Organism-on-a-Chip Models for Neurobehavioral Screening of Disease

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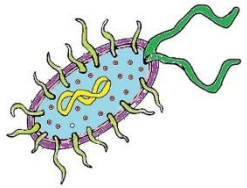
Our Research Interest



Environment / Water / Beverage / Food

Disease Pathology
Toxicology
Drug Discovery

Model Organisms for Human Disease Investigations

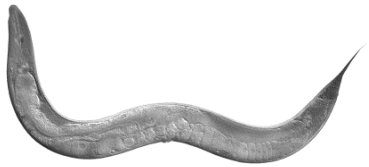
- µ-organisms & µ-particles
- D. rerio or zebrafish
- Drosophila or Fruit Fly
- C. elegans or Worm

MICROFLUIDICS
Materials
Microfab
Application

- Electrical
- Chemical & Acoustic
- Electrical, Chemical
- Magnetic, Inertial,
- Viscoelastic

Environmental cues

HEALTH & SAFETY



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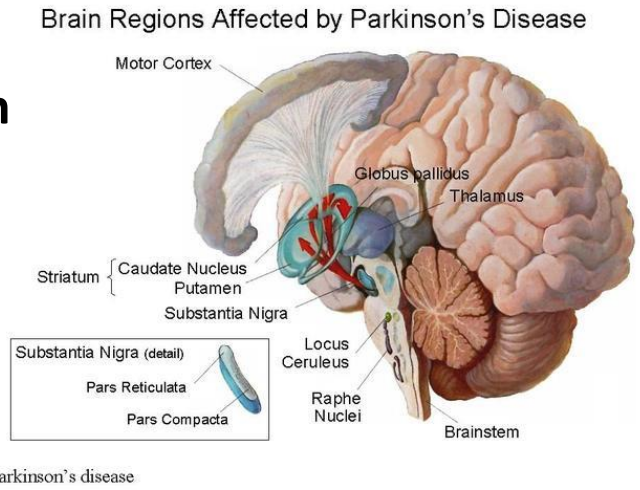
Outline of the Talk

- **Disease** - Parkinson's disease (PD) as an example
- **Drug discovery** process and issues
- **Disease models** and associated challenges
- **Microfluidics** to study disease models and search for therapeutics
    - **Organism-on-a-chip** technology (focus on **electrotaxis for PD**)
        - *Caenorhabditis elegans (Worm)*
        - *Danio rerio (zebrafish)*
        - *Drosophila melanogaster (Fruit Fly)*
- **Summary**

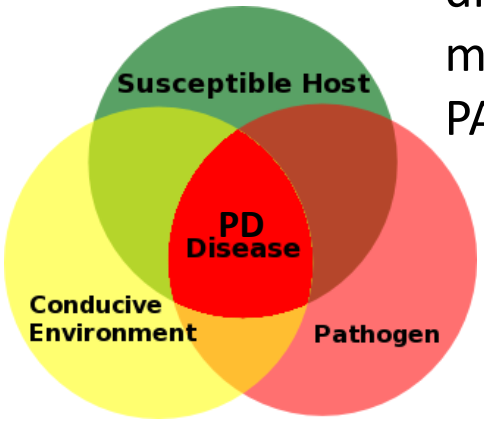
Parkinson's Disease (PD)

Degeneration of neurons in substantia nigra (region of the brain that controls movement and balance)



Parkinsonian neurotoxins:

- 6-hydroxydopamine (6-OHDA)
- 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)
- Rotenone
- Paraquat



Parkinson's disease genetic basis:
Familial cases of Parkinson disease can be caused by mutations in the LRRK2, PARK2, PARK7, PINK1, or SNCA genes.

Bove et al, NeuroRx. 2005 Jul; 2(3): 484–494.

http://www.calgarycmmc.com

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Parkinson's Disease Pathological Hallmark

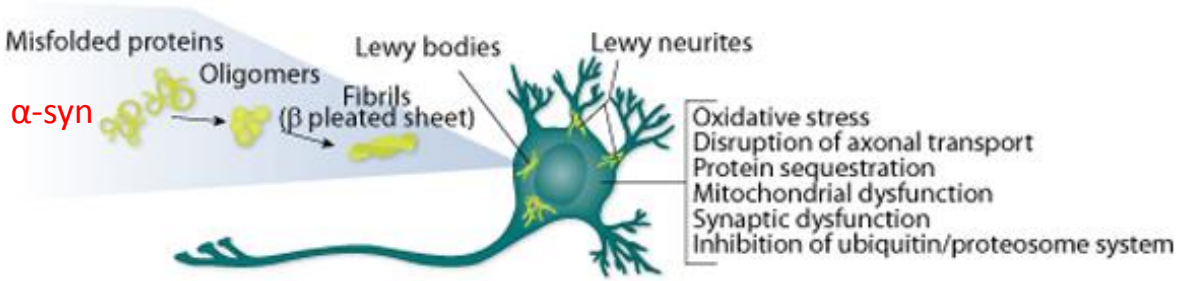
DAnergic neurons in the substantia nigra produce a chemical messenger called **dopamine**.

Dopamine **transmits signals within the brain** to produce smooth physical movements.

DAnergic neurons degenerate or die in many **PD** patients.

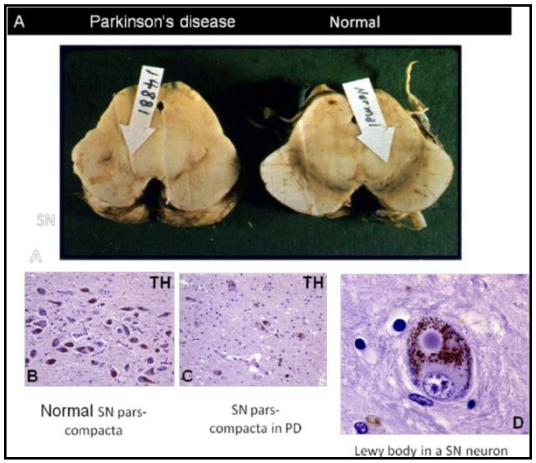
Communication between the brain and muscles **weakens**.

Brain becomes **unable to control muscle movement**.



PD Pathological Hallmark:
Accumulation of Protein α-synuclein in DN and formation of Lewy bodies

Need for drugs to prevent or suppress α-synuclein accumulation



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Mandel et. al. ISBN 978-1-60692-737-3

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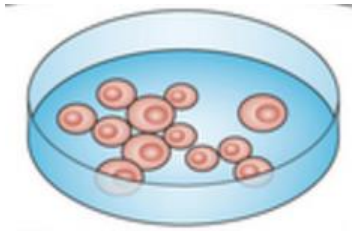
In-vitro and *in-vivo* Disease Models

Humans cannot be directly used in Drug Discovery due to **ethical issues** and **complexity**

in-vitro



Two-dimensional cell cultures

50,000 chemicals

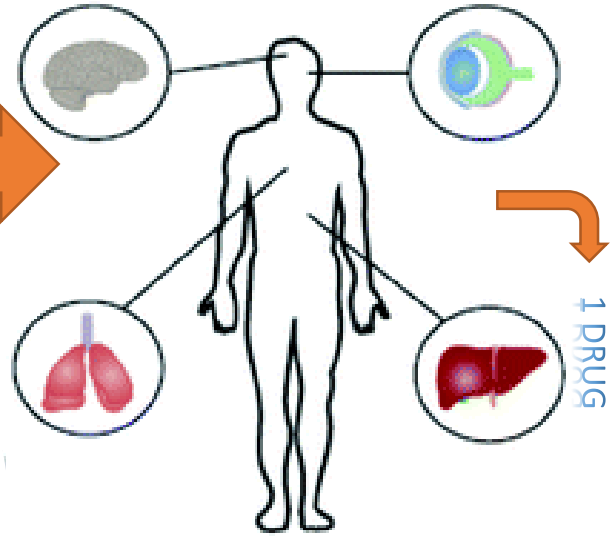
500 chemicals

in-vivo

Animal Models:
Mice, Monkey, Cats, Dogs

5 chemicals

Clinical



1 DRUG

Jong, Nature Reviews Cancer, 2014, 481–493

http://i.dailymail.co.uk/

Jackson & Lu, Integr. Biol., 2016,8, 672-683

Over-simplistic lacking many features of microenvironments like extracellular matrix (ECM) and dynamic signaling

Ethically limited, low throughput, expensive, don't mimic all aspects of disease

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Filling the Gap

Physiologically relevant tissue and organs

3D cell cultures

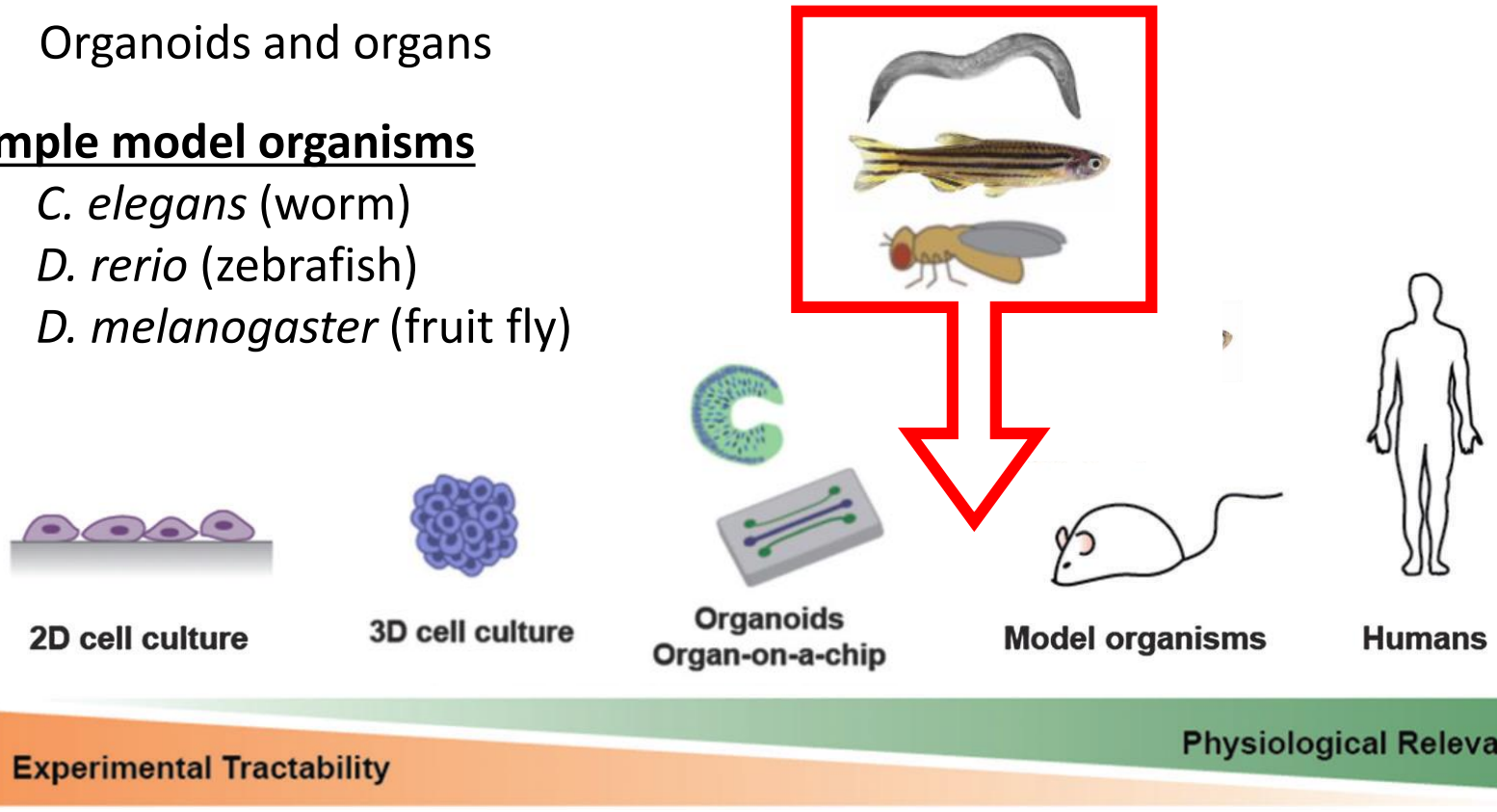
Organoids and organs

Simple model organisms

C. elegans (worm)

D. rerio (zebrafish)

D. melanogaster (fruit fly)



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https://kidscancer.uchicago.edu

http://post.queensu.ca

Jackson & Lu, *Integr. Biol.*, 2016,8, 672-683

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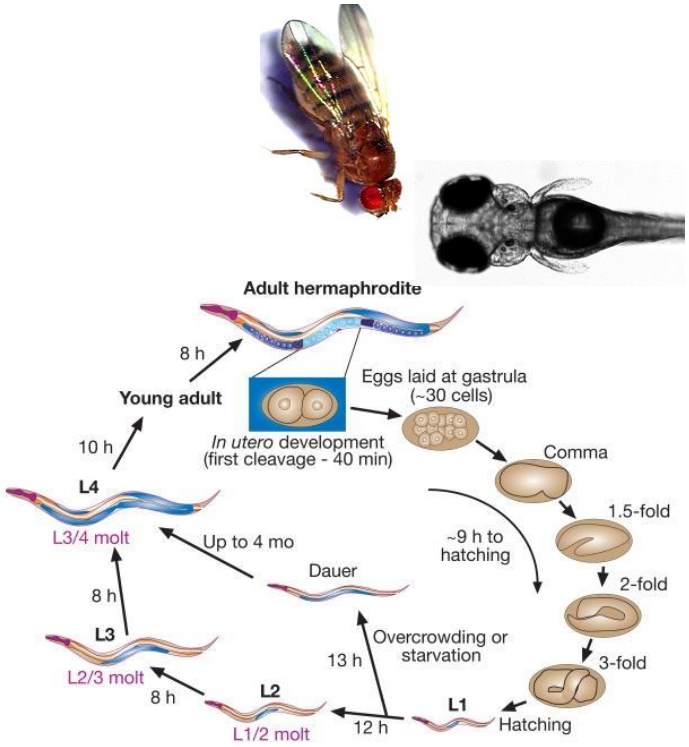
Advantages of Model Organisms

- High genetic similarity to human (fully sequenced)
- Well-mapped and simple cellular system and neuronal network
- Small size (µm-mm) and easy to grow
- Transparent bodies addressable with fluorescent tags
- Short life cycle (hrs – days) and low cost
- Modeled for human diseases
  - Pathway studies
  - Drug screening



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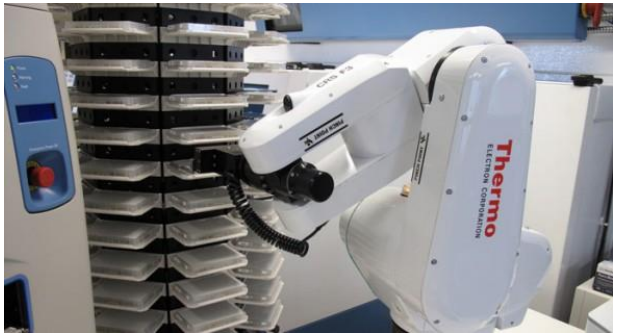
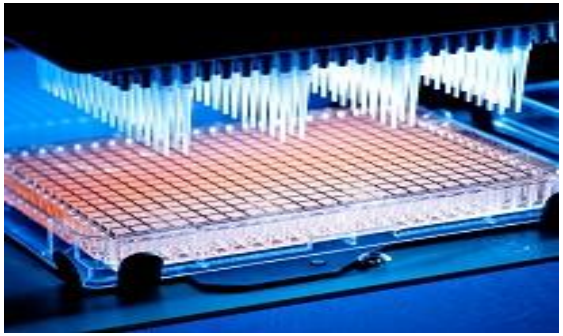
Microfluidics for Whole Organism Studies

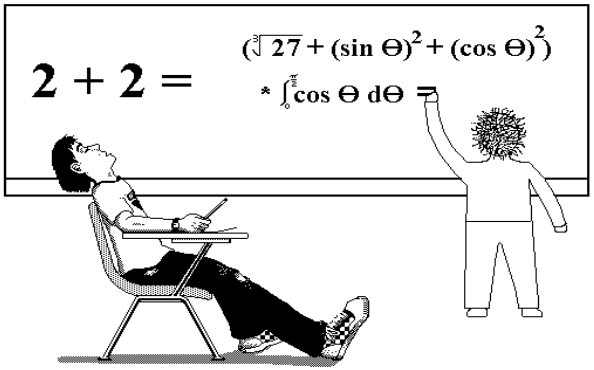
AUTOMATION





HIGH THROUGHPUT SCREENING

SIMPLICITY



LOW COST

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http://news.vanderbilt.edu
http://australianbusinessclinic.com

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http://www.criver.com

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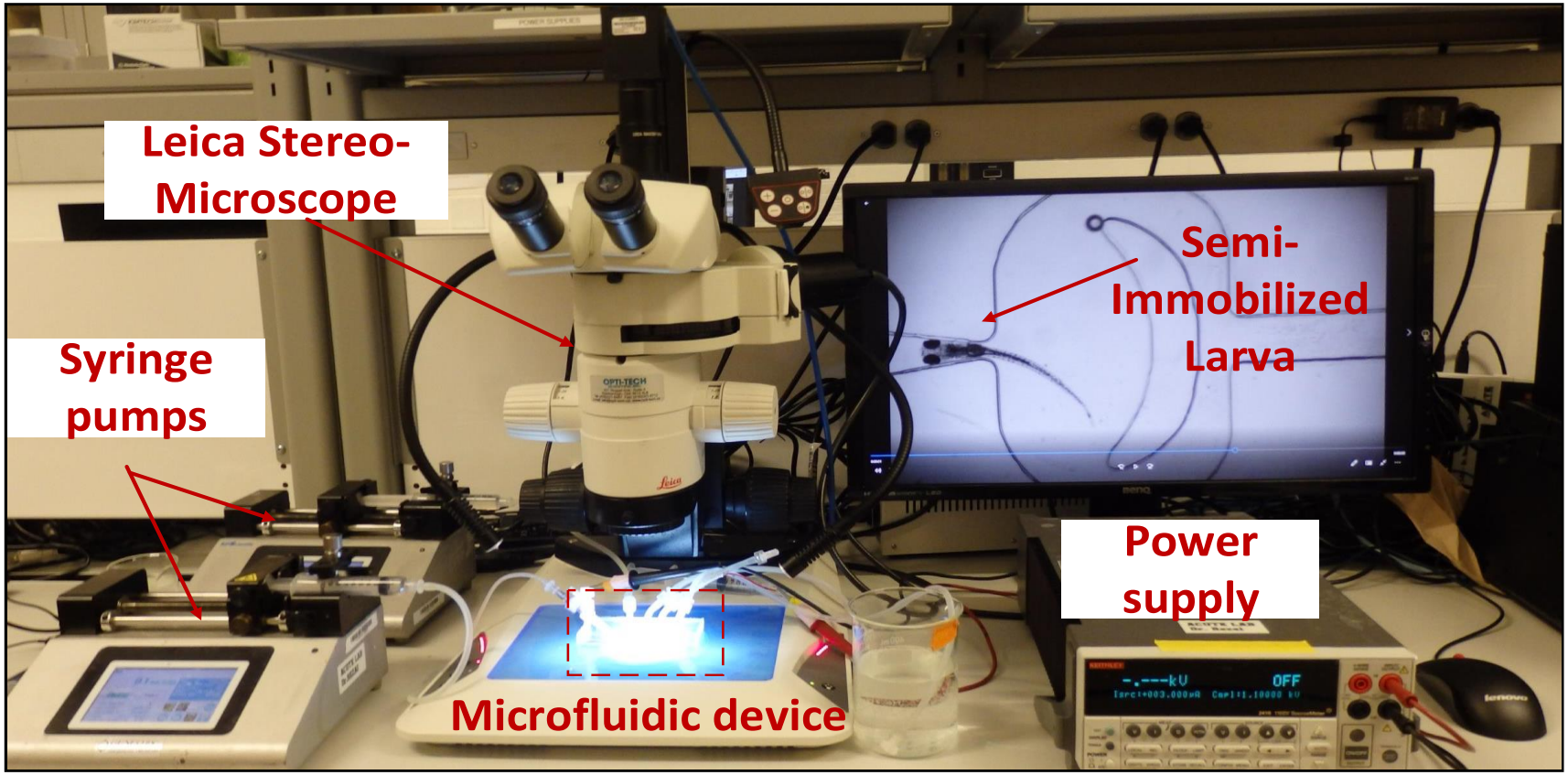
Experimental Setup



On-chip *C. elegans* Assays

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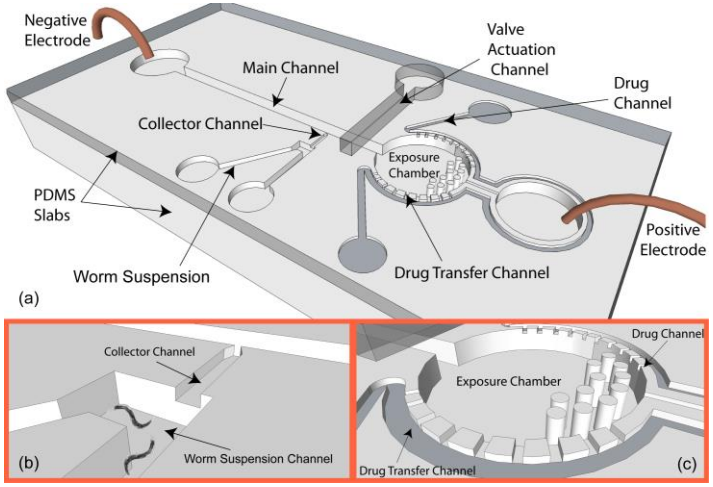
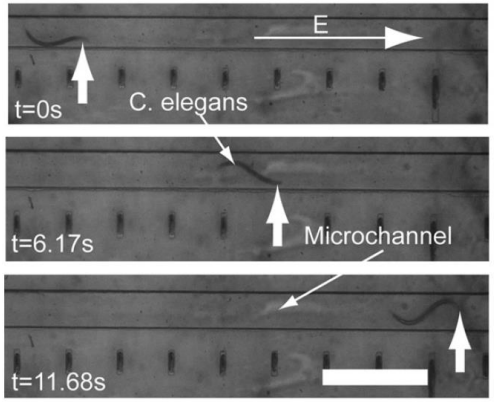
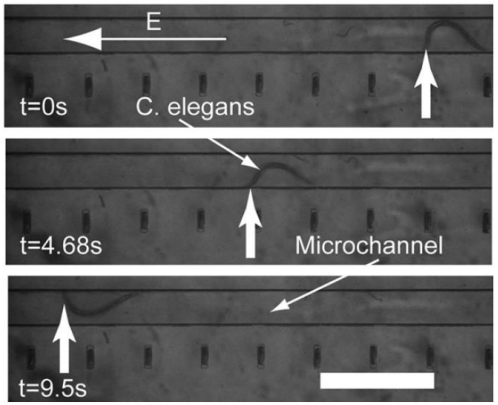
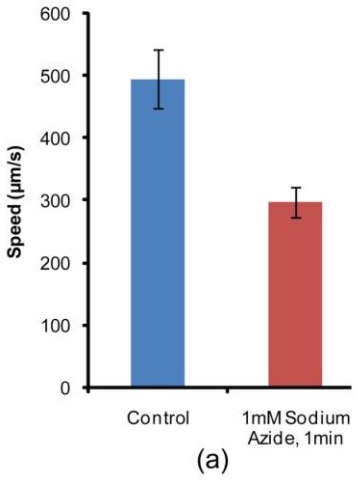
C. elegans Electrotaxis & Chemical Screening

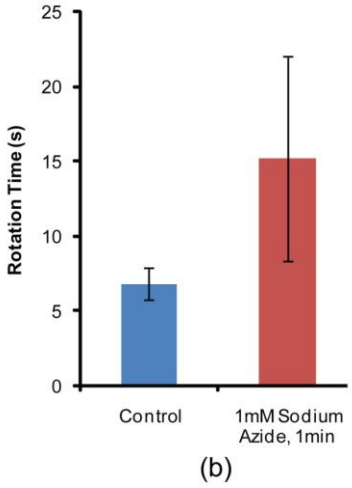
Lab on a chip to screen *C. elegans* using electrotaxis (response to electric signals)

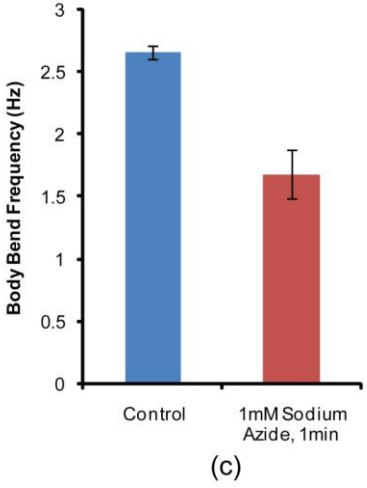












- ❑Toxicology
- ❑Drug Screening
- ❑Biological Pathway Investigations

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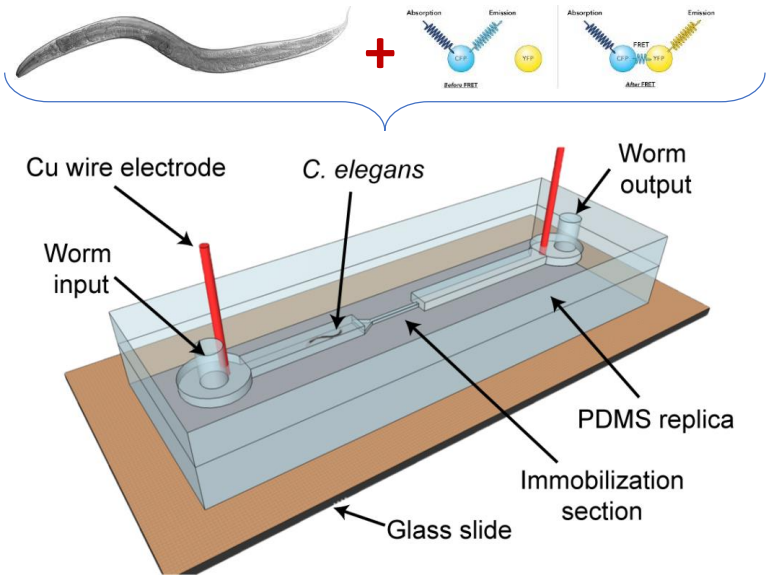
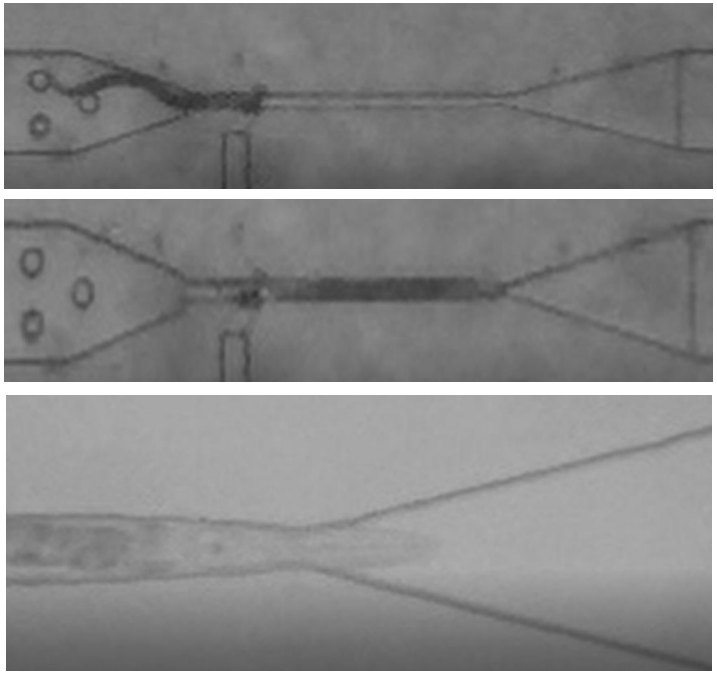
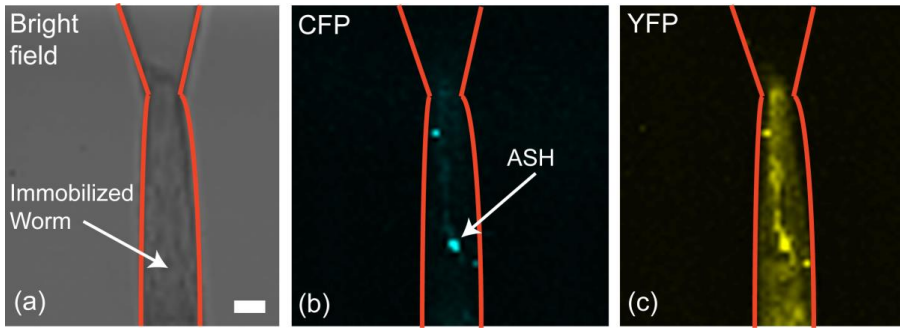
C. elegans Electrotactic Neuronal Assay

FRET Imaging of Neurons Transient Response to Electric Field

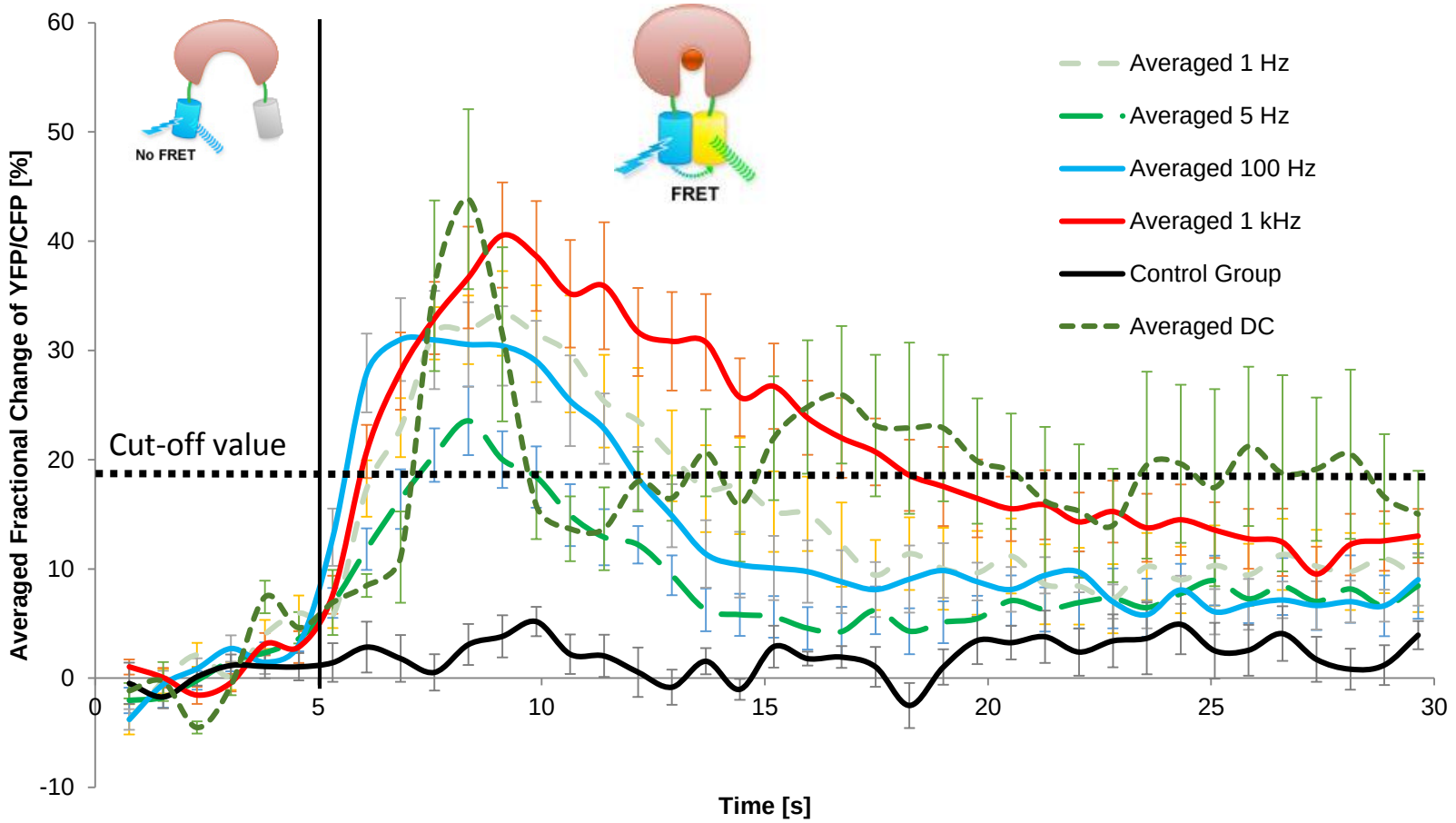






$$\frac{YFP}{CFP}$$

C. elegans Electrotactic Neuronal Assay



Correlated with Electrotactic Behavior

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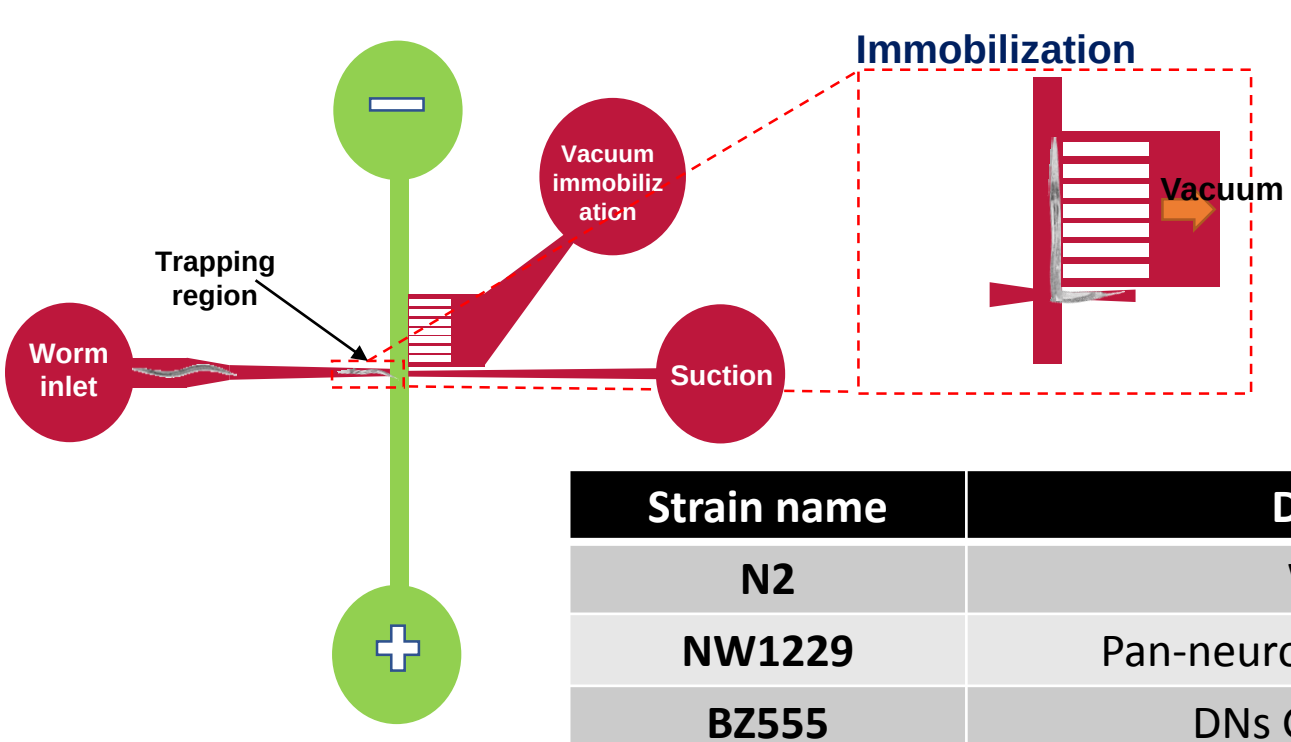
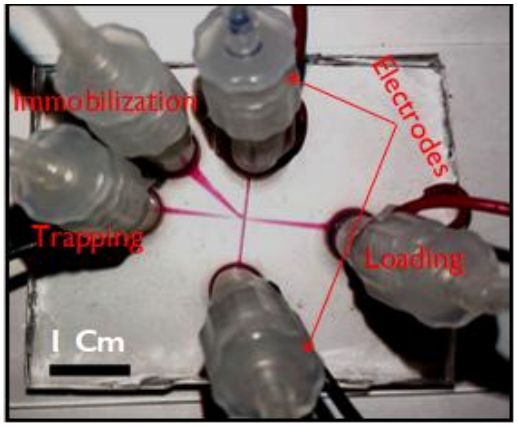
C. elegans Electrotactic PD Model





Strain name	Description
N2	Wild-Type
NW1229	Pan-neuronal GFP expression
BZ555	DNs GFP expression
NL5901	α-Syn overexpression in the muscle cells

$$\textit{Electrotaxis Time Index (ETI\%)} = \frac{\textit{Time facing the cathode}}{\textit{Total time exposed to current}}$$

Turning time (S)= Time needed to turn towards cathode

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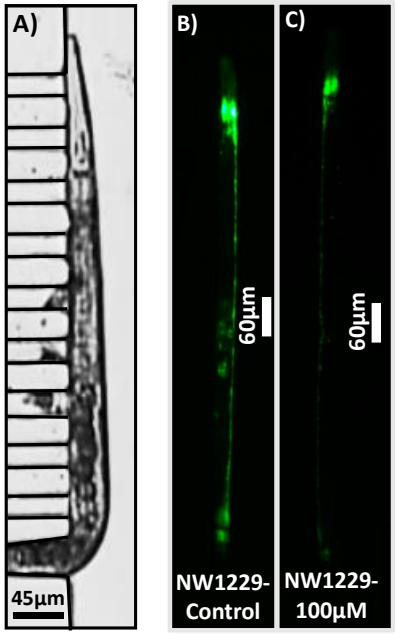
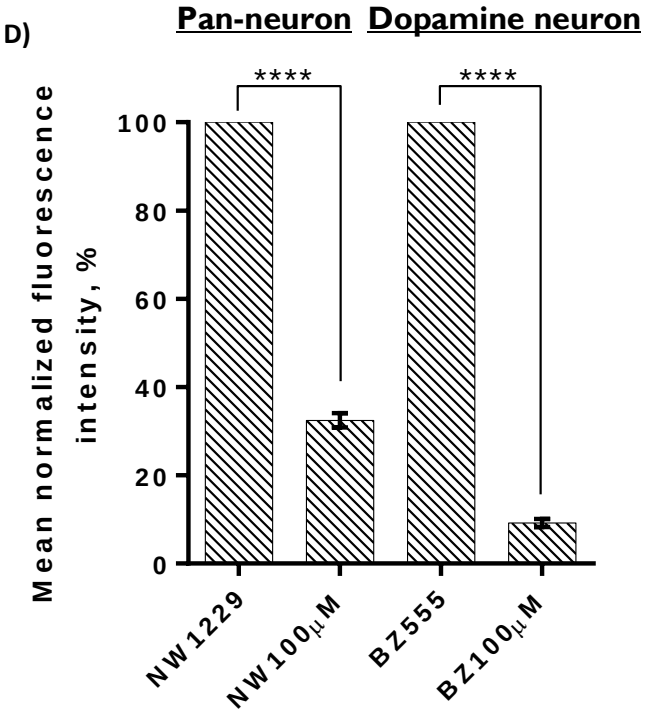
C. elegans Electrotactic PD Model

6-OHDA induced neurotoxicity and neuron degeneration





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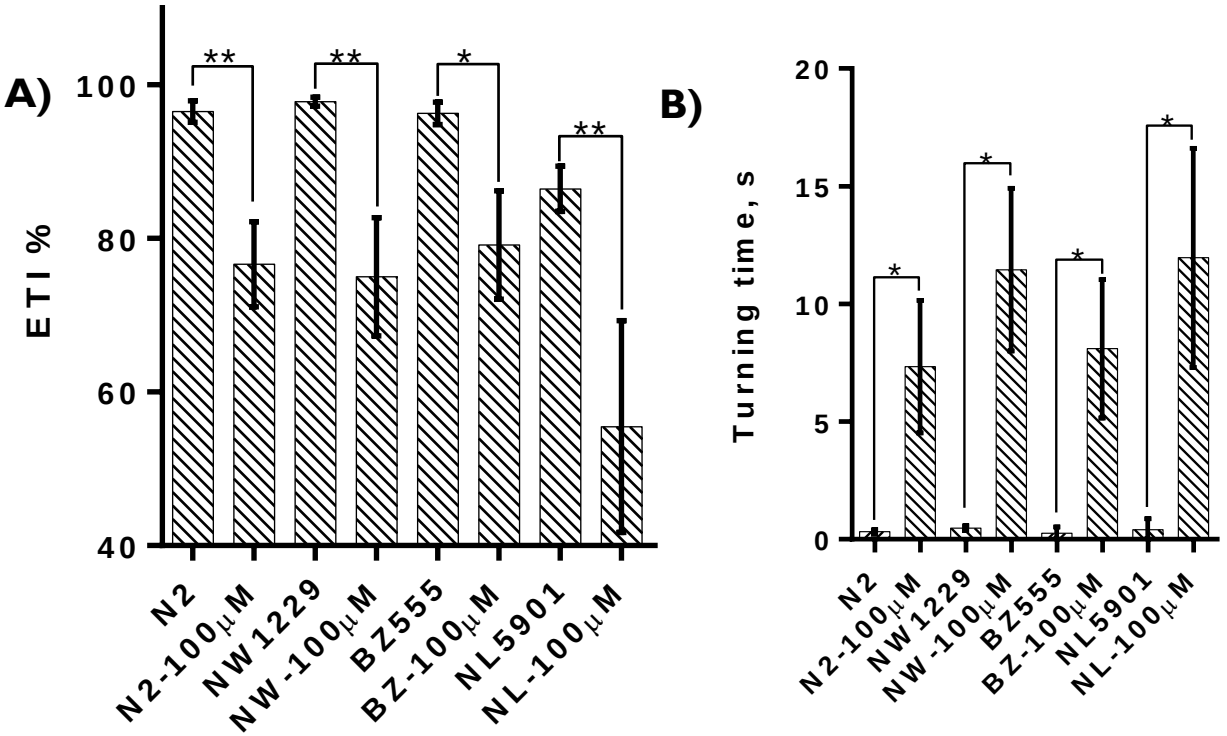
C. elegans Electrotactic PD Model

6-OHDA induced electrotactic movement impairment



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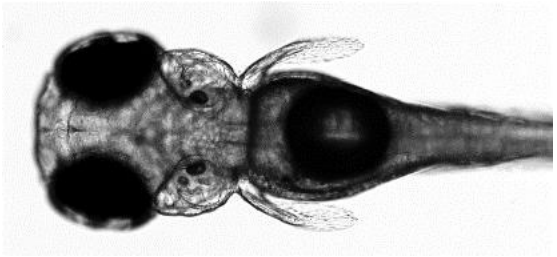
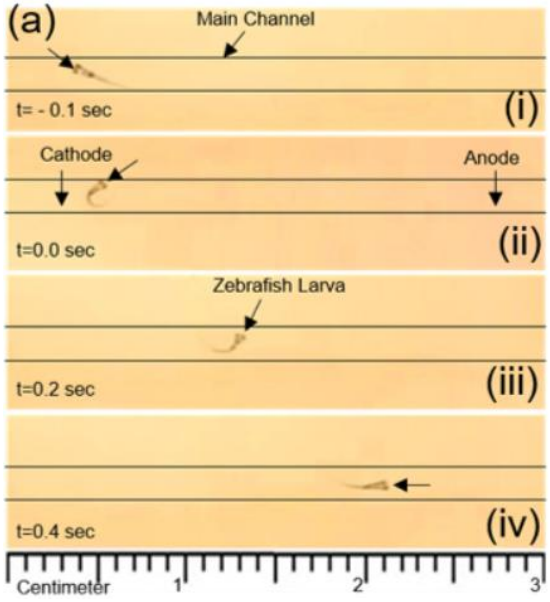
On-chip Zebrafish Assays

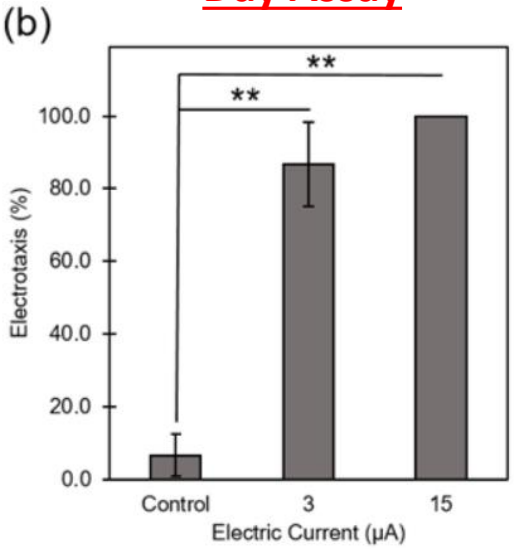


Zebrafish Electrotaxis Involves Dopamine Pathway

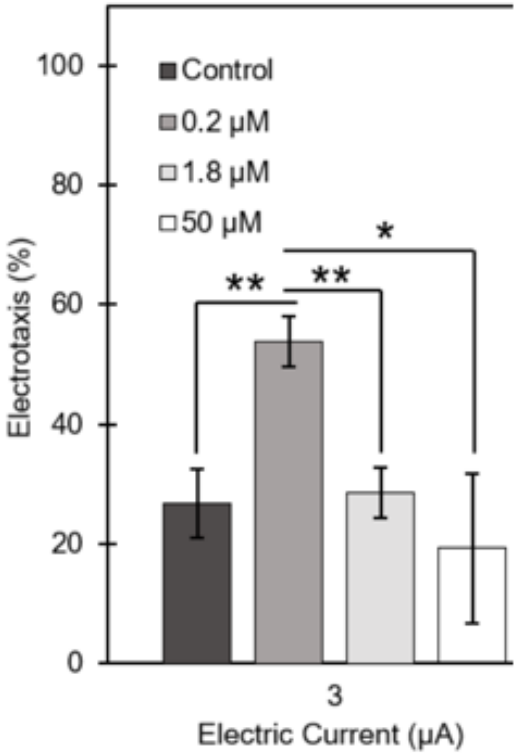
Apomorphine is a dopamine agonist that activates D-like receptors and is used in treating PD



Day Assay



Night Assay



Zebrafish electrotaxis is mediated with dopaminergic pathways

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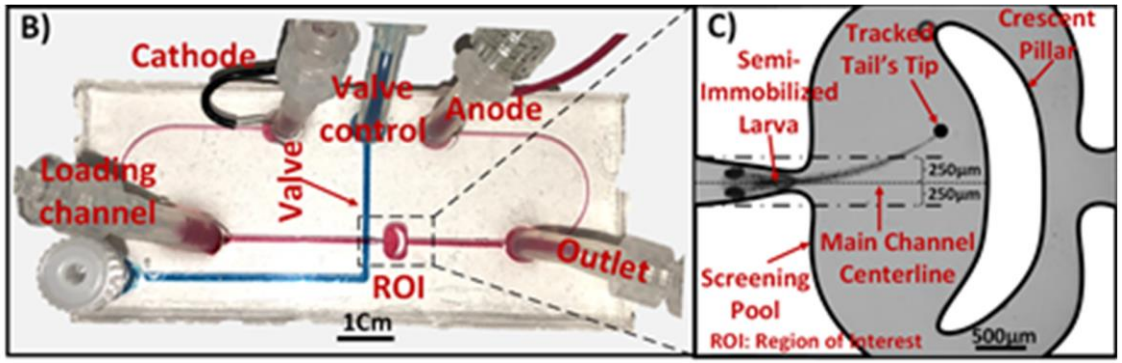
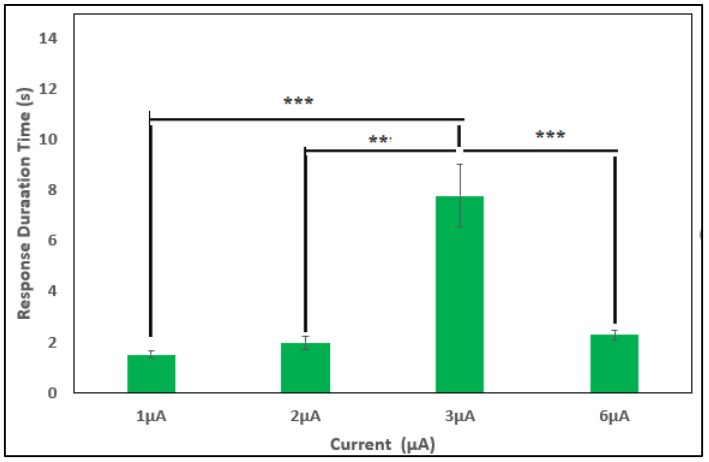
Zebrafish Electrotaxis Phenotypic Study



Response Duration Time (RDT)

The beginning of tail motion until the larva stopped moving

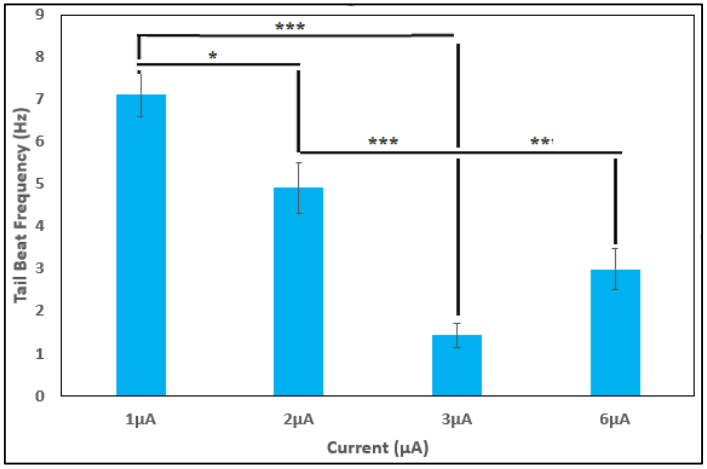


$$RDT \propto \frac{1}{TBF}$$

$$I = 3\mu A$$

Tail Beat frequency (TBF)

$$\frac{\text{The number of full cycles}}{\textit{Response duration} \text{ time}}$$



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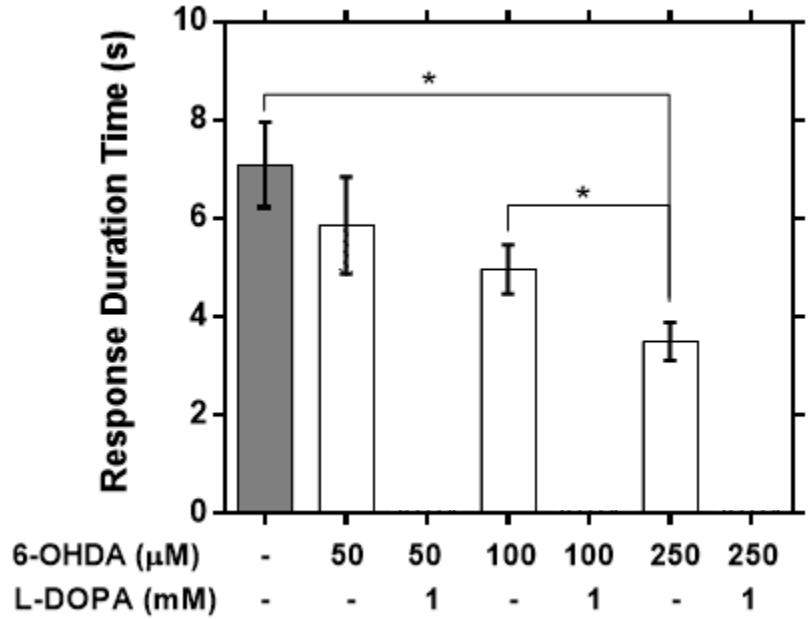
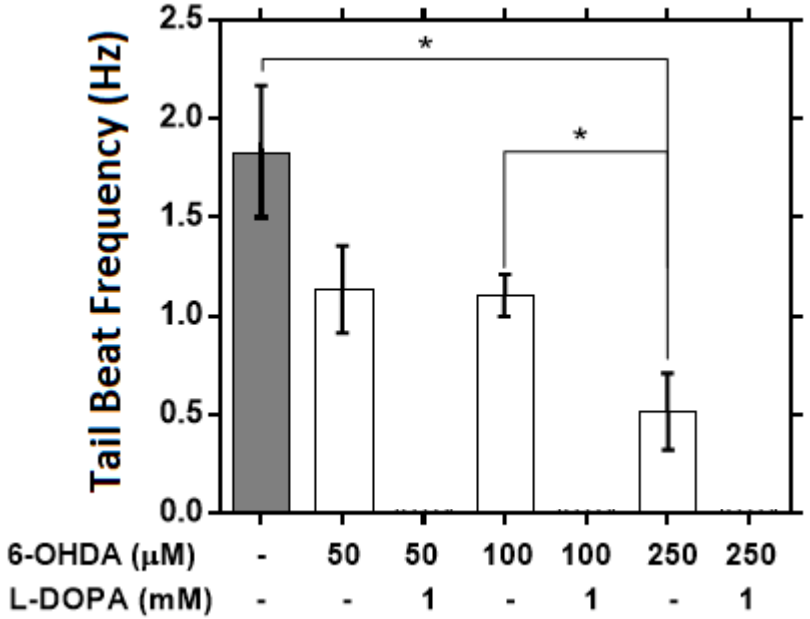
Zebrafish Electrotactic PD Model

Effect of 6-OHDA on electrotaxis RDT



Effect of 6-OHDA on electrotaxis TBF



- Exposing to 50-250μM 6-OHDA reduced RDT by 17%-50.6%.
- TBF was reduced by 37.9%-72% after exposure to 50-250μM 6-OHDA.
- Co-treatment with 1mM Levodopa did not rescue electrotaxis, except mildly at high-dose 6-OHDA.

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Summary

- In drug discovery, **disease targets** must be identified to screen for **lead chemicals** and **therapeutics**
- It's **difficult and unethical** to study **human** subjects, hence the need for alternate disease models → **Cell culture models**
- Current cell culture assays do not mimic *in-vivo* **physiology** and disease **pathology**, and are also **labour- and cost-intensive**
- **3D cell** cultures and small **model organisms** studied in **microfluidic** devices provide unprecedented platforms to study disease and look for drugs
- **Organisms** can provide a more **systemic** platform for drug discovery
- **Microfluidic electrotaxis** is a robust tool for neuronal and behavioural screening of *C. elegans* and zebrafish
- Advantages provided: Automation, Integration, HTS, resulting in **faster** development of drugs with **lower cost**

Acknowledgement

Thank you for your attention

Lab Members

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- Ramtin Ardeshiri (MSc)
- Amirreza Peimani (MSc)
- Khaled Youssef (PhD)
- Arezoo Khalili (PhD)
- Alireza Zabihi Hesari (PhD)

And many more …

FUNDING

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MINISTRY OF AGRICULTURE, FOOD AND RURAL AFFAIRS

Academic Collaborators

- Prof. Mei Zhen (U of T)
- Prof. Arthur Hilliker (York)
- Prof. G. Zoidl (York)
- Prof. Terry Kubiseski (York)
- Prof. Anurag Tandon (U of T)
- Prof. Bhagwatu Gupta (McMaster)

Industrial & Governmental Collaborators

- Public Health Agency of Canada
- Conestoga-Rovers & Associations – now GHD
- BTNX Inc.
- Dairy Quality Inc.

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