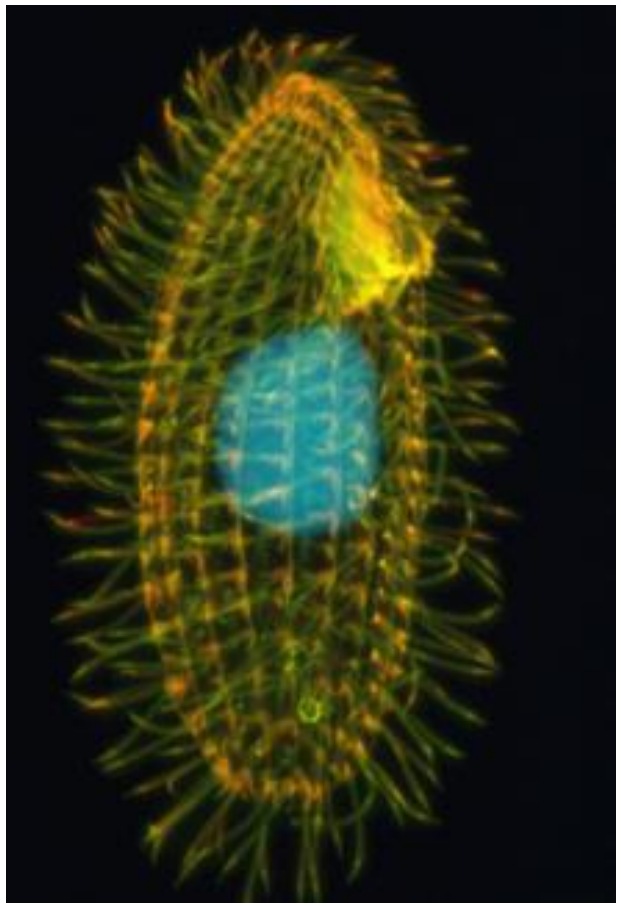
BEHAVIORAL EFFECTS OF CHEMOREPELLENTS ON MUTANT AND WILD-TYPE TETRAHYMENA *THERMOPHILA*

Original Research and Presentation By: Ananya Govindarajan

INTRODUCTION:

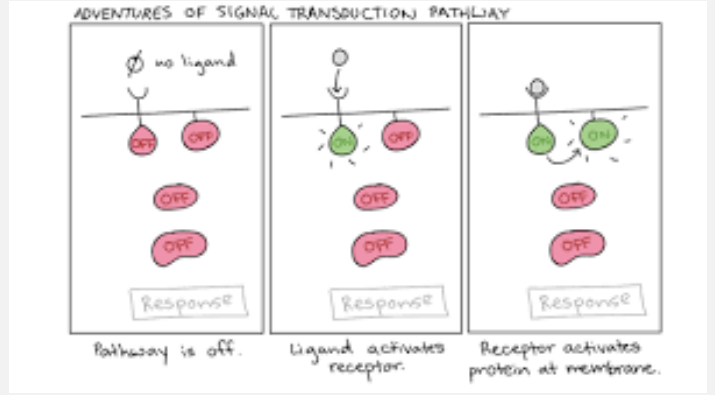
- Tetrahymena *thermophila* are **model** organisms for signal transduction pathways/cell signaling **(2)**
- Belong to Protozoa kingdom **(2,3)**
- Single-cell organisms
- Eukaryotic ciliate organism
- Used to model human neurons based on **sensory** input



https://en.wikipedia.org/wiki/Tetrahymena

INTRODUCTION:

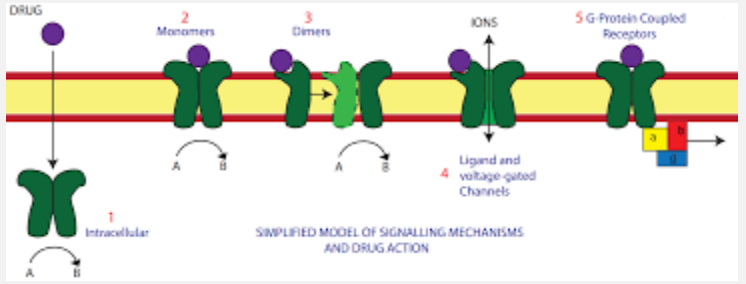
- **Cell Transduction: "Cell Signaling" (12)**
  - How cells communicate
  - Involves a pathway
    - Pathways: **not** a channel or path; some type of cellular action/response
    - Ex. Cellular movement; release of a substance, etc.
    - 3 Main steps involved:
      - (1) Ligand is released; (2) Ligand binds to receptor; (3) Cellular signaling/response



https://www.google.com/url?sa=i&url=https%3A%2F%2Fwww.khanacademy.org%2Fscience%2Fbiology%2Fcell-signaling%2Fmechanisms-of-cell-signaling%2Fa%2Fintracellular-signal-transduction&psig=AOvVaw0wPoPN3Cp-L8VE-n44Bm4J&ust=1598297357955000&source=images&cd=vfe&ved=2ahUKEwi9pbPjh7LrAhWDA98KHYs BB6 YQr4kDegUIARDYAQ

INTRODUCTION:

- Cells have **many** types of receptors/communicate differently **(12)**
  - Intracellular receptors
    - **Specific** to particular DNA segment
    - Cell-Surface Receptors: extracellularly located
      - G-Protein Coupled Receptors
      - Ligand-Gated Ion Channels
      - Tyrosine Kinase Receptors

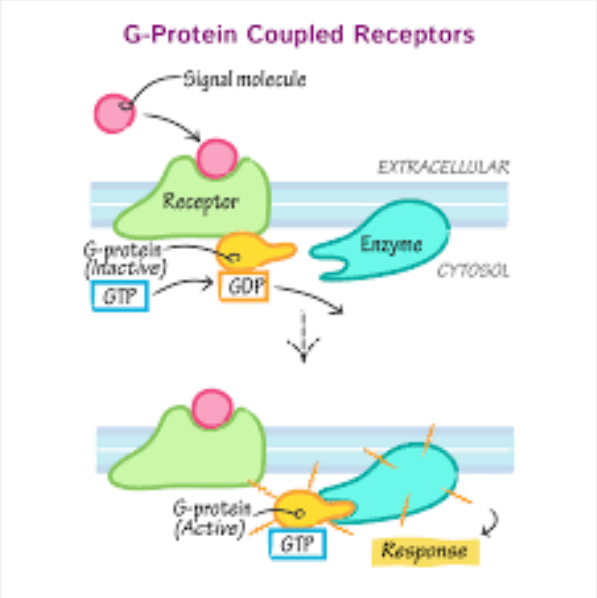


https://www.google.com/url?sa=i&url=http%3A%2F%2Fwww.partone.lifeinthefastlane.com%2Freceptor_types.html&psig=AOvVaw2m4RkmRIyp886Lry_rUCrM&ust=1609206297306000&source=images&cd=vfe&ved=2ahUKEwjbiIXfxu_tAhUBFt8KHZu8DfQQr4kDegUIARDHAQ

INTRODUCTION:

G-Protein Coupled Receptors: (17)

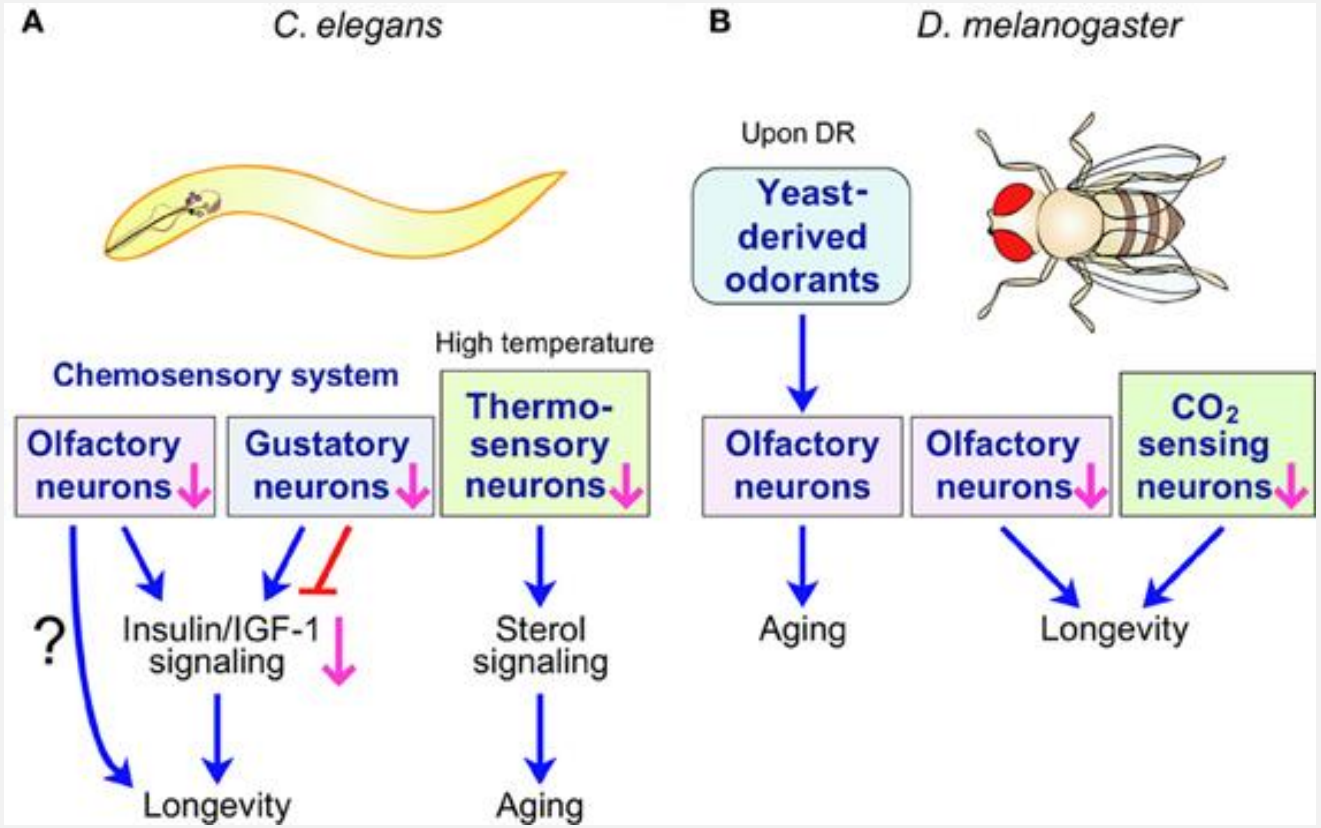
- Most **diverse** type of receptor
  - Intracellular and extracellular structures
  - 7 different protein structures
  - Hundreds of GPCRs
  - GTP vs. GDP
    - Activate g-proteins ⟶ cellular response



https://drawittoknowit.com/course/cell-biology/glossary/cellular-anatomy-physiology/g-coupled-receptor

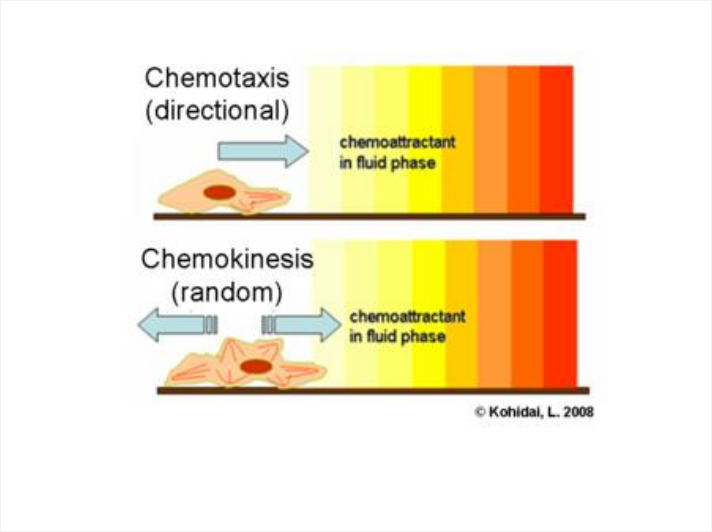
INTRODUCTION:

- **Chemosensory Pathways:** Cell responses to chemical stimuli **(17)**
  - Chemorepellent vs. Chemoattractant
  - **All** chemosensory pathways controlled by GPCRs
- GPCRs controlling these pathways require ligands
  - Lock and Key Model

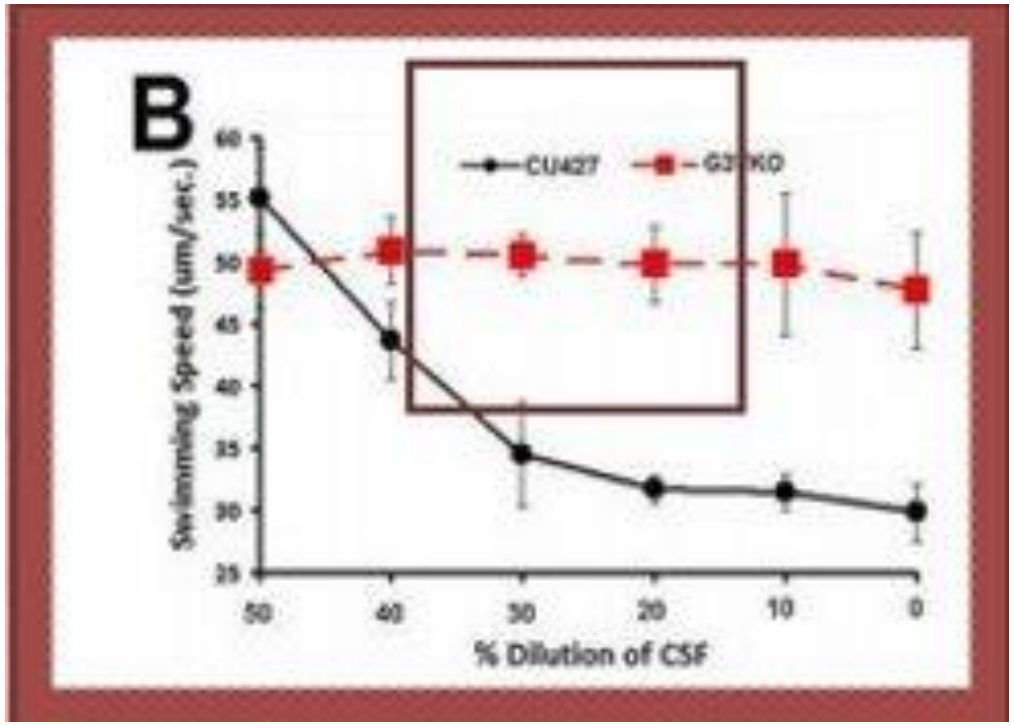


INTRODUCTION:

- **Oxidants** are known chemorepellents **(9)**
- Reduced forms of chemicals have lessened effects
- Chemorepellents cause depolarizations and ARs
  - Avoidance Reactions (ARs)
  - Backwards swimming (chemotaxis)
  - Decreased swimming speed/chemokinesis



https://en.wikipedia.org/wiki/Chemokinesis



Zhou et al. 2017

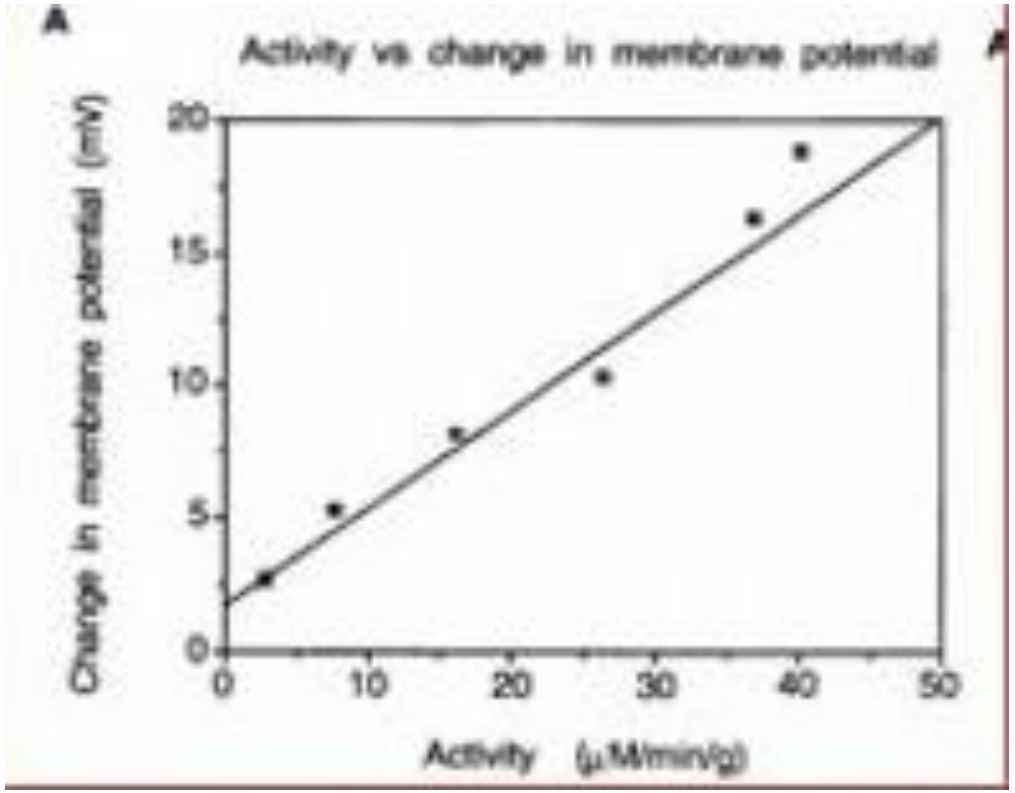
LITERATURE REVIEW:

- **Zhou & Hennessey (2017)** (17)
  - Tested Tetrahymena *thermophila* cells in CSF
  - Contained powerful chemorepellent
  - G37 gene (mutant) responsible for response
  - G37 increased responsiveness; G37 KO responsiveness was lowered
  - G37 receptor plays role in viability/motility when high density of cells present
  - Unknown what was contained in CSF

LITERATURE REVIEW:

- **Hennessey et al. (1994)** (9)
  - Forward swimming is caused by hyperpolarization
  - Depolarization results in a slower swimming speed of the ciliate
  - Chemotaxis vs. Chemokinesis
  - Ions are most potent chemorepellents
  - Similar responses in *Paramecium*

- **Rasmussen et al. (1984)** (14)
  - Tetrahymena population declines and reproduction decreases as iron increases
  - Iron's oxidized form (ferric) is essential; proteins are bound to it in humans



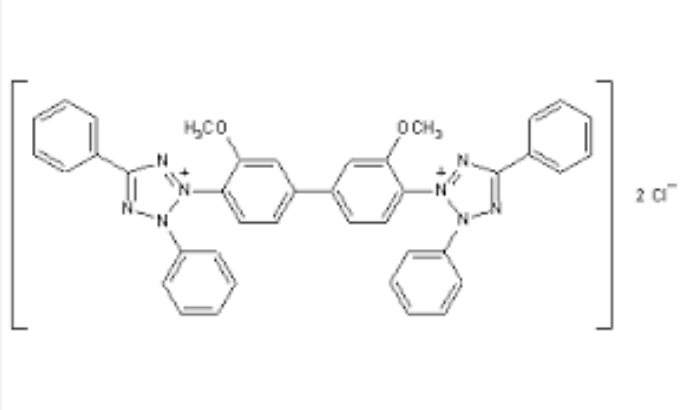
LITERATURE REVIEW:

- **Francis & Hennessey (1994)** (4)
  - All test chemicals elicit ARs
  - Millimolar concentrations repel
  - Depolarizations present for all substances
  - Non-permeable oxidants **are** chemorepellents in *Paramecium*

LITERATURE REVIEW:

- **Ems et al (2020)** (4,10)
  - Ferrous sulfate (reduced form of iron)
  - Factor in metabolic processes
  - Cellular respiration in cell cultures
  - DNA synthesis
  - Electron transport
  - Oxygen transport

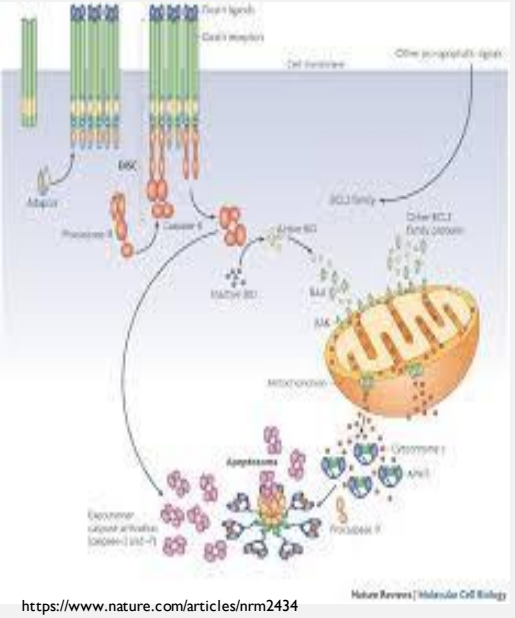
- **Riss et al. (2013)** (15)
  - Tetrazolium blue is an oxidant necessary for viability assays by using redox potential
  - Organic chloride salt



https://www.drugfuture.com/chemdata/Tetrazolium-Blue.html

LITERATURE REVIEW:

- **Garrido et al. (2006)** (6)
  - Cytochrome C: activation of proteases
  - ATP formation and electron formation
- **Aghdai et. al (2013)** (1)
  - DTT are reductants for sulfide bonds
- **Halliwell et al. (2000)** (7)
- Hydrogen peroxide ability to readily cross cell membranes
- Can result in high oxidative stress if concentration is too high in cell culture



https://www.nature.com/articles/nrm2434

GAP IN THE RESEARCH

- G37 gene encoding for GPCR has **unknown** functions in presence of different chemicals
- Behavioral effects of chemorepellents on G37 cells and wild-type cells in response to oxidants
- **Physiological** differences between G37 and wild-type cells

PURPOSE OF RESEARCH:

Goals of Research:

- Understand the effects of different test chemicals on G37 (mutant) Tetrahymena *thermophila* cells.
  - Test Chemicals: FeSO4, FeCl3, Cytochrome C, DTT, Hydrogen Peroxide, Tetrazolium Blue
- Determine the swimming speeds of cells under the influence of different concentrations of test chemicals
  - Determine concentration of test chemicals that preserve cell viability
- Understand the effects of oxidants vs. reduced chemicals on G37 and CU427 cells

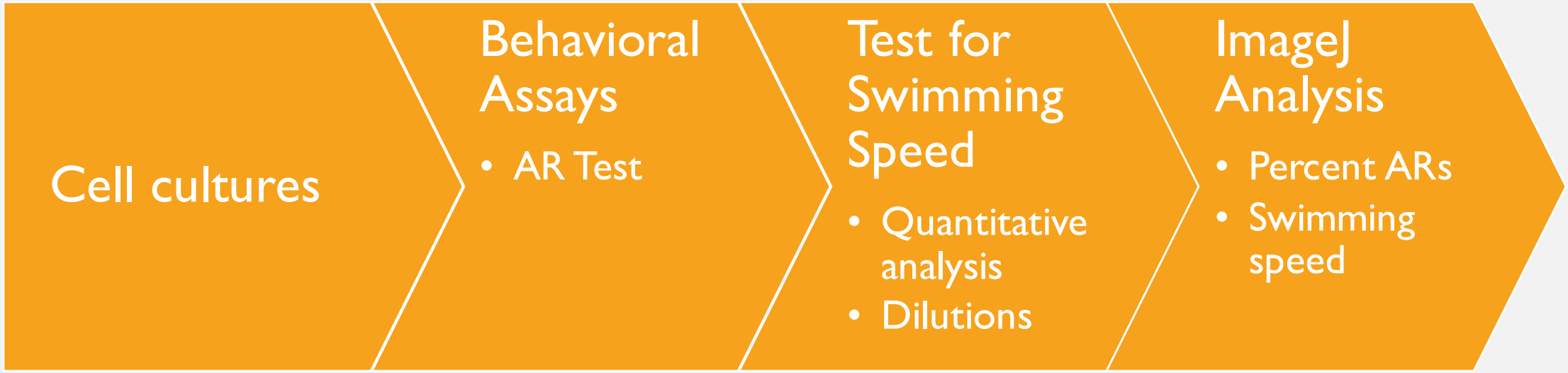
Hypothesis:

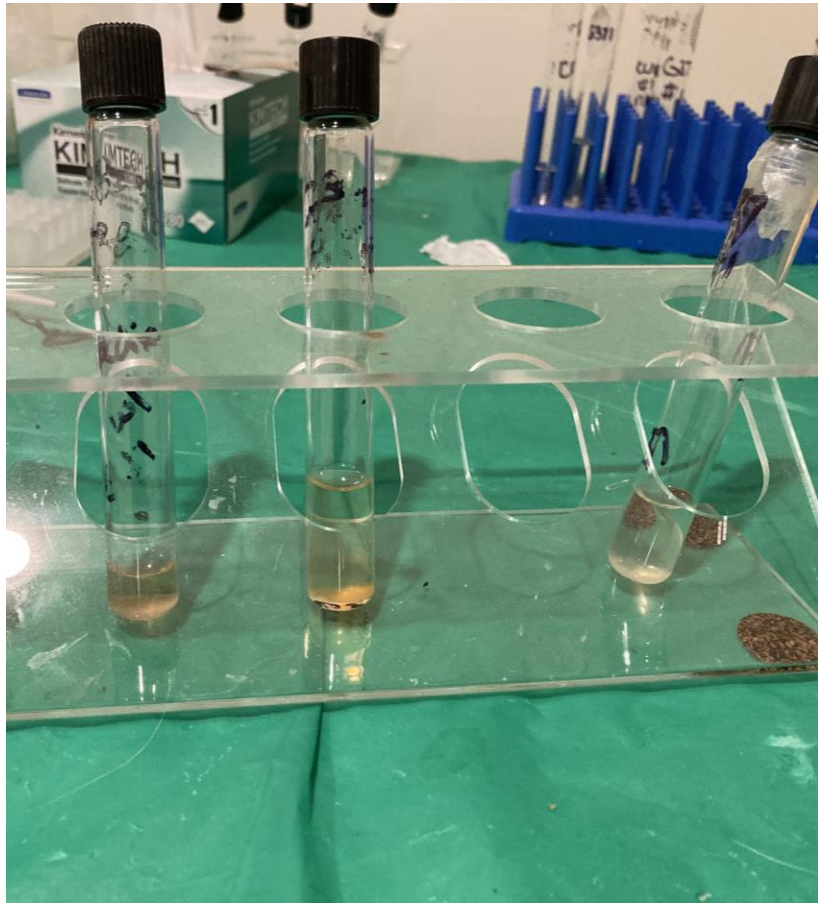
G37 cells will elicit ARs for all test chemicals and will need a lower concentration/higher dilution for viability due to heightened receptor

METHODOLOGY

METHODOLOGY:

- **Overview**





METHODOLOGY:

Cell Cultures:

- Cultured in axenic media via sterile transfers
  - Media: Yeast nucleic extract, glucose, proteose peptone
- Grown at slanted position for 3-4 days before testing
- Washed in buffer solution of MOPS, TRIS, CaCl2
- Centrifuged at 3,000 RPM for 1 minute

METHODOLOGY:

Behavioral Assays:

- Centrifuged cells and suspended 1 mL of cells in 1,000 µL of control solution
- Rest for 15 minutes before assays
- 2 µL of test solution in 8 µL of cells
  - 5 mM FeCl3
  - 5 mM FeSO4
  - 5 mM NBT, cytochrome C, hydrogen peroxide,
- Recorded via Motic camera and software for 10 seconds
  - Uploaded to ImageJ Fiji for analysis

METHODOLOGY:

Swimming Speed/Dilutions:

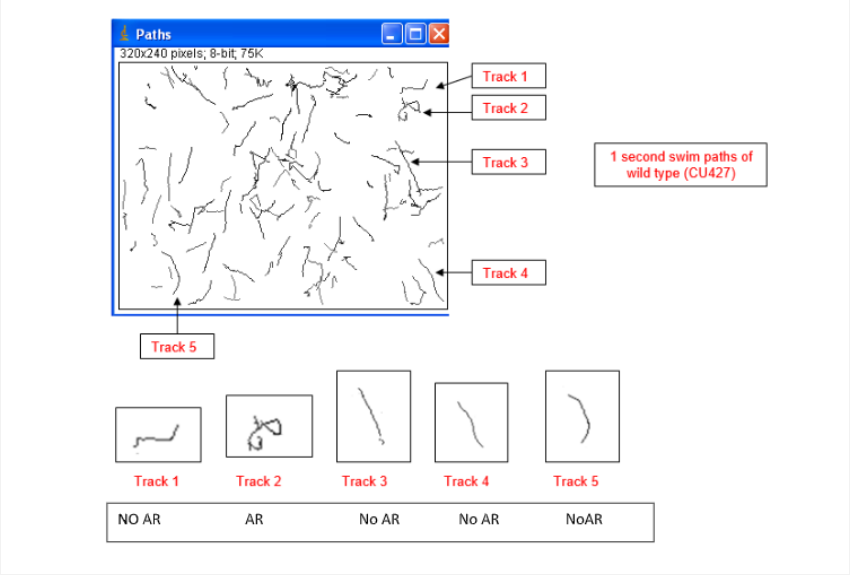
Started with initial solution of 5 mM and tested

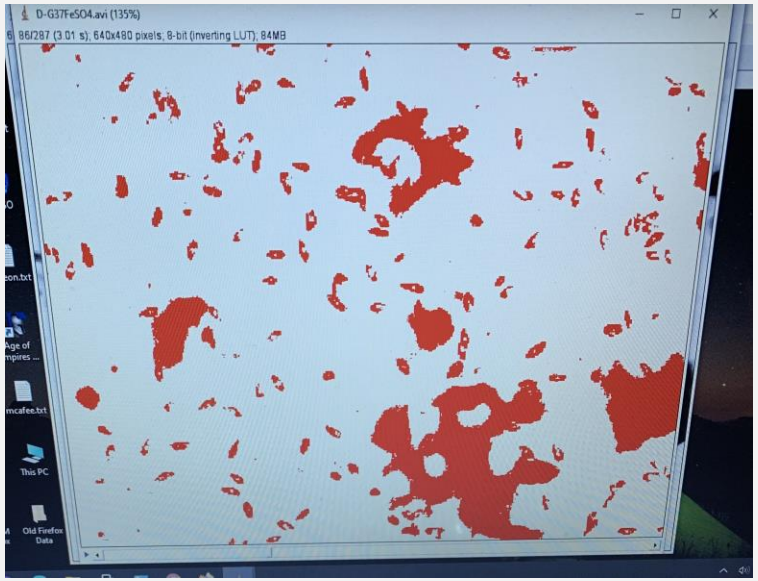
Diluted 1:10, 1:100, 1:1000, 1:10,000

Observed for ARs physically

Recorded for 10-15 seconds for ImageJ speed analysis

Helps indicate viable environment for cells





METHODOLOGY:

Data Analysis

Trials repeated three times

Focused on 5-10 cells per recording

Tracked physically and follow swimming paths

- Tally if exhibiting AR behavior (ARs, backwards swimming, decreased swimming speed, whirling in place, etc.)
- Averaged results of trials for test chemicals
- ImageJ analysis for tracks
- Compiled graphs based on percentages

RESULTS:

CURRENT RESULTS:

- **All trials** exhibited reliable AR behavior percentages
- 82.2% of G37 cells exhibited ARs
- 68.0% CU427 exhibited ARs
- Tested in 5mM ferrous sulfate

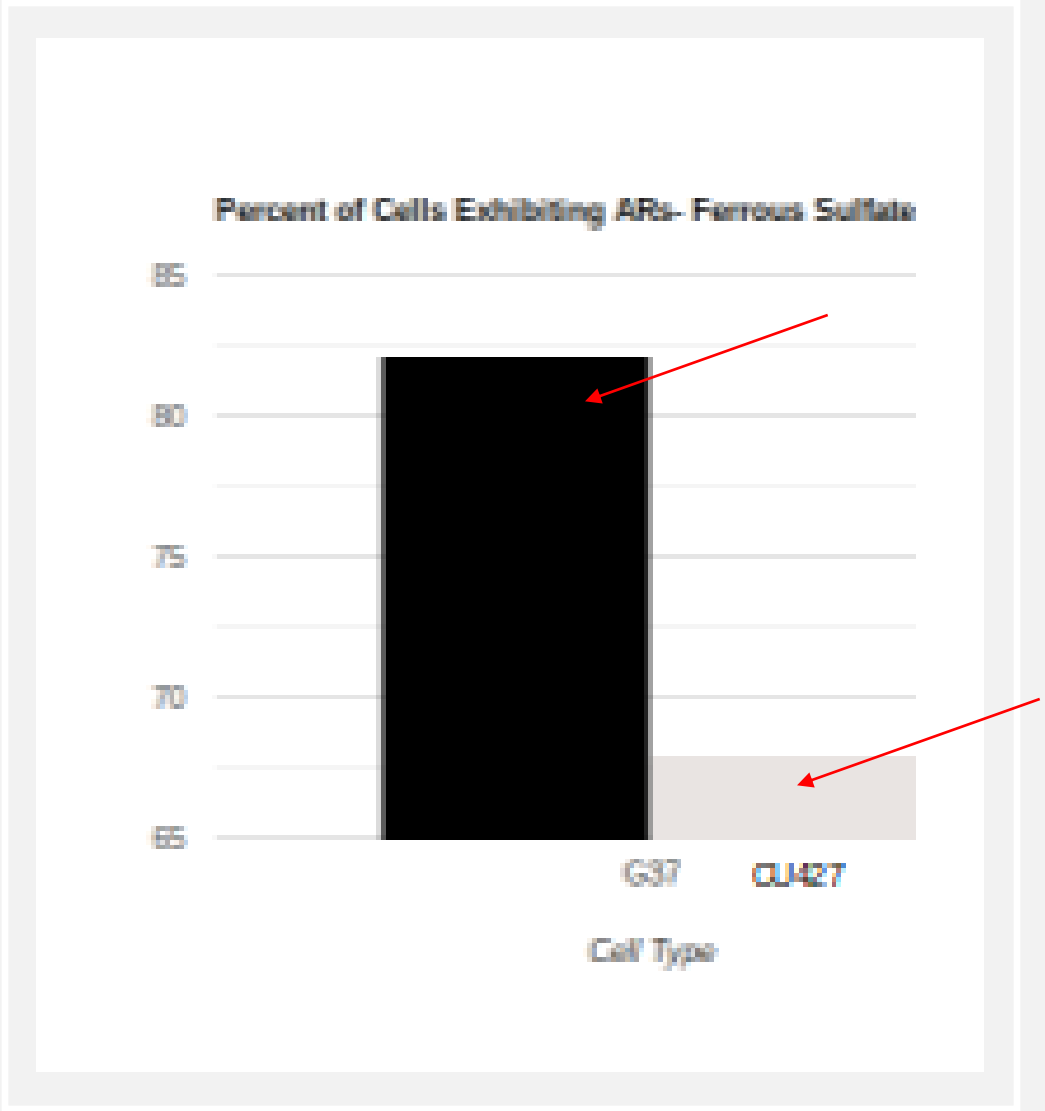


Fig. 1: Percentage of cells exhibiting ARs when exposed to ferrous sulfate

CURRENT RESULTS:

- Cells showed reliable percentage of ARs in **all trials**
- 86.0% G37 cells exhibited ARs
- 38.0% CU427 cells exhibited ARs

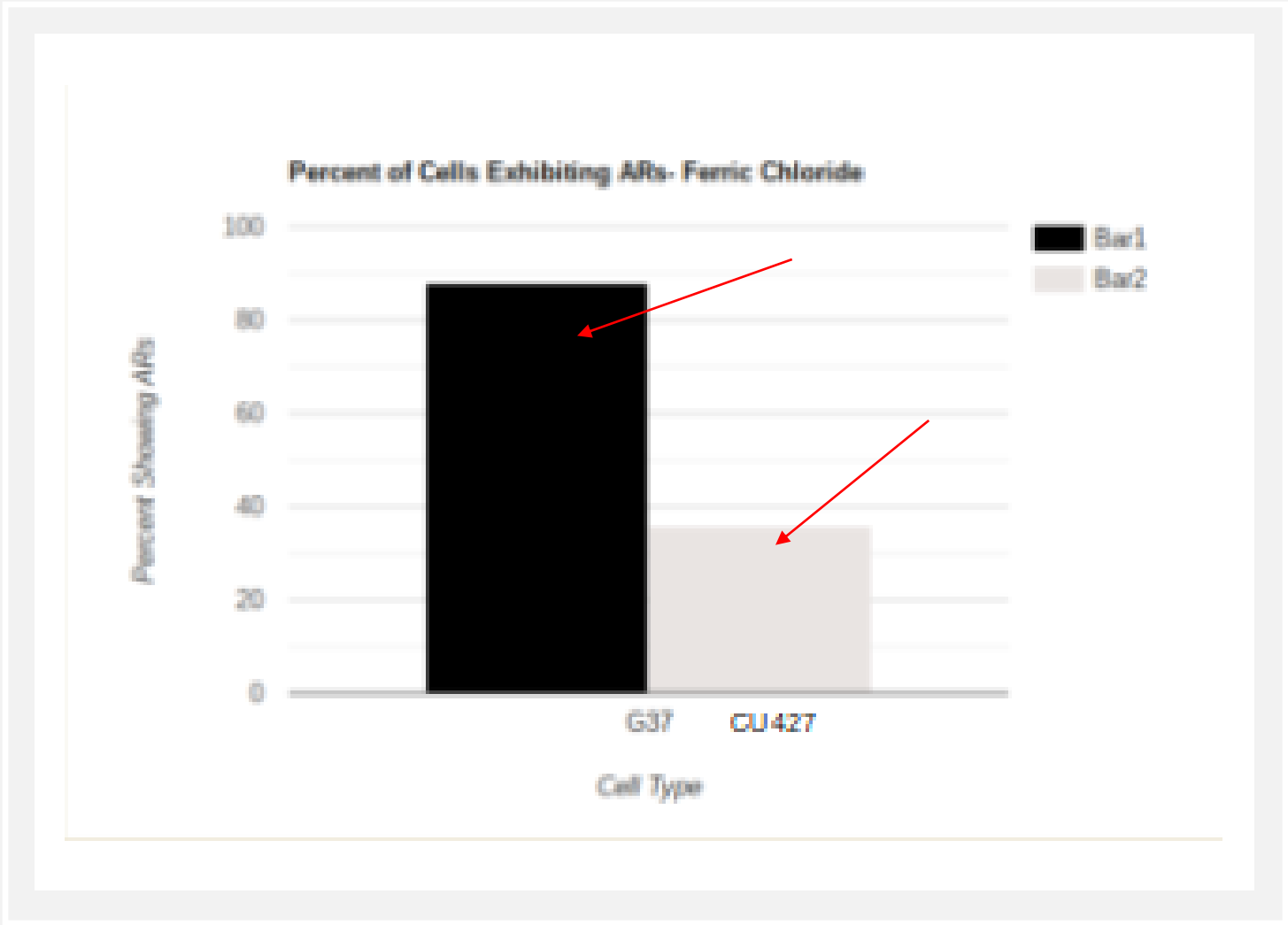
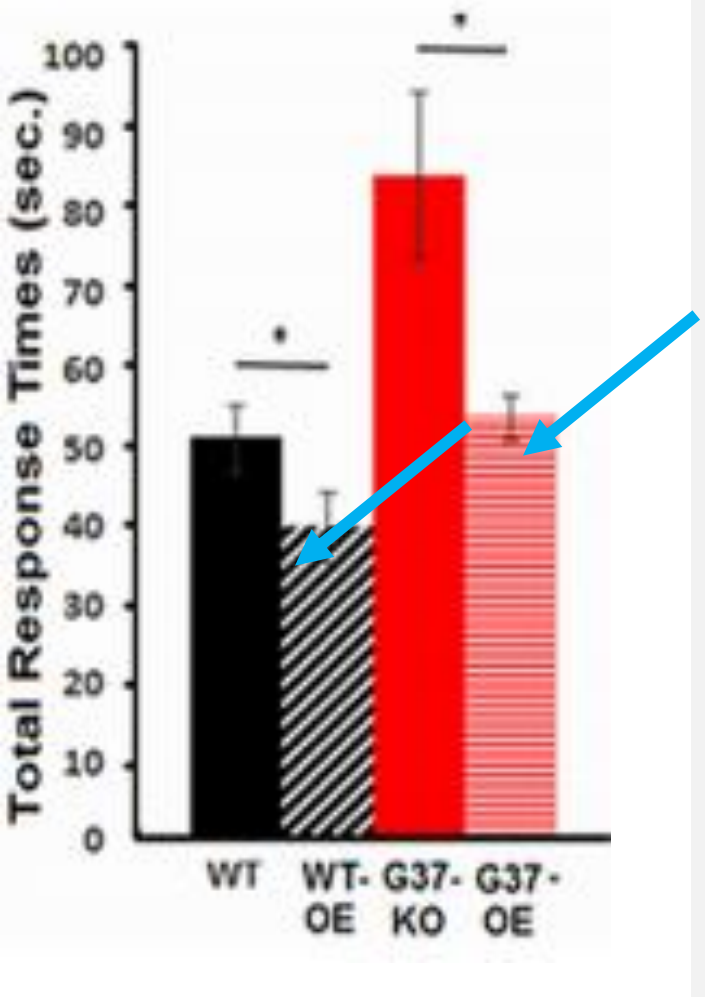


Fig. 2: Percentage of cells exhibiting ARs in response to ferric chloride

PREDICTED RESULTS:

Based on Zhou and Hennessey, 2017 (17)

- G37 cells will be less responsive to oxidants
  - Hydrogen peroxide
  - Tetrazolium Blue
  - Cytochrome C

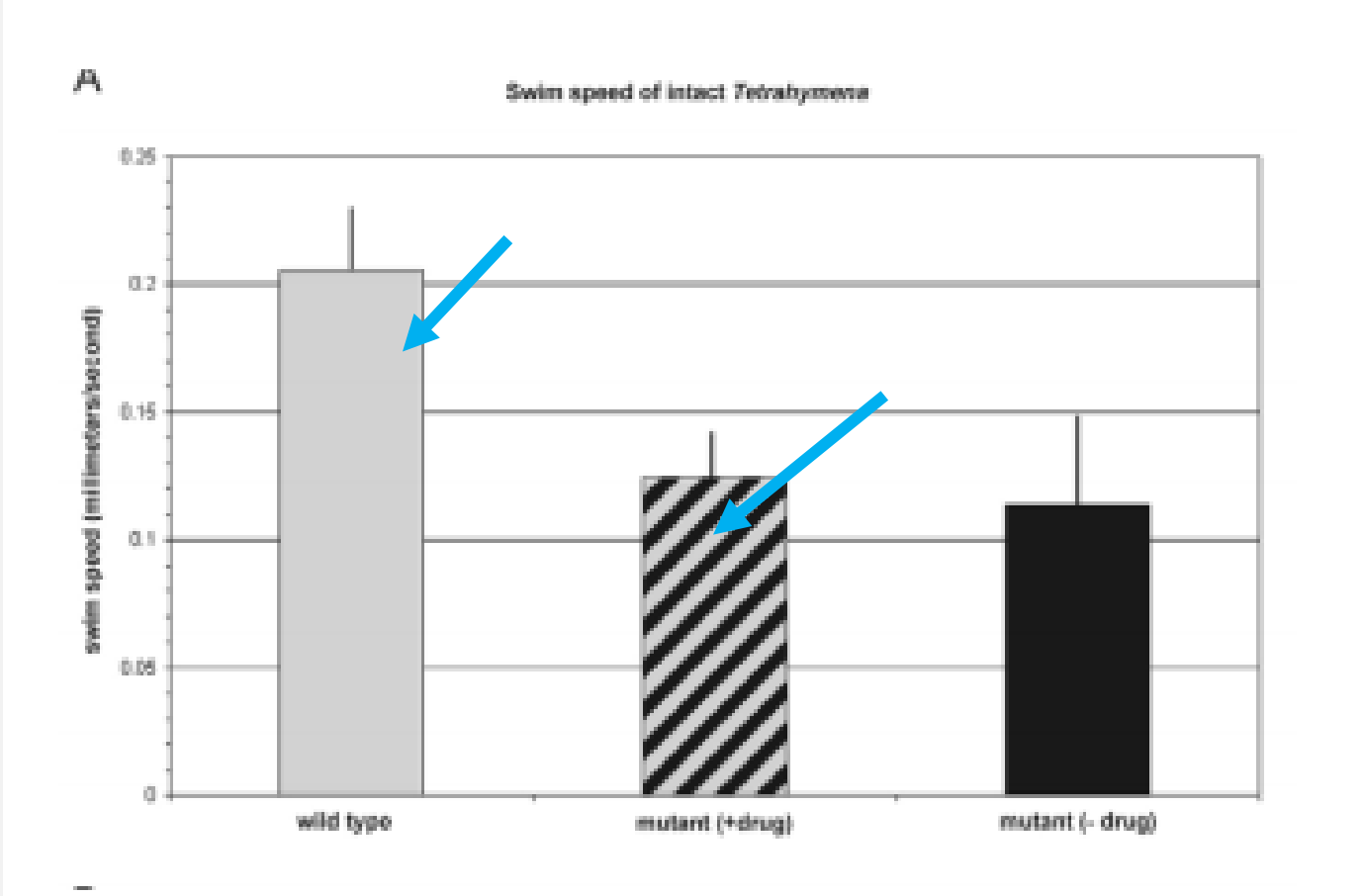


Zhou and Hennessey, 2017

PREDICTED RESULTS:

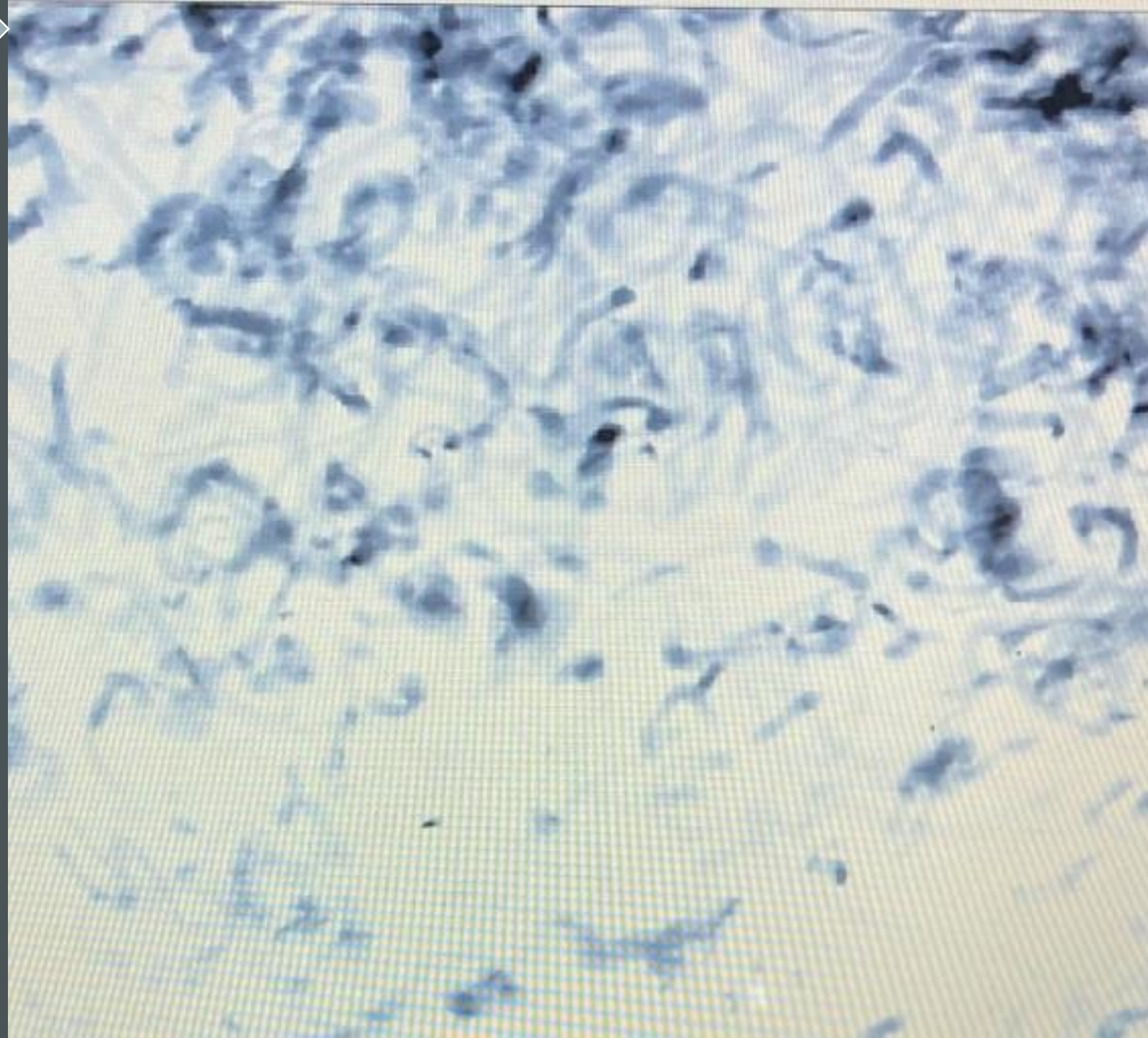
Based on results of Hennessey et al. 2007 (21)

- G37 exhibit lower swim speeds to reductants
  - DTT
    - WT: .2 mm/s
    - Mutant: .13 mm/s



DISCUSSION:

- G37 cells are more responsive to iron solutions
  - Both reduced and oxidized
  - Exhibited most in ferric chloride- oxidant
- Oxidants have greater effect on G37 receptor
  - Possibly due to structure interactions
  - Unclear structure
  - Iron is effective chemorepellent

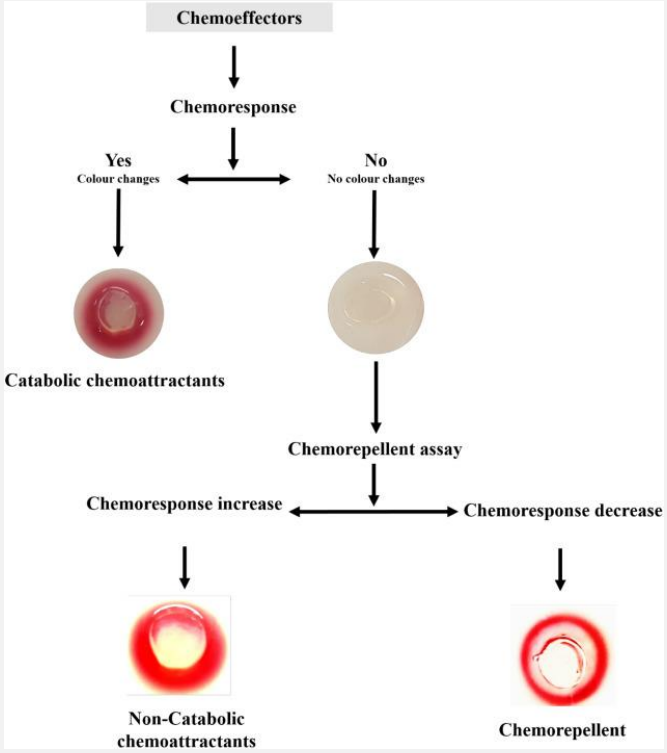


DISCUSSION: PREDICTED RESULTS

- All other non-iron oxidants had negligible effect on G37
  - NBT, hydrogen peroxide, cytochrome C
  - Longer response times
- Reductant DTT affects G37; **not** considerably
  - Lower swim speeds
- Indicative of iron-based receptor
  - Not all non-permeable oxidants/reductants affect G37 receptor
  - Unsure of other reliable G37 chemorepellents

SIGNIFICANCE & LIMITATIONS

- **Limitations:**
  - Resources
  - Imaging Quality
  - Time
- **Significance:**
  - Evolutionary history of neuron
  - Models neuronal response
  - Insight on critical survival pathway



https://www.sciencedirect.com/science/article/abs/pii/S0167701218300976

CONCLUSION:

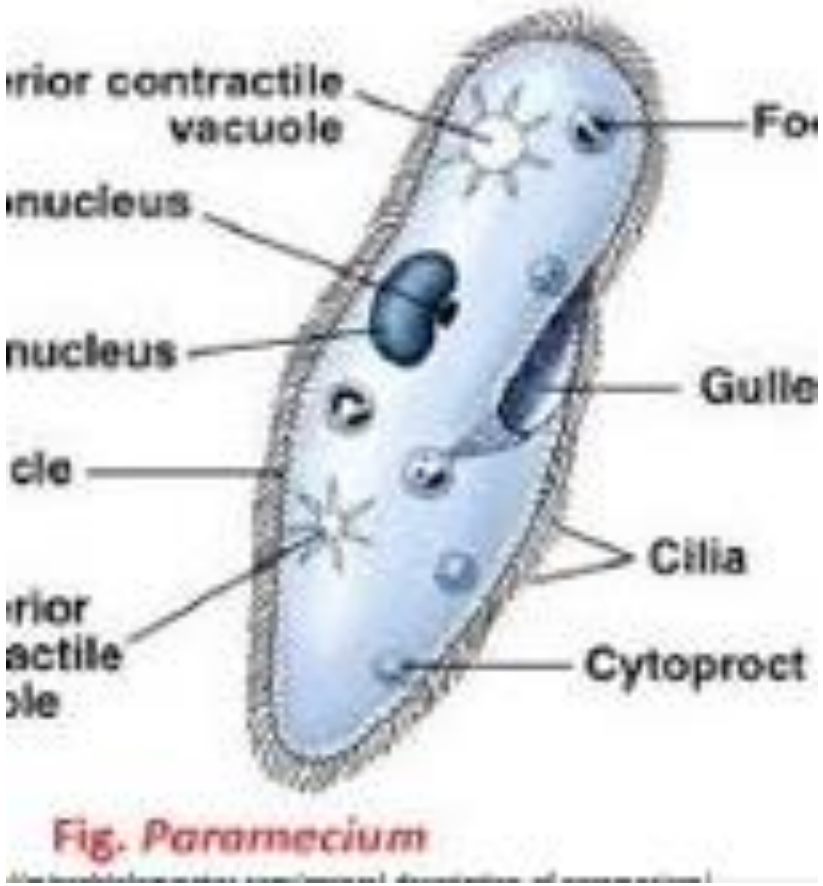
CONCLUSION

Purpose: Determine effects of chemorepellents on G37 receptor compared to wild-type

Methodology: Cell cultures, Behavioral assays-5 test chemicals, ImageJ analysis

Results: Iron-higher G37 ARs; non-iron oxidants/reductants: less G37 ARs/swimming speed

Significance: Novel receptor is affected by iron (both forms); insight on pathways; can be applied to other organisms



https://www.wesleylife.org/memory-care/get-the-facts/

FUTURE RESEARCH:

- Chemoattractants effect on G37
- Other test chemicals-oxidants and reductants
- Changes in membrane potential of G37 & CU427
- GPCR3 structure/response to chemical stimuli
- Responses in *Paramecium* mutant genes

ACKNOWLEDGMENTS:

Dr. Todd Hennessey

University at Buffalo & Department of Arts and Sciences

Ms. Gillian Rinaldo

Science Research Peers

Friends and Family

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