UNIVERSITY of WASHINGTON

ACT Project 3: Translational Pharmacoepidemiology

Project Leads: Shelly Gray, PharmD, MS; Jessica Young, PhD

Presenting today: Jessica Young, PhD; Shelly Gray, PharmD, MS; Tiara Schwarze-Taufiq, BS

What is Translational Pharmacoepidemiology?

Combining population-based observational studies with *in vitro* cellular models to uncover mechanisms by which medications taken by older adults could lead to dementia.

Why is this important?

- Older adults use a wide range of medications that may have off-target effects.
- Observational studies cannot tell whether it is the drug itself or the condition for which it was prescribed for that increases dementia risk.
- This concept is known as ”Confounding by Indication”

How are we addressing this in Project 3?

Aim 1: Deploy a human stem cell-based molecular assay to directly test mechanisms of neurotoxicity from AChs and address confounding by indication.

Aim 2: To determine comparative associations of AHTs with dementia and AD using neuropathology and neuroimaging outcomes. Test cellular mechanisms of neuroprotection.

Focus on Aim 1

Aim 1: Deploy a human stem cell-based molecular assay to directly test mechanisms of neurotoxicity from AChs and address confounding by indication.

ACh Background and Rationale

Original Investigation

Cumulative Use of Strong Anticholinergics and Incident Dementia
A Prospective Cohort Study

Shelly L. Gray, PharmD, MS; Melissa L. Anderson, MS; Sascha Dublin, MD, PhD; Joseph T. Hanlon, PharmD, MS; Rebecca Hubbard, PhD; Rod Walker, MS; Onchee Yu, MS; Paul K. Crane, MD, MPH; Eric B. Larson, MD, MPH

Gray et al. *JAMA Intern Med* 2015; 175:401-407.

AC Exposure is Associated with Dementia and Alzheimer's Disease

ACh Use	Dementia HR (95% CI)	AD HR (95% CI)
No use	*1*	*1*
< 90 TSDD	0.92 (0.74-1.16)	0.95 (0.74-1.23)
90 – 365 TSDD	1.19 (0.94-1.51)	1.15 (0.88-1.51)
365 – 1095 TSDD	1.23 (0.94-1.62)	1.30 (0.96-1.76)
> 1095 TSDD	1.54 (1.21-1.96)	1.63 (1.24-2.14)

Adjusted for age, study cohort, sex, education, hypertension, diabetes, smoking, stroke, coronary heart disease, body mass index, exercise, self-rated health, depression, Parkinsons disease, benzodiazepines

Gray SL et al. JAMA Intern Med 2015; 175(3):401-407.

Dementia risk may vary by ACh medication class

Anticholinergic drugs and risk of dementia: case-control study

Kathryn Richardson,[1] Chris Fox,[2] Ian Maidment,[3] Nicholas Steel,[2] Yoon K Loke,[2] Antony Arthur,[1] Phyo K Myint,[4] Carlota M Grossi,[1] Katharina Mattishent,[2] Kathleen Bennett,[5] Noll L Campbell,[6] Malaz Boustani,[7] Louise Robinson,[8] Carol Brayne,[9] Fiona E Matthews,[10] George M Savva[1]

- ✓ Antidepressants
- ✓ Bladder antimuscarinics
- ✓ Antiparkinson drugs

- ⊘ Antihistamines, antispasmodics, antipsychotics,

Anticholinergic Drug Exposure and the Risk of Dementia

A Nested Case-Control Study

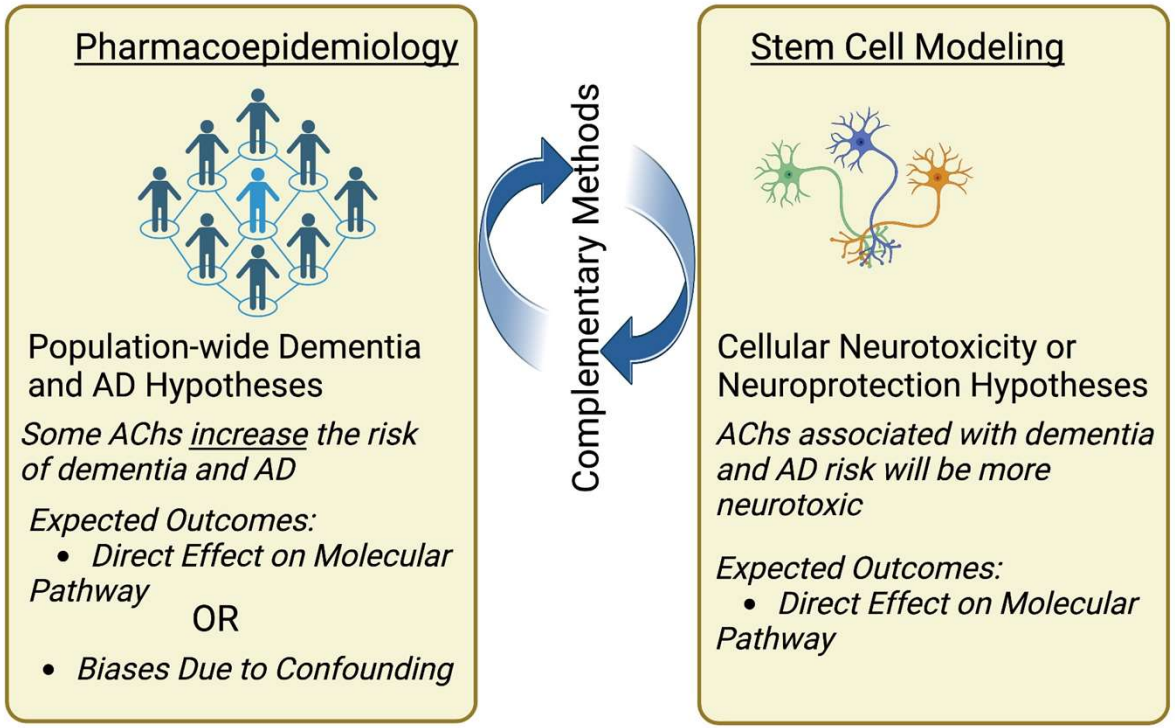
Carol A. C. Coupland, PhD,[✉1] Trevor Hill, MSc,[1] Tom Dening, MD,[2] Richard Morriss, MD,[2] Michael Moore, MSc,[3] and Julia Hippisley-Cox, MD[1,4]

- ✓ Antidepressants
- ✓ Bladder antimuscarinics
- ✓ Antiparkinson drugs
- ✓ Antipsychotics, antiepileptics

- ⊘ Antihistamines, skeletal muscle relaxants, gastrointestinal antispasmodics

Richardson K et. al. BMJ 2018;361:k1315 | doi: 10.1136/bmj.k1315
Coupland et al. JAMA Intern Med. 2019;179(8):1084-1093

Model



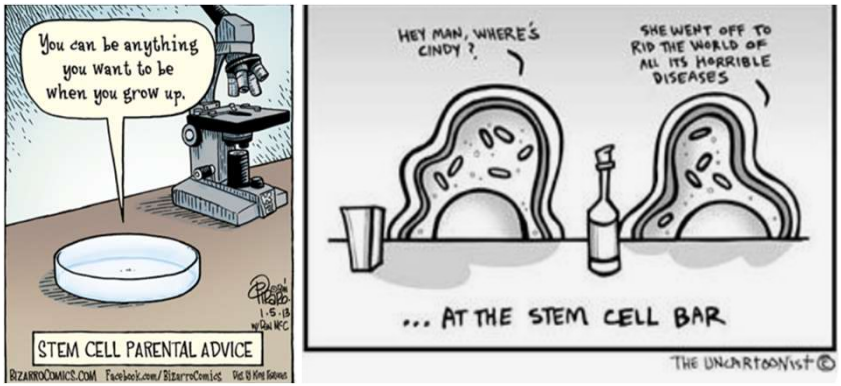
Possible Cellular Mechanisms:

1. Downstream events from Antagonism of ACh receptors.

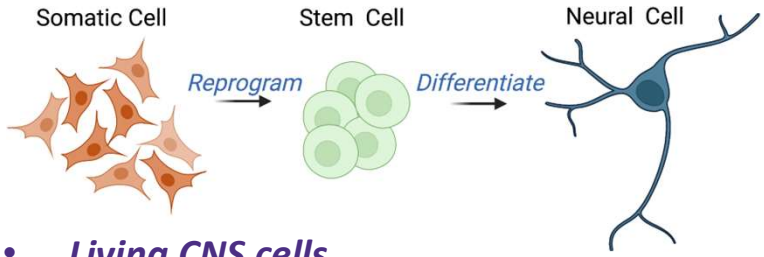
2. Off-target effects of drugs
   *Can still affect pathway related to AD.

We will test this using human induced pluripotent stem cells (hiPSCs)

- These are somatic cells, taken from a patient with a disease.
- They have been “reprogrammed” to a stem-cell state.
- They can become any cell type of the body.



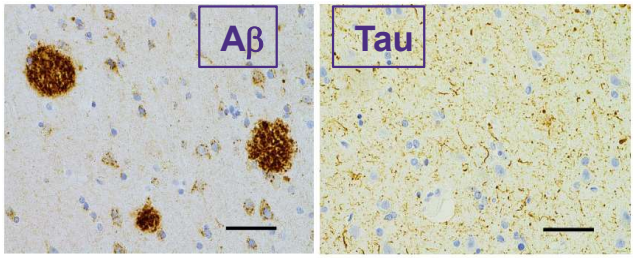
Human Induced Pluripotent Stem Cells



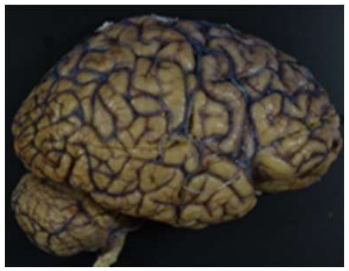
- ***Living CNS cells***
- ***Human Genetics***
- ***Reductionist model that can directly test the effect of the drug***

The process to generate hiPSC-derived neurons

- We take leptomeningeal tissue collected at autopsy from ACT subjects
- These subjects have a neuropathological diagnosis of AD or no-AD.
- In the lab, we dissect this tissue and culture it as a primary leptomeningeal cell line

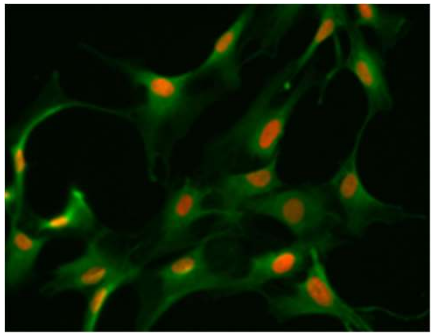


In collaboration with Neuropathology Core: Dr. Keene's group



~ 2 weeks

Primary leptomeningeal cells



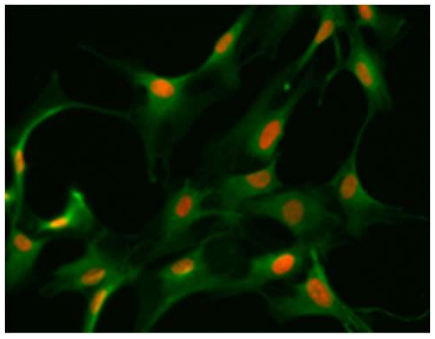
Rose…Keene, Young, JNEN 2018.

The process to generate hiPSC-derived neurons

- We reprogram these cells by transfecting them with four factors
  - OCT3/4
  - KLF4
  - SOX2
  - L-MYC

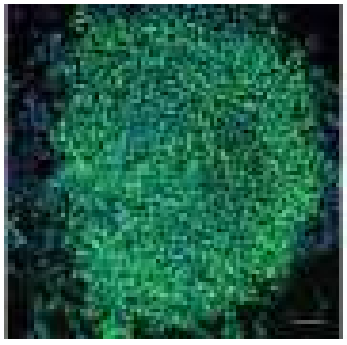
Pluripotency factors: mimic gene expression found in an embryo
Nobel Price 2012

Primary leptomeningeal cells



~30 days

hiPSC colony: Can become any cell type



The process to generate hiPSC-derived neurons

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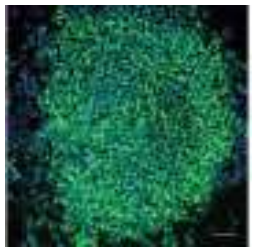
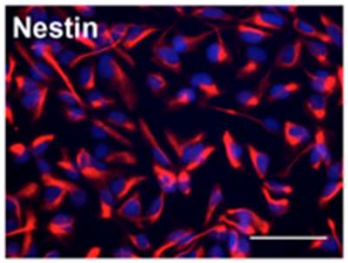


12 days

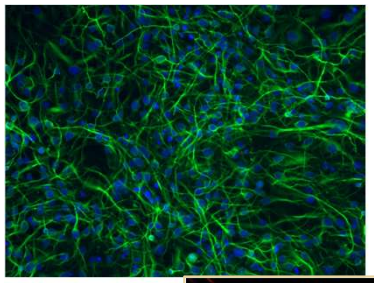
Promote neuroectoderm
and neural progenitor cells

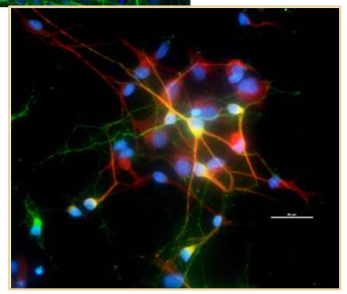
Neural progenitor cells



60 days

Promote excitatory
cortical neurons





Stem Cell Rationale

> These cells have the genetic background of the ACT participant from whom they were derived

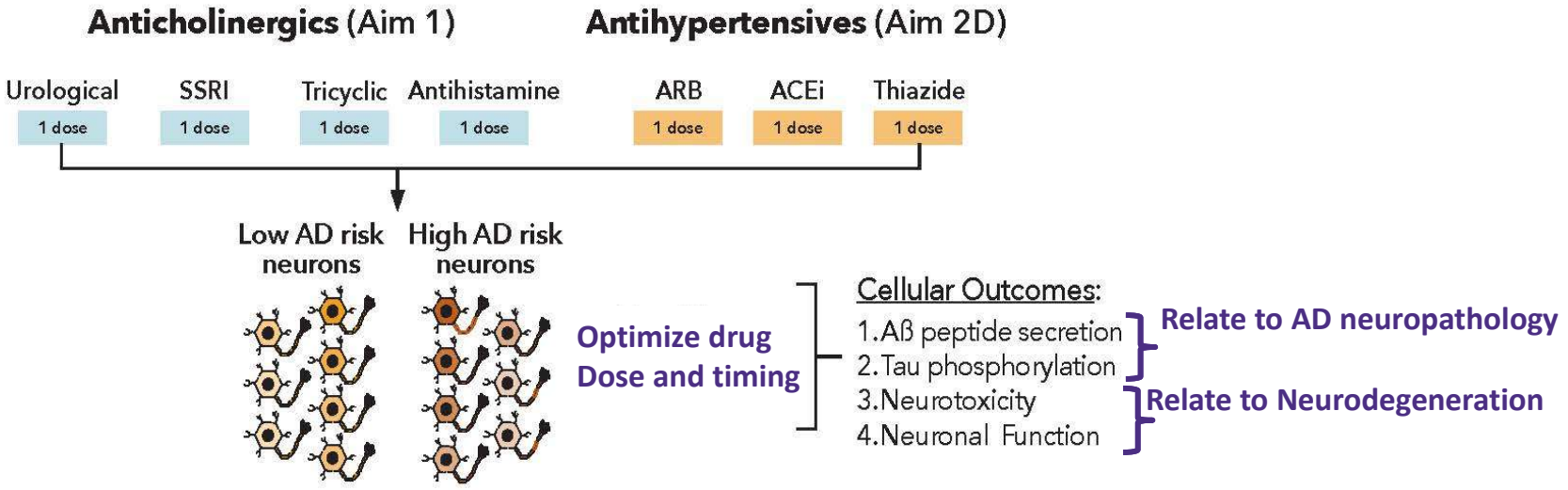
> The cells can be used to test the direct effects of a drug and understand the mechanism of action at the cellular level.

> Conditions that may contribute to confounding by indication are removed

> We anticipate that these experiments combined with the observational studies in ACT will provide clarity to the ACh and AHT hypotheses.

Overall Project

- Development of a bioassay that measures relevant AD cellular phenotypes after treatment with a drug.
- We will use cell lines generated from ACT patients with high and low AD risk.
- We will measure four cellular outcomes.



Understanding Cholinergic Signaling Pathways in Neurons

	Muscarinic	Nicotinic
Signalling Type	Metabotropic (G-protein coupled)	Ionotropic
Subtypes	M1-M5	N1, N2
Localization	M1-M5 all found in brain Also found in heart, intestine, and bladder	N1– peripheral/muscle N2– central/neuronal
Relevance to brain	M1– learning and higher cognitive processes All– many; BBB permeability, synaptic plasticity	Neurotransmitter release, small subset of fast excitatory transmission, neuroinflammation

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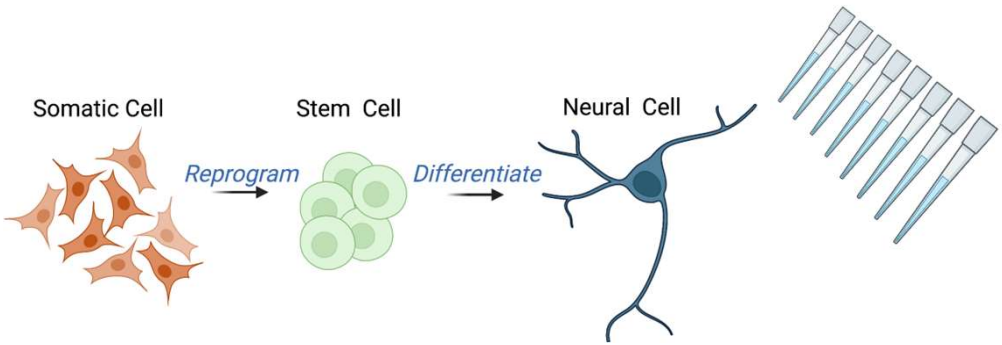
Hypotheses about what blocking these receptors might do

- Blockage of normal and pathological tau uptake in neurons
- Altered equilibrium between amyloidogenic and non-amyloidogenic APP processing
- Animal studies suggest that muscarinic antagonism may decrease both short and long-term potentiation

Proof-of-Concept experiment



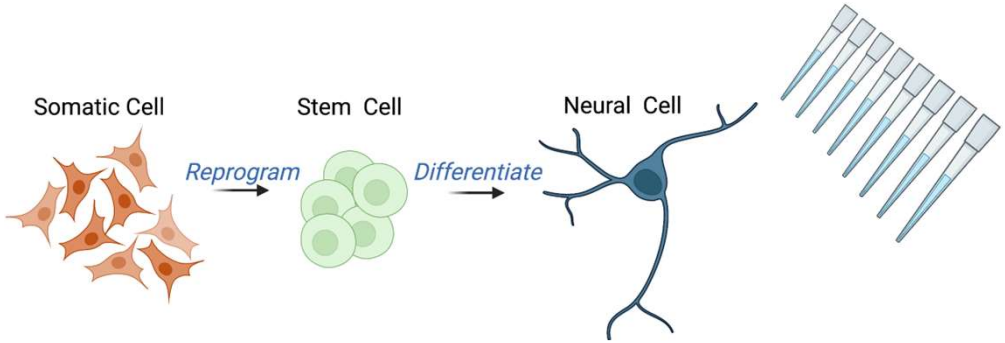
Add ACh treatments:
8 drugs
2 doses
2 timepoints

Measure:
Cytotoxicity,
Aβ

WT Neurons

AD Neurons (APP Swedish Mutation)

Proof-of-Concept experiment



WT Neurons

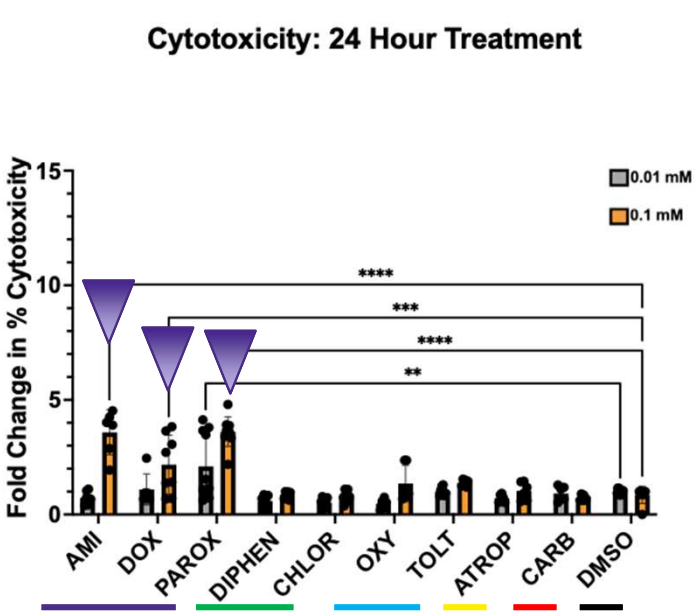
AD Neurons (APP Swedish Mutation)

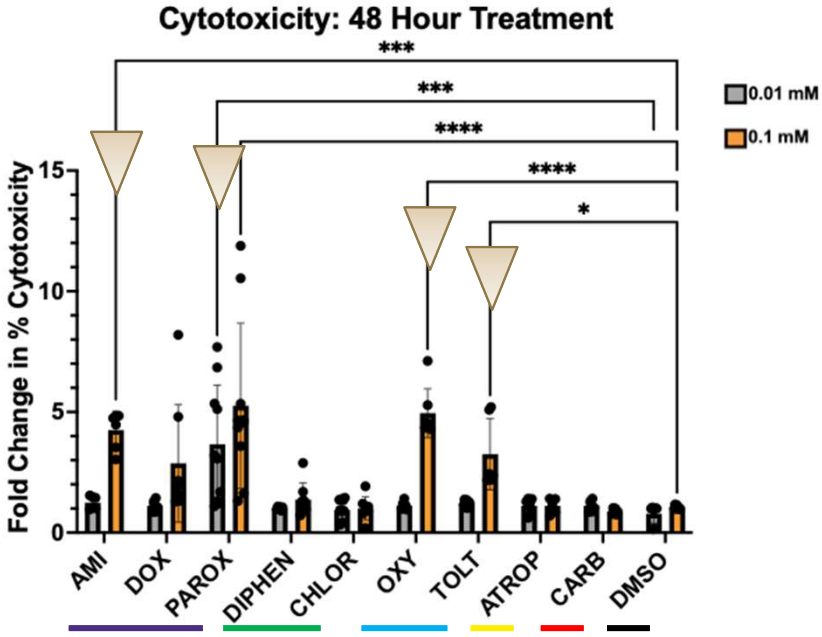
Drugs tested:

- Antidepressants:
  - Amitriptyline
  - Doxepin
  - Paroxetine
- Antihistamines
  - Diphenhydramine
  - Chlorpheniramine
- Bladder antimuscarinics
  - Oxybutynin
  - Tolterodine
- Antispasmodics
  - Atropine

Results

> Cytotoxicity

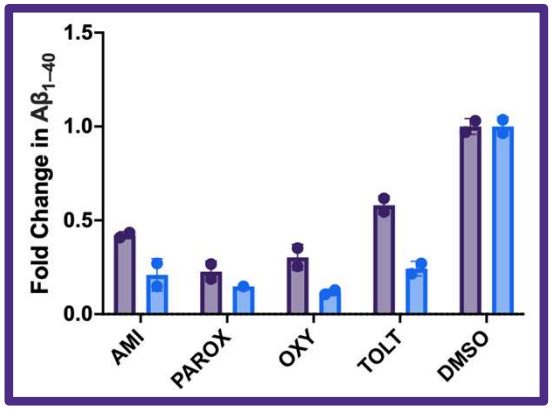


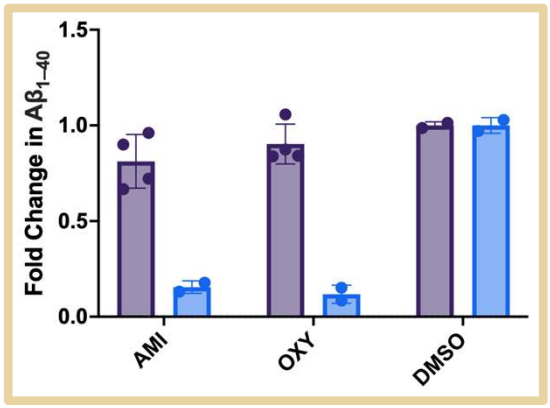


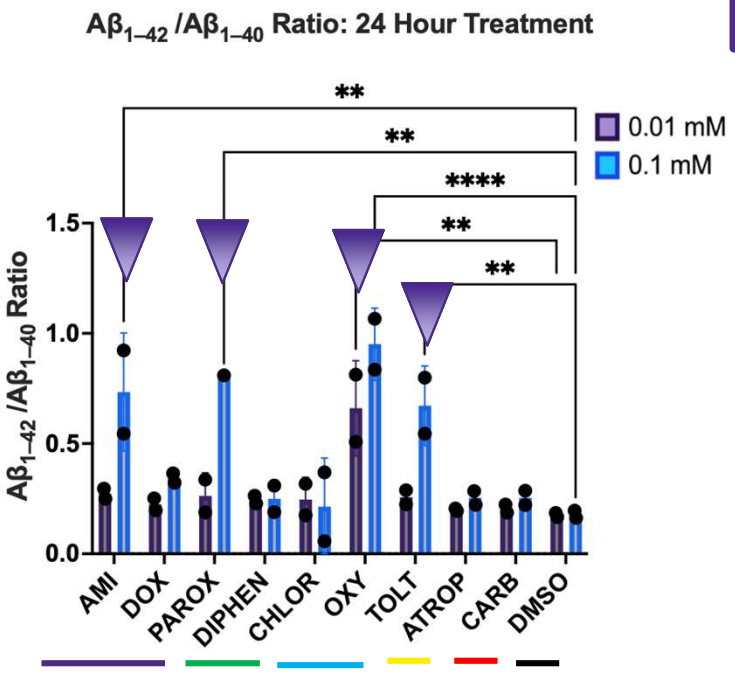
Antidepressants
Antihistamines
Bladder antimuscarinics
Antispasmodics
Cholinergic agonist
Vehicle control

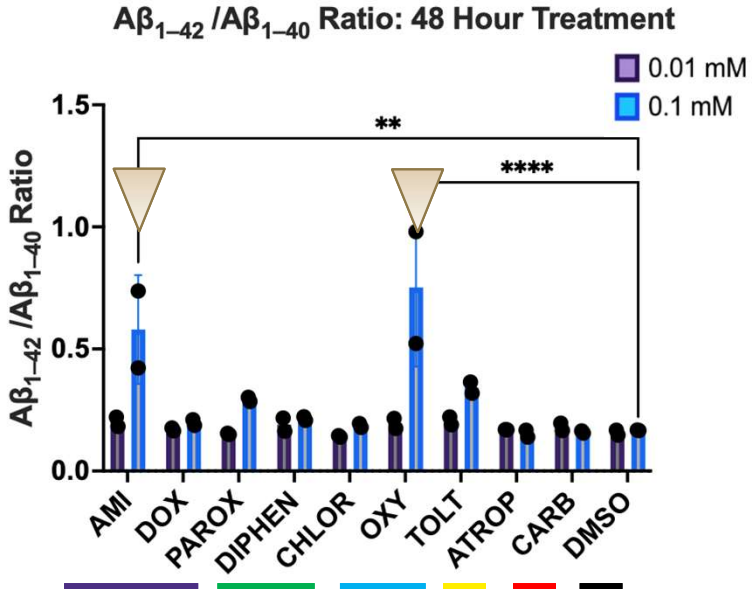
Results

> Amyloid Beta









Antidepressants
Antihistamines
Bladder antimuscarinics
Antispasmodics
Cholinergic agonist
Vehicle control

Results

> Summary

Table 1.	Association found in observational studies	Neurotoxicity in stem cell-derived neurons
Antidepressants	Yes: Positive Association	
Amitriptyline		Dose-dependent
Doxepin		Dose & time dependent
Paroxetine		Dose & time dependent
Antihistamines	No	
Diphenhydramine		None
Chlorpheniramine		None
Bladder antimuscarinics	Yes: Positive Association	
Oxybutynin		Dose & time dependent
Tolterodine		None
Antispasmodics	No	
Atropine		None

Dose and time-dependent effects

- Examining dose and time dependence may clarify nuances in how drugs exert effects on AD phenotypes.

- Differences in molecular pathways that lead to neurotoxicity or changes in APP processing may occur at specific concentrations or exposure periods.

Conclusions

> Cytotoxicity differs by class and between individual drugs of the same class

- Antidepressants and bladder antimuscarinics demonstrated toxicity while antihistamines and antispasmodics did not, matching population study findings

> Drugs demonstrating toxicity increased the ratio of secreted AB42/40 in a dose- and time-dependent manner

Future work

- **Testing in ACT participant cell lines**
  - **23 hiPSC lines generated**
  - **12 AD/11CTL: Neuropathological Diagnosis**
  - **10 Male/13 Female**

Future work

- Experiments testing engagement of pathways involved in muscarinic antagonism and/or off-target effects of each drug
- Assays for different proteins involved in amyloid processing
- Dose-response for drugs demonstrating toxicity

Project Team:

UW

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