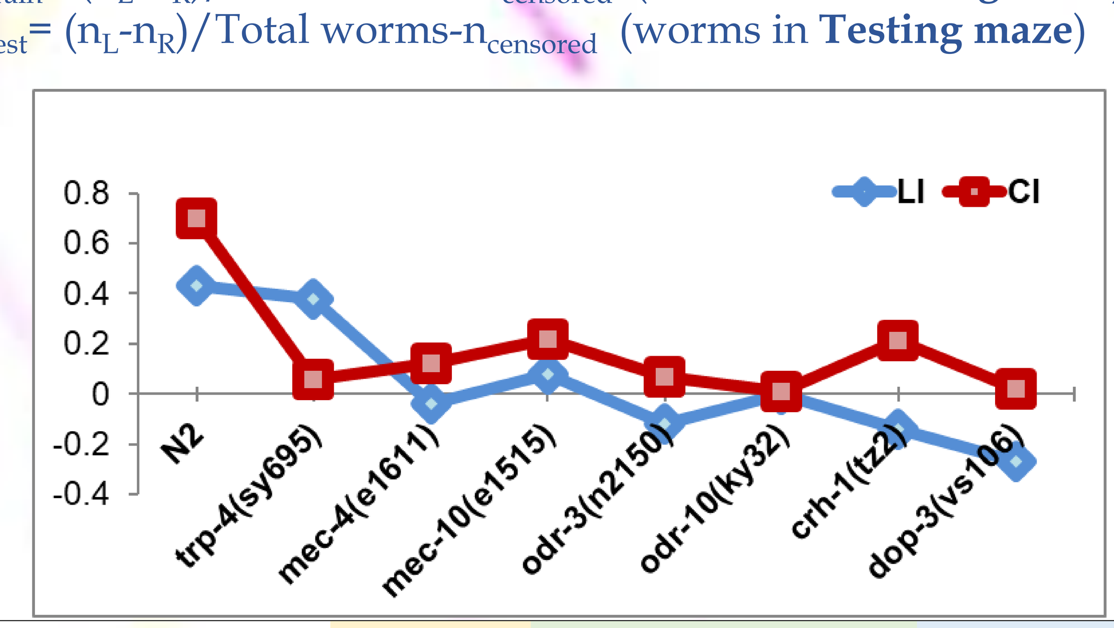



| *p*-values | Control (empty) 60°/120° | Training/Testing,60°/120° |
|---|---|---|
| $SI_{60/120}$ to $SI_{90/90}$ | **0.04,** n=75, 8% | |
| $CI_{60/120}$ to $SI_{90/90}$ | | **<0.001,** n=88, 9% |
| $CI_{60/120}$ to $CI_{90/90}$ | | **<0.001,** n=88, 9% |
| $CI_{60/120}$ to $SI_{60/120}$ | | **<0.001,** n=88, 9% |
| $LI_{60/120}$ to $SI_{90/90}$ | | **0.003, n**=45, 13% |
| $LI_{60/120}$ to $LI_{90/90}$ | | 0.57, n=45, 13% |
| $LI_{60/120}$ to $SI_{60/120}$ | | **<0.001,** n=45, 13% |

Absence of tactile input compromises navigation and learning ability



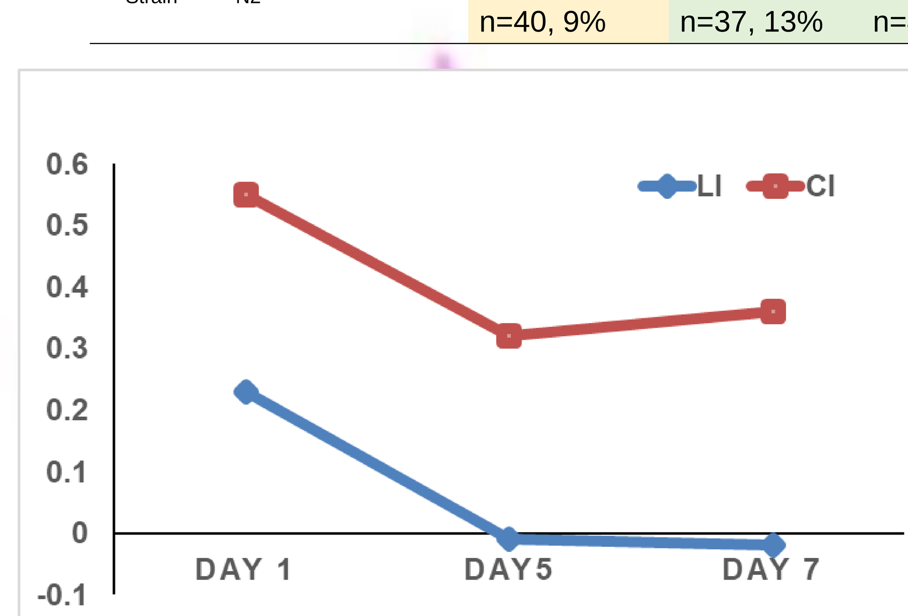

| *p*-values | Smooth maze |
|---|---|
| $SI_{Smooth}$ to $SI_{Basic}$ | 0.77, n=45, 10% |
| $CI_{Smooth}$ to $SI_{Basic}$ | **<0.001,** n=103, 16% |
| $CI_{Smooth}$ to $SI_{Smooth}$ | **0.001,** n=103, 16% |
| $CI_{Smooth}$ to $CI_{Basic}$ | **<0.001,** n=103, 16% |
| $LI_{Smooth}$ to $SI_{Basic}$ | 0.64, n=40, 18% |
| $LI_{Smooth}$ to $SI_{Smooth}$ | 0.65, n=40, 18% |
| $LI_{Smooth}$ to $LI_{Basic}$ | **0.01,** n=40, 18% |




Molecular mechanism:
P,C&M: required for food location
C&M: required for learning
CREB-like factor & dopamine signaling involved

Proprioception
Mechanosensation
Chemosensation
Signaling molecules

| *p*-values | N2 (wild type) | *trp-4* (sy695) | *mec-4* (e1611) | *mec-10* (e1515) | *odr-3* (n2150) | *odr-10* (ky32) | *crh-1* (tz2) | *dop-3* (vs106) |
|---|---|---|---|---|---|---|---|---|
| $CI_{Strain}$ to $SI_{Naive}$ | **<0.001** n=116,12% | 0.48 n=111, 5% | 0.21 n=96, 6% | **0.047** n=88, 8% | 0.44 n=95, 10% | 0.83 n=85, 7% | **0.02** n=105,13% | 0.73 n=109, 9% |
| $CI_{Strain}$ to $CI_{N2}$ | | **<0.001** n=111, 5% | **<0.001** n=96, 6% | **<0.001** n=88, 8% | **<0.001** n=95, 10% | **<0.001** n=85, 7% | **<0.001** n=105,13% | **<0.001** n=109, 9% |
| $LI_{Strain}$ to $SI_{Naive}$ | **<0.001** n=70, 5% | **0.02** n=40, 9% | 1 n=37, 13% | 0.63 n=40, 11% | 0.64 n=40, 16% | 1 n=39, 9% | 0.44 n=40, 16% | 0.16 n=40, 7% |
| $LI_{Strain}$to $LI_{N2}$ | | 0.39 n=40, 9% | **0.001** n=37, 13% | **0.014** n=40, 11% | **<0.001** n=40, 16% | **0.003** n=39, 9% | **<0.001** n=40, 16% | **<0.001** n=40, 7% |




The differential effect of aging:
Learning ability declines first

| *p*-values | Day 1 | Day 5 | Day 7 |
|---|---|---|---|
| $CI_{Dayx}$ to $SI_{Naive\_Day1}$ | **<0.001** n=120, 5% | **0.0061** n=76, 5% | **0.002** n=64, 3% |
| $CI_{Dayx}$ to $CI_{Day1}$ | | **0.01** n=76, 5% | **0.03** n=64, 3% |
| $LI_{Dayx}$ to $SI_{Naive\_Day1}$ | **<0.001** n=62, 13% | 1 n=41, 10% | 1.12 n=40, 2% |
| $LI_{Dayx}$ to $LI_{Day1}$ | | **0.03** n=41, 10% | **0.02** n=40, 2% |

## Statistical Analysis

Comparisons were made by the binomial probability distribution test in MATLAB R2016b (Mathworks, USA), using the binocdf and binopdf functions of Statistics and Machine Learning Toolbox. Differences are statistically significant when *p*-value<0.05.

## Conclusions

- First-time evidence of space-related **navigational learning in *C. elegans***
- Learning observed after a single training session and is detected very shortly after
- **Learning is a multisensory behavior** and leads to biased decision making
- Worm-Maze is suitable for running behavioral assays to monitor decision making, learning and sensory integration in worms
- **Aging has a differential effect on worms' behaviors**, implying that aging does not affect all neurons and neuronal circuits uniformly

## Acknowledgments

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