UNIVERSITY of WASHINGTON

ISCRM

# Evaluating the Impact of Antihypertensive and Anticholinergic Medications on Dementia Prognosis using Pharmacoepidemiology and Stem Cell-Based Approach


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# Project 3 Objectives

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

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

# Angiotensin II–Stimulating Antihypertensives and Dementia-related Neuropathology

# Background

Antihypertensives (AHT) can be categorized according to their activity at the type 2 and 4 angiotensin II receptors.

## Ang II Stimulating AHT

- Angiotensin II receptor blockers (ARBs)
- Dihydropyridine calcium channel blockers
- Thiazide diuretics

## Ang II Inhibiting AHT

- Angiotensin-converting enzyme inhibitors (ACE-Is)
- β-blockers
- Non-dihydropyridine calcium channel blockers

# Background

- Observational studies support the angiotensin II hypothesis where AngII stimulating AHTs are associated with lower dementia risk compared with inhibiting AHTs.
- Use of Ang II stimulating AHTs was associated with:
  - 45% lower incidence rate of dementia over 6.7 years of follow-up.
  - 24% lower incidence rate of probable dementia or amnestic mild cognitive impairment (MCI) over 4.8 years of follow-up in the Systolic Blood Pressure Intervention Trial (SPRINT).

JAMA Netw Open. 2022; 5(1):e2145319
*Neurology*. 2021;96(1):e67
*JAMA Netw Open.* 2023;6(1):e2249370
Lancet Reg Health Eur. 2024 May 15;42:100927

# Sample and Eligibility

- ACT participants with ≥ 1 person-year (PY) of stimulating or inhibiting AHT exposure
- ≥ 1 biennial follow-up visit.
- ≥ 80% of the follow-up period with continuous KPWA enrollment
- Consented to brain autopsy, had died, and with neuropathological outcomes
- Had blood pressure data in years of AHT use
- Sample is 756

# Outcomes

| Primary |
| --- |
| CERAD (moderate/frequent) |
| Braak stage (V or VI) |
| LATE present (2 or 3) |
| Cerebral microinfarcts (any) |
| ADNC (intermediate/high) |

| Secondary |
| --- |
| Thal phase (3-5) |
| Cerebral amyloid angiopathy (any) |
| Atherosclerosis (mod to severe) |
| Arteriosclerosis (mod to severe) |
| Macroscopic infarcts (any) |

# Antihypertensive Use

- Person-year (PY) of stimulating and inhibiting AHTs in two sources:

**Pre-1977**

Manual review and abstraction of paper-based medical records which captures the year and name of each AHT.

**One PY** defined as one mention of AHT in a calendar year

**Since 1977**

Electronically in KPWA automated pharmacy dispensing data.

**One PY** defined as two fills in a calendar year

- Modeled cumulative PY of stimulating and inhibiting AHTs
  - total PY of use (primary, continuous exposure)
  - long-term use (secondary, binary exposure) defined as ≥15 years of cumulative exposure
- Follow-up is from earliest fill to death

# Statistical Analysis

- Modeled binary and continuous outcomes with multivariable modified Poisson and linear models, respectively
- Adjusted for demographic and clinical characteristics, including average SBP and DBP during follow-up, with attention paid to confounding by indication (atrial fibrillation, myocardial infarction, diabetes, stroke)
- Accounted for selection bias using augmented inverse probability weighting.

# Characteristics (n=756)

| Characteristics | N (%) |
|---|---|
| Age at death, mean (SD) | 89 (6) |
| Female | 440 (58) |
| White | 710 (94) |
| **History of comorbidities at time of death** | |
| Diabetes | 245 (32) |
| Stroke | 429 (57) |
| Myocardial infarction | 417 (55) |
| Atrial fibrillation | 429 (57) |
| Coronary artery disease | 456 (60) |

# Exposure and Blood Pressure across follow-up

| | |
|---|---|
| **Antihypertensive medication, mean (SD) total PY of use** | |
| Stimulating medications | 9.3 (10.1) |
| Inhibiting medications | 12.2 (9.2) |
| **Type of AngII antihypertensive, n (%)** | |
| Stimulating only | 50 (7) |
| Inhibiting only | 172 (23) |
| Both | 534 (71) |
| **Blood Pressure, mean (SD)** | 429 (57) |
| Average annual systolic blood pressure, mmHg | 136 (13) |
| Average annual diastolic blood pressure, mmHg | 74 (7) |

# Primary Neuropathologic Outcomes

| Outcomes | N | Outcome prevalence N (%) | Cumulative PY of exposure RR (95% CI) | Long-term use (≥ 15 yrs) RR (95% CI) |
|---|---|---|---|---|
| CERAD (moderate/frequent) | 756 | 413 (55) | 0.99 (0.97-1.01) | 0.83 (0.61-1.12) |
| Braak stage (V or VI) | 750 | 288 (38) | 0.98 (0.96-1.00) | 0.68 (0.46-1.03) |
| LATE present (2 or 3) | 736 | 207 (28) | 0.97 (0.95-1.00)* | 0.57 (0.35-0.94)* |
| Cerebral microinfarcts (any) | 753 | 377 (50) | 1.00 (0.98-1.01) | 1.15 (0.85-1.55) |
| ADNC (intermediate/high) | 742 | 431 (58) | 0.99 (0.97-1.00) | 0.72 (0.54-0.96) |

Model adjusted for ACT study cohort, age at death, age at first known AHT use, sex, average annual diastolic and systolic blood pressure, history of atrial fibrillation, diabetes, myocardial infarction, and stroke any time prior to death. Age and blood pressure were modeled as natural cubic splines with two knots at the tertile.

*$p<0.05$

# Secondary Neuropathologic Outcomes

| Outcomes | N | Prevalence N (%) | Cumulative PY of exposure RR (95% CI) | Long-term exposure (≥ 15 yrs) RR (95% CI) |
|---|---|---|---|---|
| Thal phase (3-5) | 748 | 501 (67) | 1.00 (0.98-1.01) | 0.96 (0.75-1.22) |
| Cerebral amyloid angiopathy (any) | 756 | 387 (51) | 1.00 (0.98-1.02) | 0.96 (0.70-1.33) |
| Atherosclerosis (mod to severe) | 745 | 554 (74) | 1.00 (0.98-1.01) | 0.94 (0.78-1.13) |
| Arteriosclerosis (mod to severe) | 661 | 508 (77) | 0.99 (0.98-1.00)* | 0.72 (0.60-0.88)** |
| Macroscopic infarcts (any) | 753 | 268 (36) | 1.00 (0.98-1.02) | 0.89 (0.60-1.31) |

Adjusted as prior model. *p<0.05, **p<.001

# Summary

- In this community-based autopsy cohort, we provide the first examination of whether Ang II stimulating compared with AngII inhibiting antihypertensives are associated with a lower risk of dementia-related neuropathology, adjusting for blood pressure throughout the follow-up period.

- We found a lower risk of LATE pathology, moderate/severe arteriolosclerosis and intermediate/high ADNC associated with exposure to Ang II stimulating AHT vs. Ang inhibiting antihypertensives.

# Human stem cell-based molecular assay to directly test mechanisms of neurotoxicity from anticholinergics (Achs)

# Background

- Overall anticholinergic burden is associated with increased risk for dementia.
- Recent studies have assessed ACh exposure according to pharmacological or therapeutic class and found inconsistent associations across these ACh classes

| Association with dementia | ACh class | Example medications |
|---|---|---|
| Yes | Antidepressants | Amitriptyline, Doxepin, Paroxetine |
| Yes | Bladder antimuscarinics | Oxybutynin, Tolterodine |
| No | Antihistamines | Chlorpheniramine, Diphenhydramine |
| Mixed | Antispasmodic | Atropine |

BMJ 2018;361:k1315; JAMA Intern Med. 2019;179(8):1084-1093

# Challenge



## Understanding these factors in patient populations can be challenging and have bias

Medical condition associated with dementia risk : Depression

Medication used to treat depression: Antidepressant

Medication associated with dementia risk: Antidepressant

***What is the underlying cause?***

# Methodology



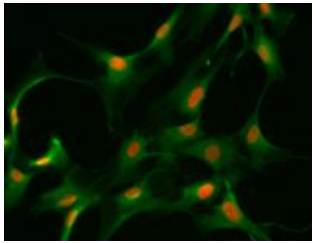
## Human Induced Pluripotent Stem Cell (hiPSCs) can help to answer this question

Post-mortem brain

ACT
ADULT CHANGES IN THOUGHT STUDY
Kaiser Permanente Washington Health Research Institute

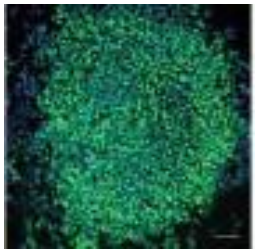
**14 ACT participants:**
7 males and 7 females

Leptomeningeal cells



*Reprogram*

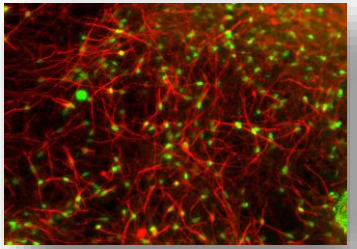
hiPSCs

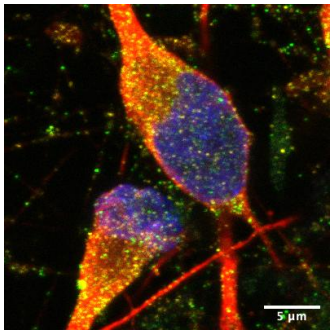


**14 hiPSC lines**

*Differentiate*

hiPSC-derived cortical neurons






Neurons express synaptic proteins and show synaptic networks

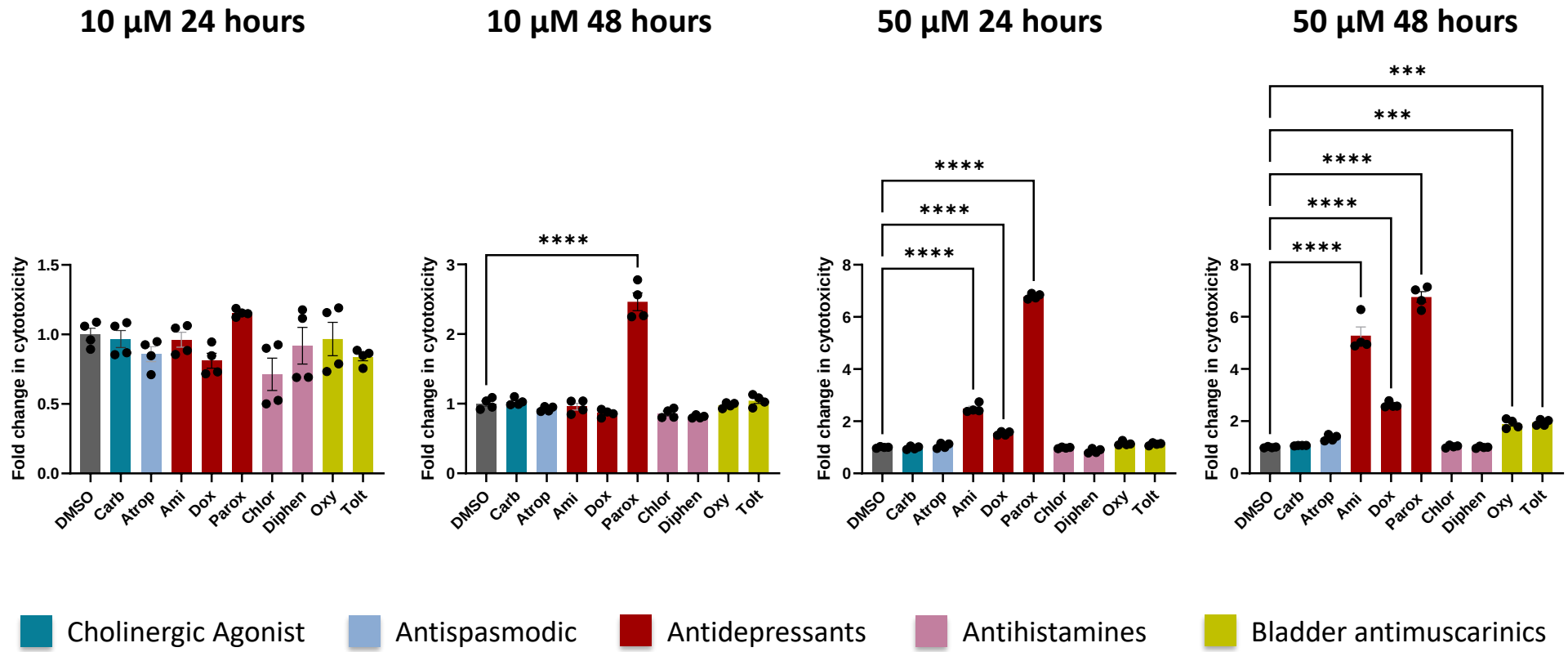
# Investigating the effects of various anticholinergics on AD-related phenotypes using hiPSC-neurons from ACT cohort

| Anticholinergic class | | Drug name |
|---|---|---|
| Cholinergic agonist control | | Carbachol |
| | | |
| ACh groups **associated with dementia** | **Antidepressants** | Amitriptyline<br>Doxepin<br>Paroxetine |
| | **Bladder antimuscarinics** | Oxybutynin<br>Tolterodine |
| ACh groups **NOT associated with dementia** | **Antihistamines** | Chlorpheniramine<br>Diphenhydramine |
| | **Antispasmodic** | Atropine |

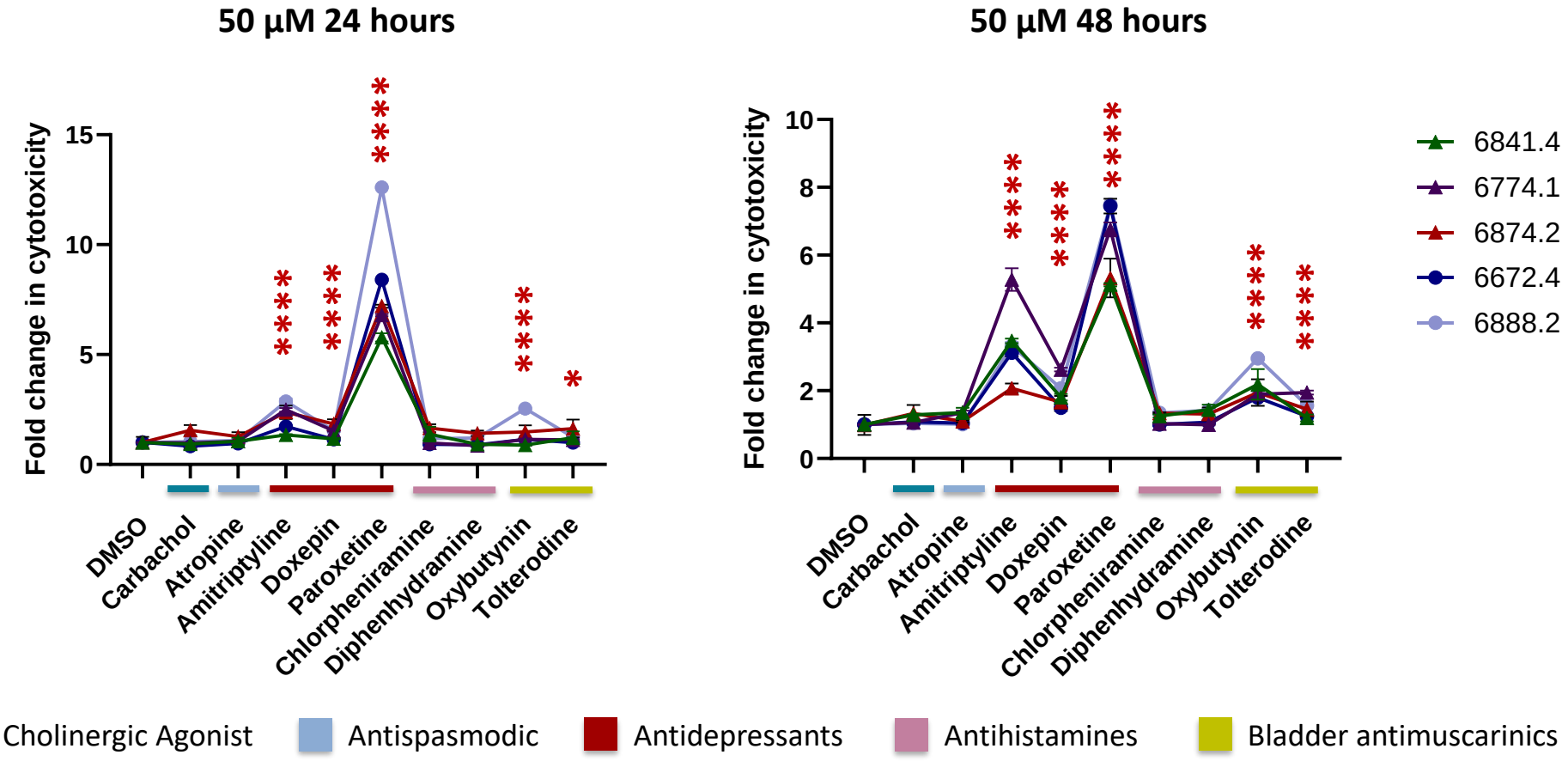
**Screening assessments for AD-related phenotypes:**

1. **Neurotoxicity level**
2. **Neuronal synaptic firing**
3. **Amyloid β peptides levels**

# Antidepressants and bladder antimuscarinics are neurotoxic in a dose and time-dependent manner



# Antidepressants and bladder antimuscarinics are consistently neurotoxic across multiple ACT cell lines

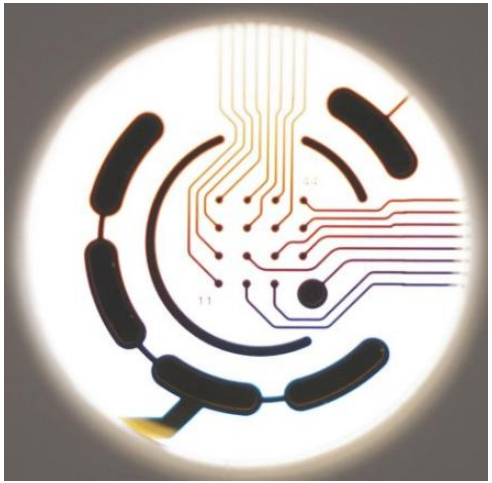


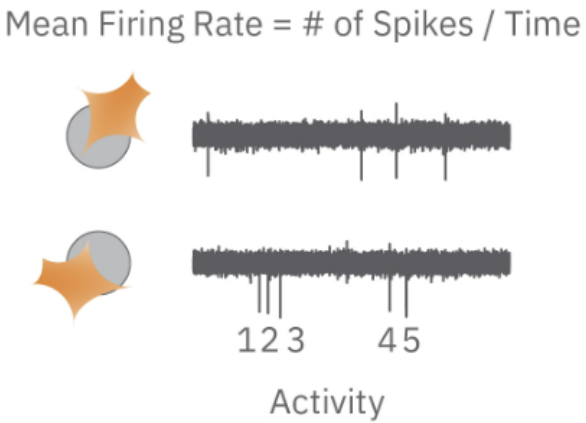
# Measuring synaptic firing activity of hiPSC-derived neurons using multi electrode arrays (MEAs)

**MEA recording:**

High-throughput method to measure neuronal firing activity of the same culture over time

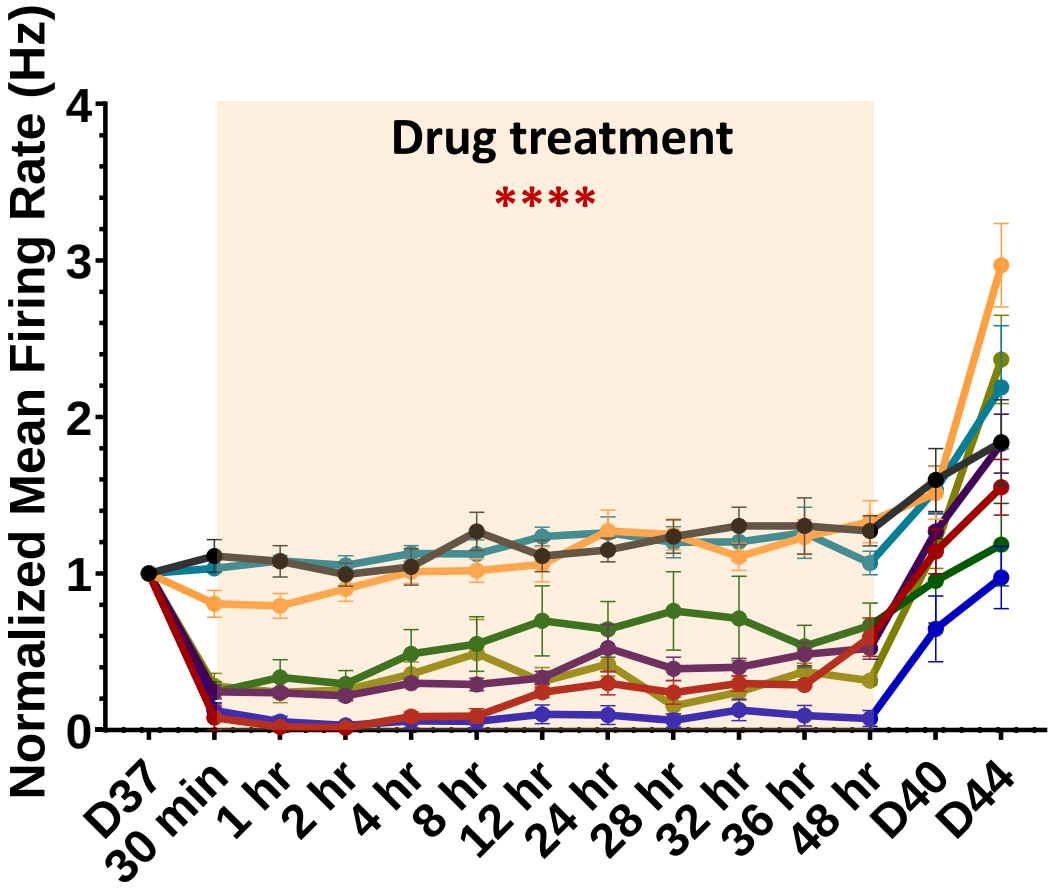
- Neuronal mean firing rate *(robustness)*
- Neuronal network burst frequency *(connectivity)*
- Network interburst interval *(regularity)*






Axion Biosystems

# Dementia-associated drugs reduce neuronal firing rate



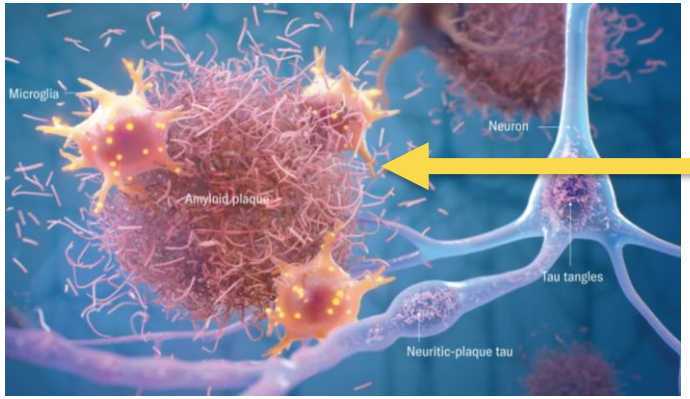

- DMSO
- Carbachol
- Atropine

Non-dementia-linked anticholinergics

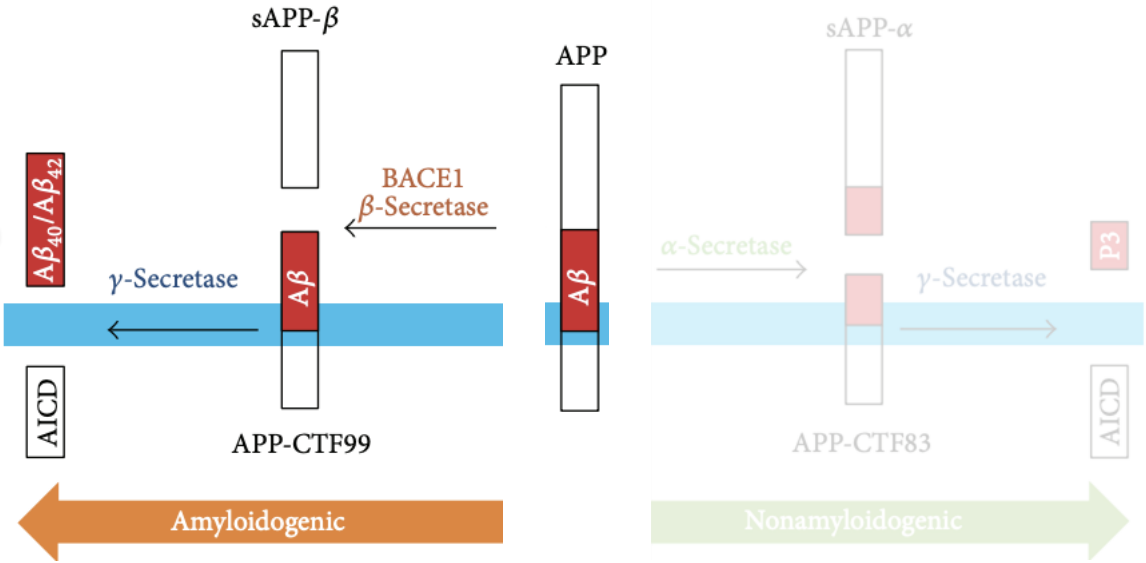
- Amitriptyline
- Doxepin
- Paroxetine
- Oxybutynin
- Tolterodine

Dementia-linked anticholinergics

# Amyloid beta (Aβ) peptides are generated from Amyloid Precursor Protein (APP) sequential cleavage process

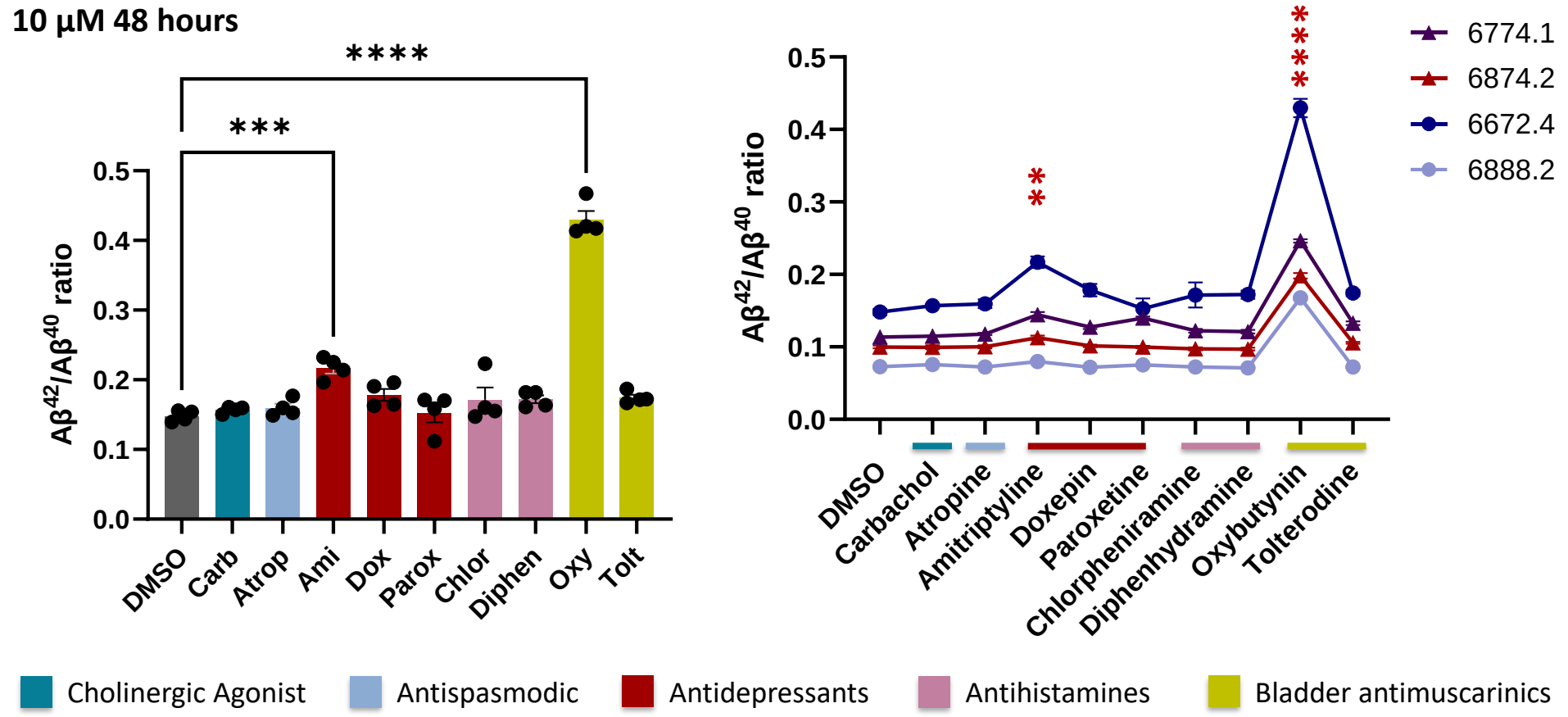



scientificamerican.com




**↑ $A\beta^{42}/A\beta^{40}$ ratio = ↑ amyloid plaque formation**

# Antidepressants and bladder antimuscarinics increase $A\beta^{42}/A\beta^{40}$ ratio

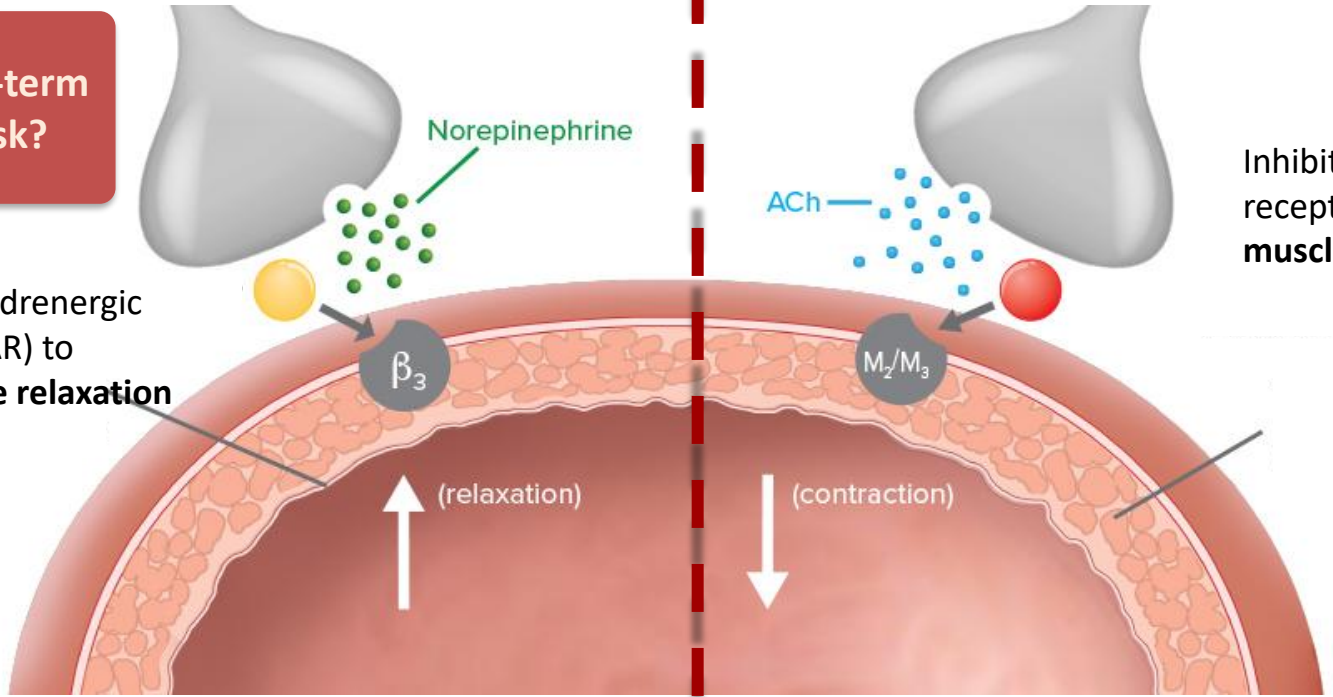


# Mirabegron is an alternative medication to bladder antimuscarinics without anticholinergic activity

## Mirabegron

## Bladder antimuscarinics (anticholinergics)

**Potential long-term dementia risk?**



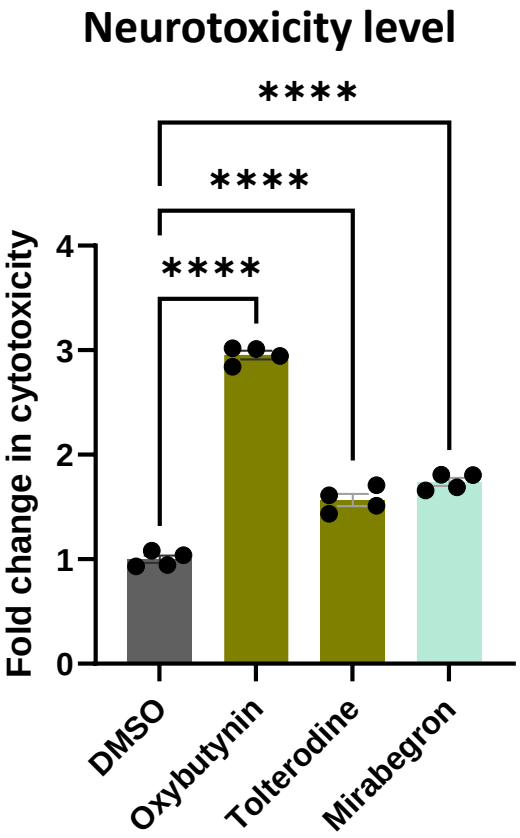

Activates $\beta_3$-Adrenergic receptor ($\beta_3$-AR) to **induce muscle relaxation**

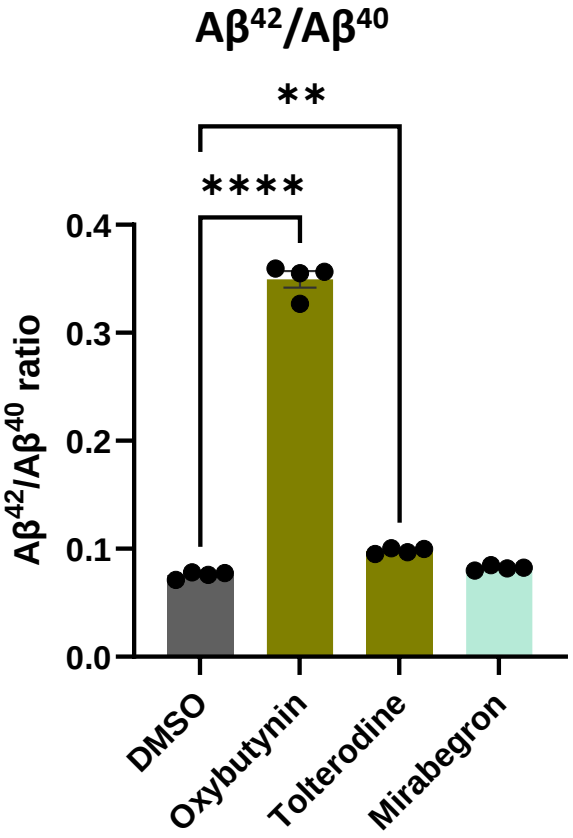
Inhibit muscarinic receptors to **prevent muscle contraction**

myrbetriqhcp.com/combination-treatment/

# Mirabegron has a modest increase of neurotoxicity but does not increase $A\beta^{42}/A\beta^{40}$ ratio

**50 μM**
**48 hours**




Bladder antimuscarinics

Non-anticholinergic OAB (Mirabegron)

# Summary



- Dementia-associated-anticholinergics – **Antidepressants** & **bladder antimuscarinics** – **induce neurotoxicity, impair neuronal synaptic firing, and increase the $A\beta^{42}/A\beta^{40}$ ratio** at these time points, corroborating the links found in the pharmacoepidemiology studies
- Non-dementia-linked-anticholinergics – **Antihistamines** & **antispasmodic** – **do not trigger these AD-related molecular neurotoxic phenotypes**
- **Non-anticholinergic** drug may have **modest neurotoxicity but does not alter amyloid peptide ratio**, suggesting a promising alternative to dementia-associated-anticholinergic medications

# Acknowledgements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NIH NIA

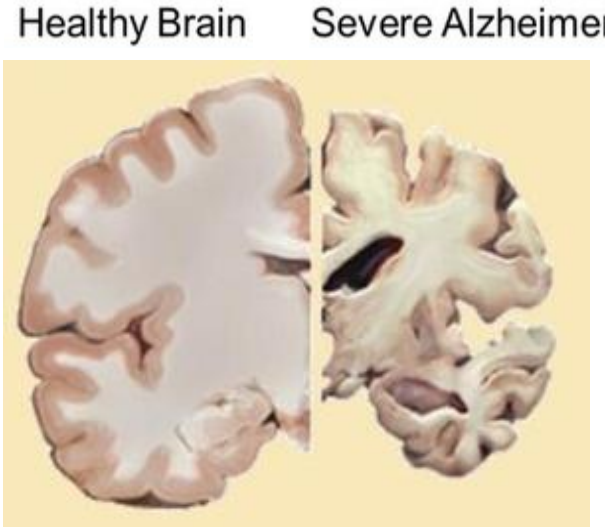
UW Medicine LABORATORY MEDICINE & PATHOLOGY

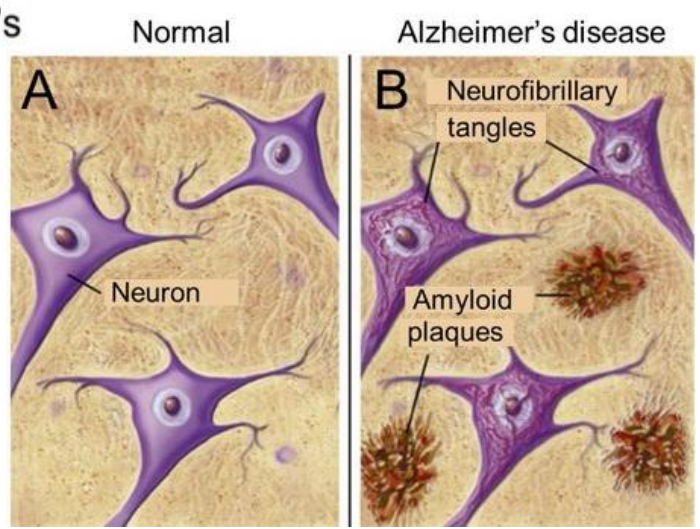
ISCRM Institute for Stem Cell and Regenerative Medicine

ACT ADULT CHANGES IN THOUGHT STUDY

Kaiser Permanente Washington Health Research Institute

# Alzheimer's disease is a dementia characterized by **neuronal loss, neurofibrillary tangles, and amyloid plaques**



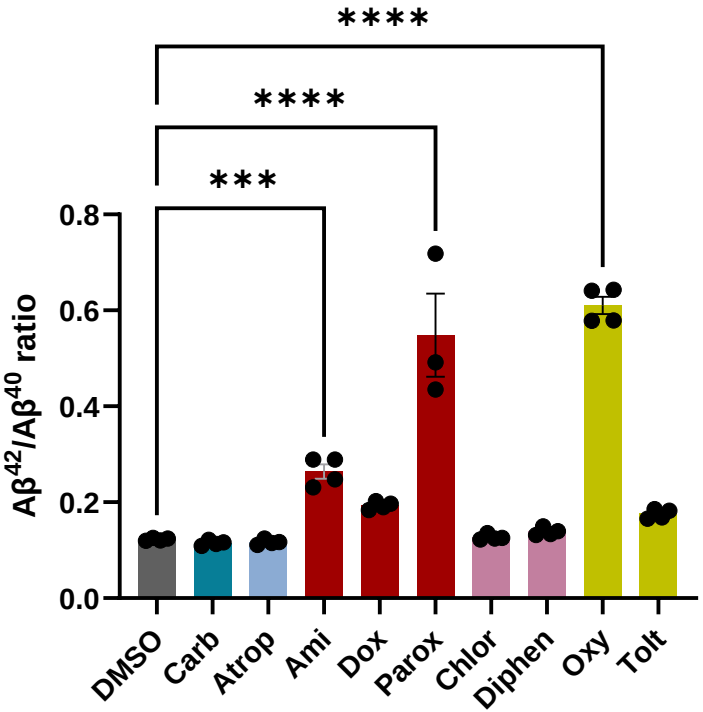

**Neuronal loss**
resulting in brain atrophy

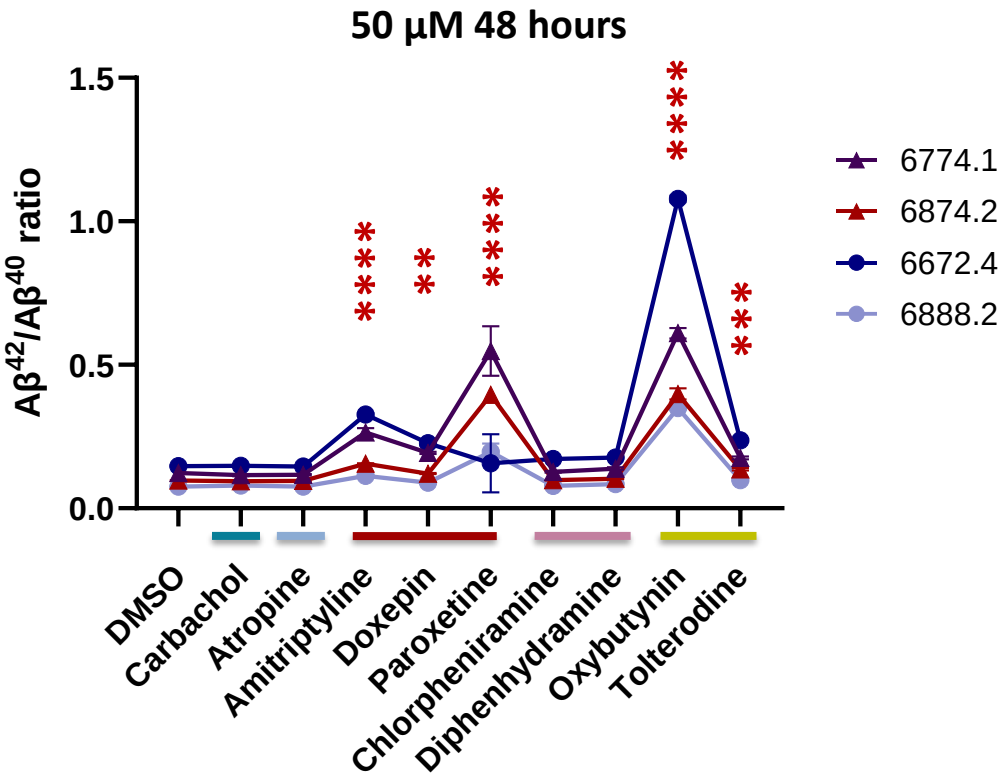
**Tau tangles**
intracellular aggregation of Tau due to its hyperphosphorylation

**Amyloid plaque**
extracellular aggregation of amyloid peptides

Loof, A.D. and Schoofs, L. 2019, *OBM Neurobio.*

# Antidepressants and bladder antimuscarinics increase $A\beta^{42}/A\beta^{40}$ ratio

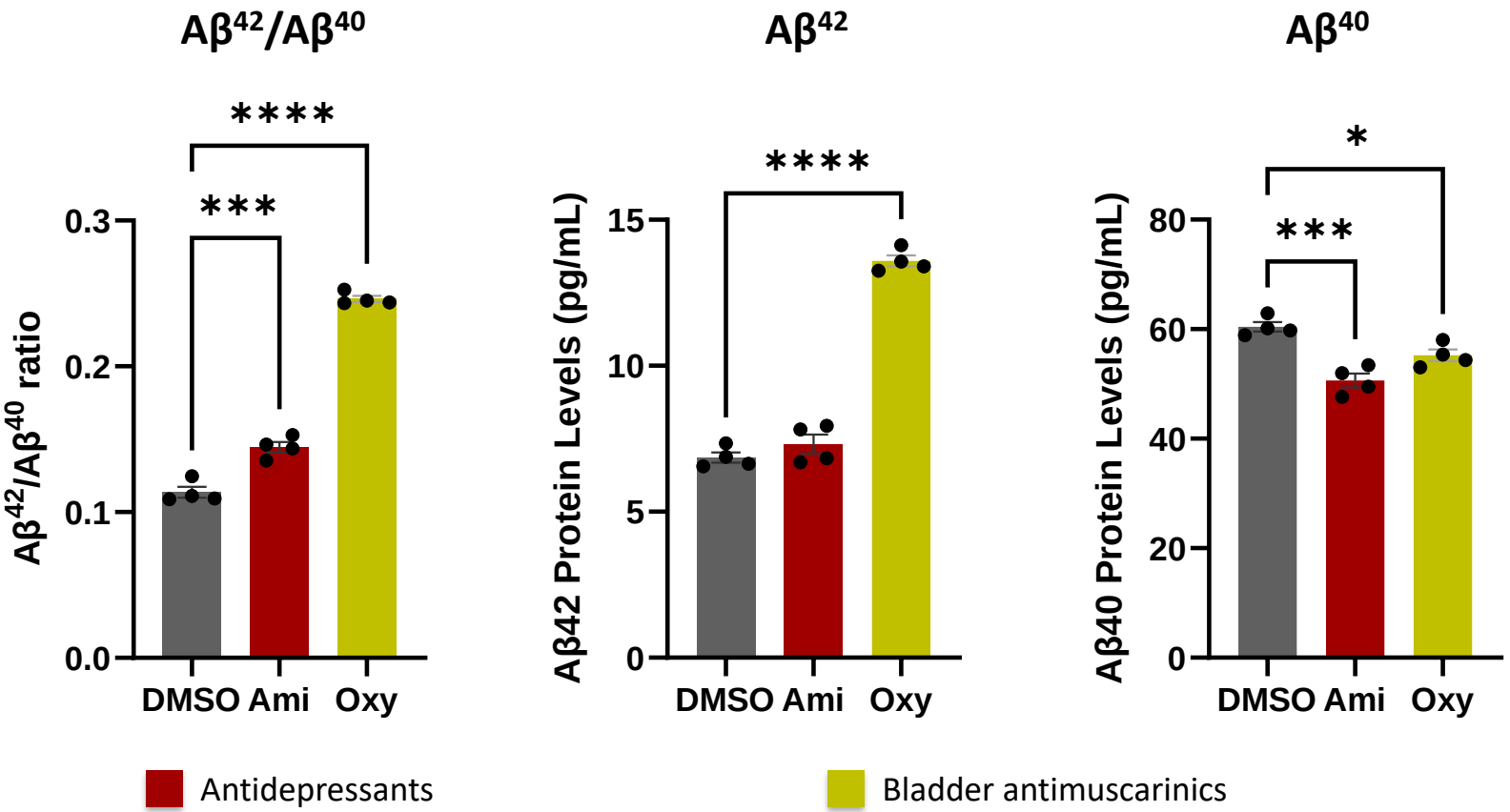


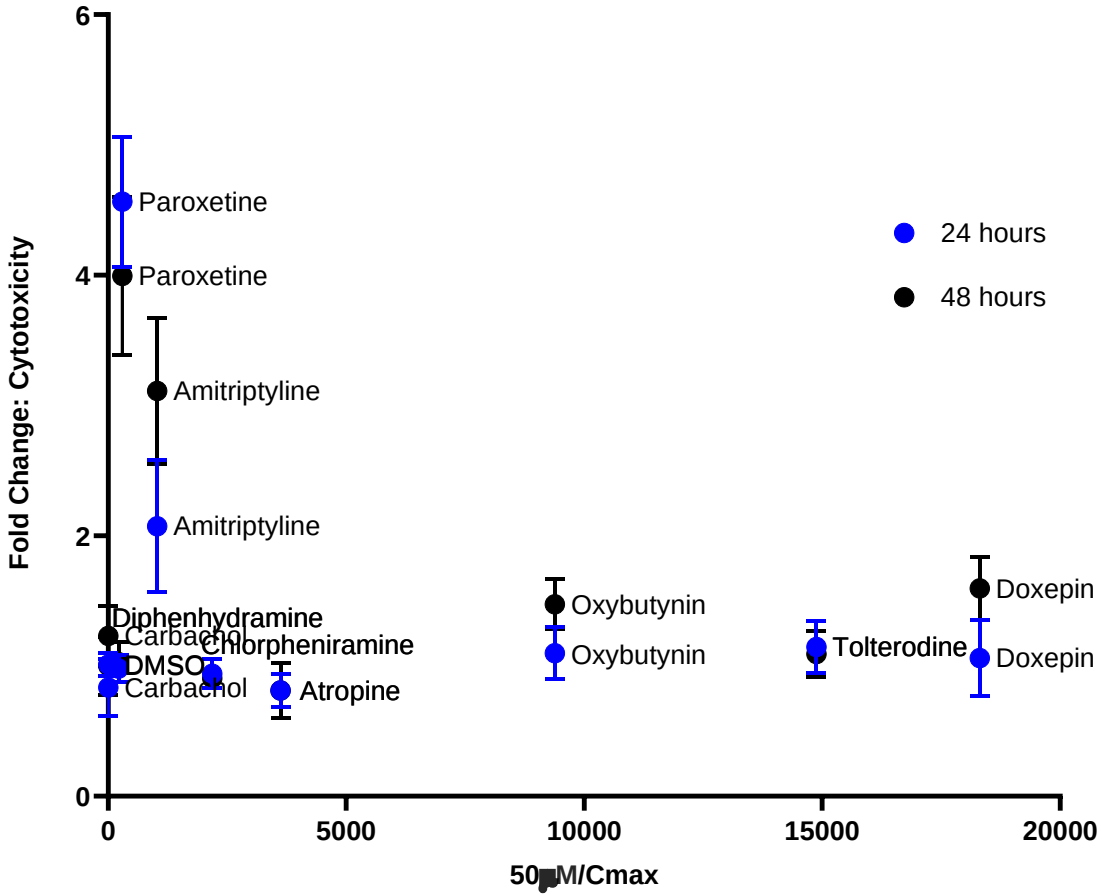




Cholinergic Agonist | Antispasmodic | Antidepressants | Antihistamines | Bladder antimuscarinics

# Amitriptyline and Oxybutynin raise $A\beta^{42}/A\beta^{40}$ ratio through distinct mechanisms

**10 µM**
**48 hours**




Fold Change: Cytotoxicity
6
4
2
0
0
5000
10000
15000
20000
50µM/Cmax
24 hours
48 hours
Paroxetine
Paroxetine
Amitriptyline
Amitriptyline
Diphenhydramine
Carbachol
Chlorpheniramine
DMSO
Carbachol
Atropine
Oxybutynin
Oxybutynin
Tolterodine
Doxepin
Doxepin

# Future Directions



- What is the **underlying mechanistic differences** across different anticholinergic classes resulting these distinct effects on AD-related phenotypes?
  - ➢Unbiased, global proteomics analyses to investigate the on- and off-target mechanisms
- How do the **antidepressants** and **bladder antimuscarinics** class affect organelle functions in the neurons?
  - ➢ Assess organelle morphology using ICC and organelle functional assays, such as Seahorse assay
- How do the **antidepressants** and **bladder antimuscarinics** class affect neuronal activity?
  - ➢Multielectrode array (MEA) analysis

## 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]

Some classes of “definite” anticholinergic drugs were associated with future dementia incidence.

- ✓ 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

**Future research should examine anticholinergic drug classes as opposed to anticholinergic effects intrinsically or summing scales for anticholinergic exposure**

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

# Introduction



Higher usage of anticholinergic medications in aging adults is correlated with an increased dementia incidence

Table 3. Association of Incident Dementia and AD With 10-Year Cumulative Anticholinergic Use[a]

| Diagnosis, TSDD[b] | Follow-up Time, Person-years | No. of Events | HR (95% CI) | |
|---|---|---|---|---|
| | | | Unadjusted[c,d] | Adjusted[d,e] |
| Dementia | | | | |
| 0 | 5618 | 136 | 1 [Reference] | 1 [Reference] |
| 1-90 | 7704 | 203 | 0.96 (0.77-1.20) | 0.92 (0.74-1.16) |
| 91-365 | 5051 | 172 | 1.31 (1.04-1.65) | 1.19 (0.94-1.51) |
| 366-1095 | 2626 | 102 | 1.39 (1.07-1.82) | 1.23 (0.94-1.62) |
| >1095 | 4022 | 184 | 1.77 (1.40-2.23) | 1.54 (1.21-1.96) |

Gray, SL et al., *JAMA Intern Med*. 2015

# Challenge



Understanding these factors in patient populations can be challenging and have bias

**Confounding by indication bias**

A bias when the reason that someone takes a medication is also the reason for an observed effect

***Can we use a simplified model to directly test the effect of these medications associated with disease on brain cells?***

- Anticholinergics [ACh]
  - Higher dose (>1095 TSDD) over 10 years associated with **1.63** greater risk for AD[1]
  - Two studies from the UK have since reported that only certain classes of AChs may increase risk.

- Antihypertensives [AHTs] - Ang II hypothesis
  - Evidence to suggest that drugs that increase angiotensin receptor activity may reduce dementia.[2]
  - *The Lancet* Commission identified mid-life hypertension as an important modifiable risk factor for dementia
  - Need for research on the comparative effectiveness of different classes of AHTs on brain health (*National Academies of Science, Engineering, and Medicine*) .[12]

van Dalen JW .2021;96(1):e67-e80

# Introduction



## Only certain types of anticholinergic drugs are correlated with dementia

*Those with *strong* anticholinergic activity are the most associated

**Table 3 | Adjusted odds ratios of dementia by prescription of an anticholinergic drug by Anticholinergic Cognitive Burden (ACB) score and drug class**

| Drug class | No of cases (%) | No of controls (%) | Odds ratio (95% CI) Adjusted at start of DEP*† | Adjusted at end of DEP*‡ |
|---|---|---|---|---|
| **ACB score of 1** | | | | |
| Analgesic | 23 871 (58.6) | 158 162 (55.7) | 1.02 (1.00 to 1.05) | 1.02 (0.99 to 1.04) |
| Antidepressant | 5958 (14.6) | 28 767 (10.1) | 1.37§ (1.32 to 1.42) | 1.25§ (1.20 to 1.30) |
| Antipsychotic | 8051 (19.7) | 50 079 (17.6) | 1.05§ (1.02 to 1.08) | 1.04 (1.01 to 1.07) |
| Cardiovascular | 27 926 (68.5) | 191 895 (67.6) | 0.97 (0.94 to 0.99) | 0.98 (0.95 to 1.01) |
| Gastrointestinal | 10 845 (26.6) | 71 814 (25.3) | 0.97 (0.94 to 0.99) | 0.96§ (0.93 to 0.99) |
| Respiratory | 9385 (23.0) | 62 787 (22.1) | 0.99 (0.97 to 1.02) | 0.99 (0.97 to 1.02) |
| Other | 11 521 (28.3) | 77 345 (27.2) | 0.95§ (0.92 to 0.97) | 0.95§ (0.92 to 0.98) |
| **ACB score of 2** | | | | |
| Analgesic | 385 (0.9) | 2337 (0.8) | 1.03 (0.92 to 1.15) | 1.03 (0.92 to 1.16) |
| Antipsychotic | 22 (0.1) | 69 (0.0) | 1.44 (0.87 to 2.36) | 1.35 (0.82 to 2.23) |
| Antiparkinson | 57 (0.1) | 141 (0.0) | 1.55§ (1.12 to 2.14) | 1.32 (0.96 to 1.82) |
| Respiratory | 19 (0.0) | 123 (0.0) | 0.89 (0.55 to 1.45) | 0.83 (0.51 to 1.36) |
| Other | 985 (2.4) | 5454 (1.9) | 1.07 (1.00 to 1.15) | 1.09 (1.01 to 1.17) |
| **ACB score of 3** | | | | |
| Antidepressant | 8823 (21.6) | 50 817 (17.9) | 1.13§ (1.10 to 1.16) | 1.11§ (1.08 to 1.14) |
| Antipsychotic | 1036 (2.5) | 5140 (1.8) | 1.09 (1.02 to 1.18) | 1.07 (1.00 to 1.16) |
| Gastrointestinal | 1817 (4.5) | 12 057 (4.2) | 0.94 (0.89 to 0.99) | 0.94 (0.89 to 0.99) |
| Antiparkinson | 270 (0.7) | 951 (0.3) | 1.45§ (1.25 to 1.68) | 1.29§ (1.11 to 1.50) |
| Respiratory | 4002 (9.8) | 25 195 (8.9) | 1.04 (1.00 to 1.08) | 1.03 (1.00 to 1.07) |
| Urological | 3261 (8.0) | 16 873 (5.9) | 1.23§ (1.18 to 1.28) | 1.18§ (1.13 to 1.23) |
| Other | 284 (0.7) | 1741 (0.6) | 0.99 (0.87 to 1.13) | 0.99 (0.87 to 1.13) |

DEP=drug exposure period
*Adjusted for age, region, any falls, any fractures, and number of doctor consultations in the 12 months before the DEP. Also adjusted for the number of prescriptions during the DEP for the following drugs not rated as anticholinergic: benzodiazepines, z drugs, antidepressants, antinausea and antivertigo preparations, antiepileptics, and antiparkinson drugs.
†Adjusted for the following variables measured at the start of the DEP: body mass index, smoking status, harmful alcohol use, depression duration (0, 0-2, 2-5, 5-10, 10-20, and >20 years), and all diagnoses listed in table 1.
‡Adjusted for the following variables measured at the end of the DEP: body mass index, smoking status, harmful alcohol use, depression duration (0, 0-5, 5-10, 10-15, 15-20, and >20 years), and all diagnoses listed in table 1.
§P<0.01.

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# Background: Angiotensin Hypothesis