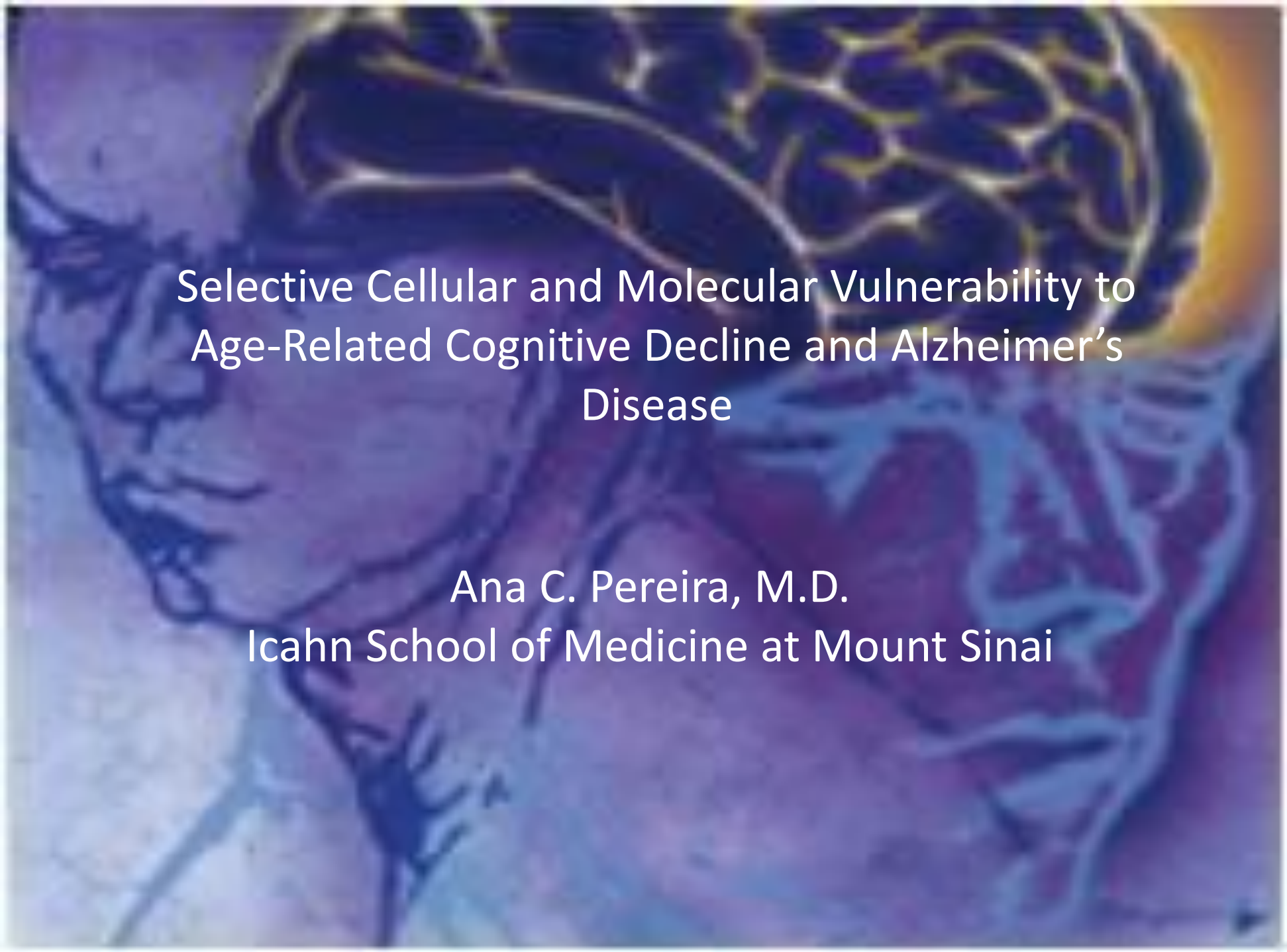
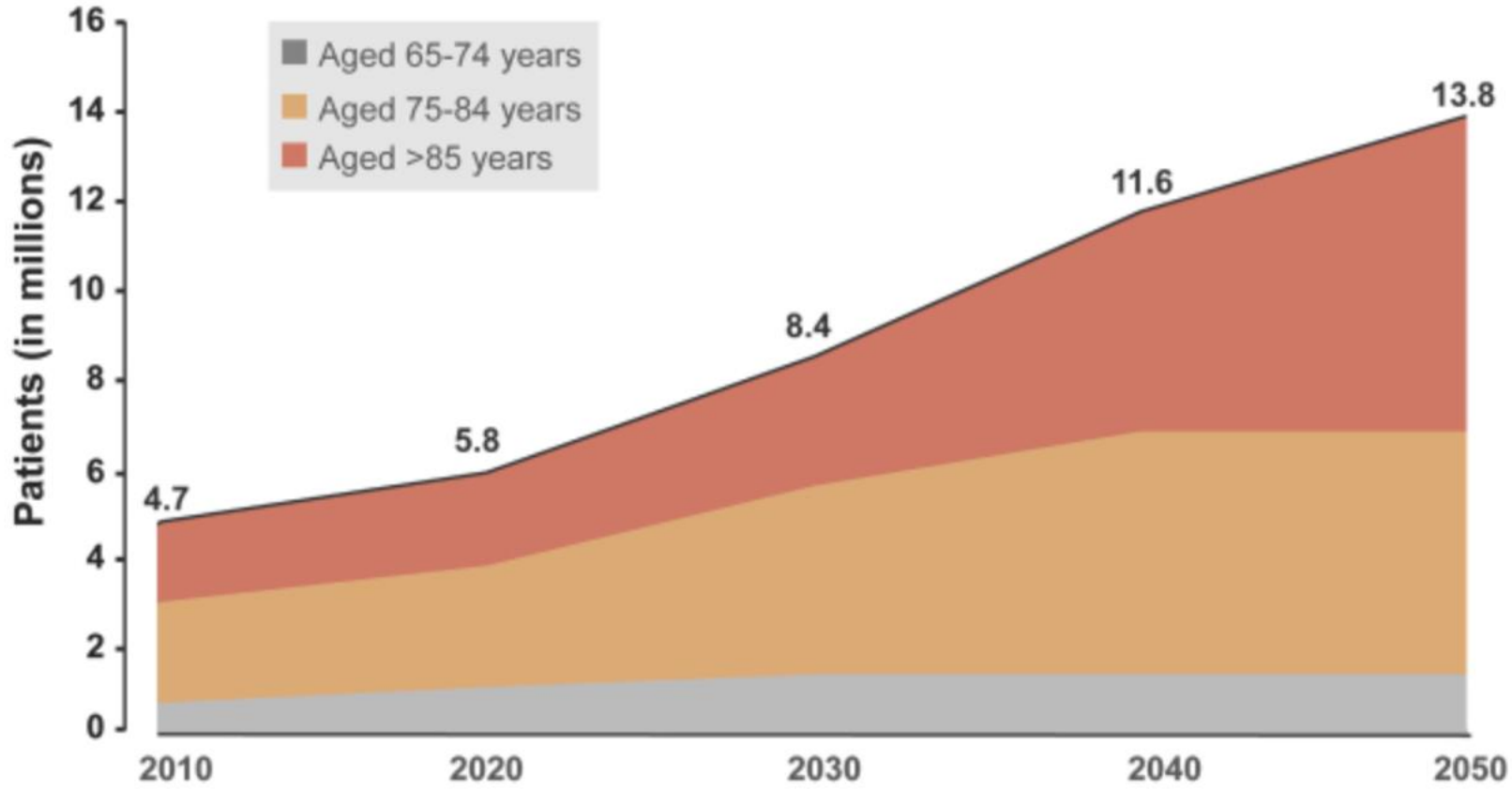
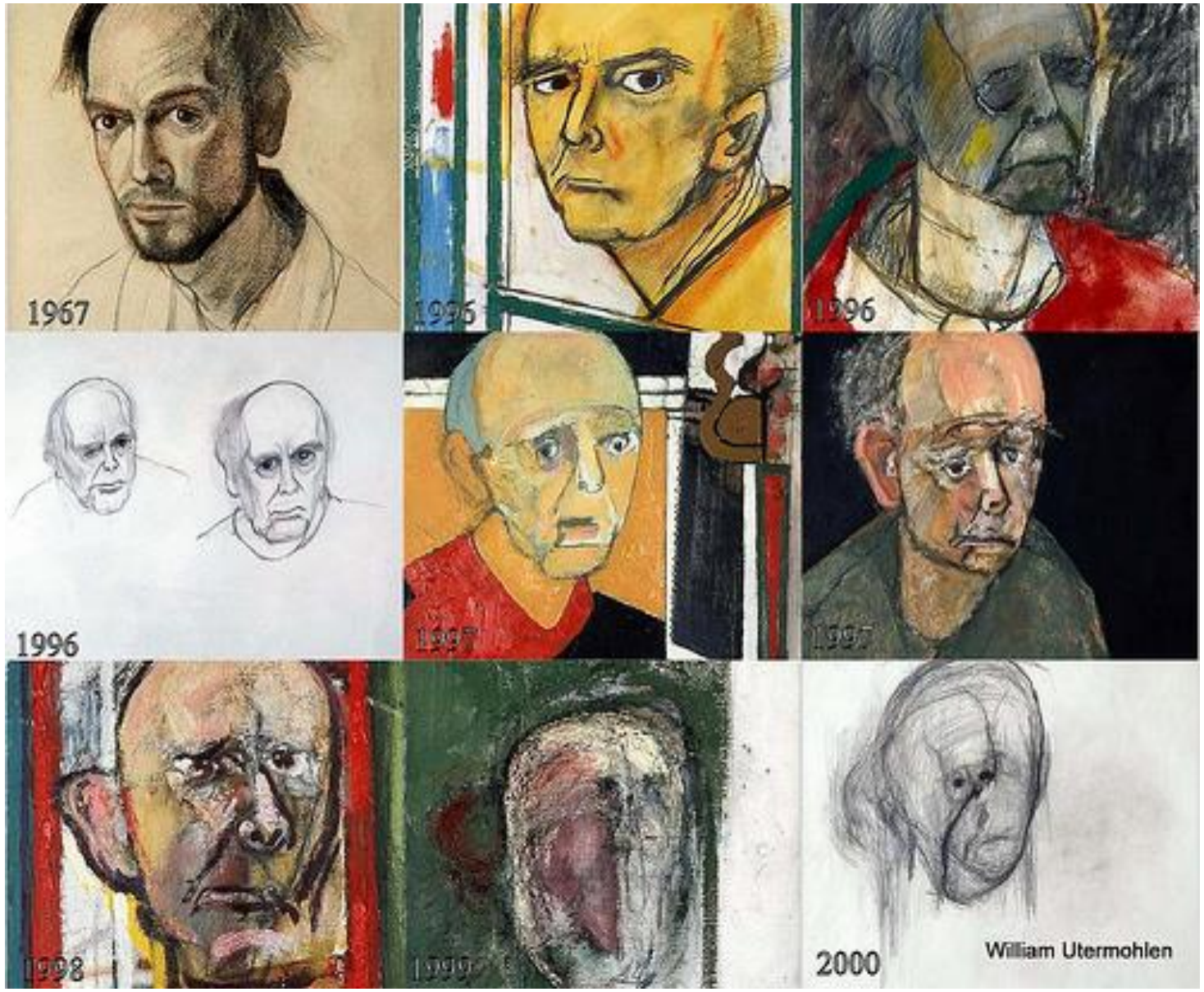


Selective Cellular and Molecular Vulnerability to Age-Related Cognitive Decline and Alzheimer's Disease

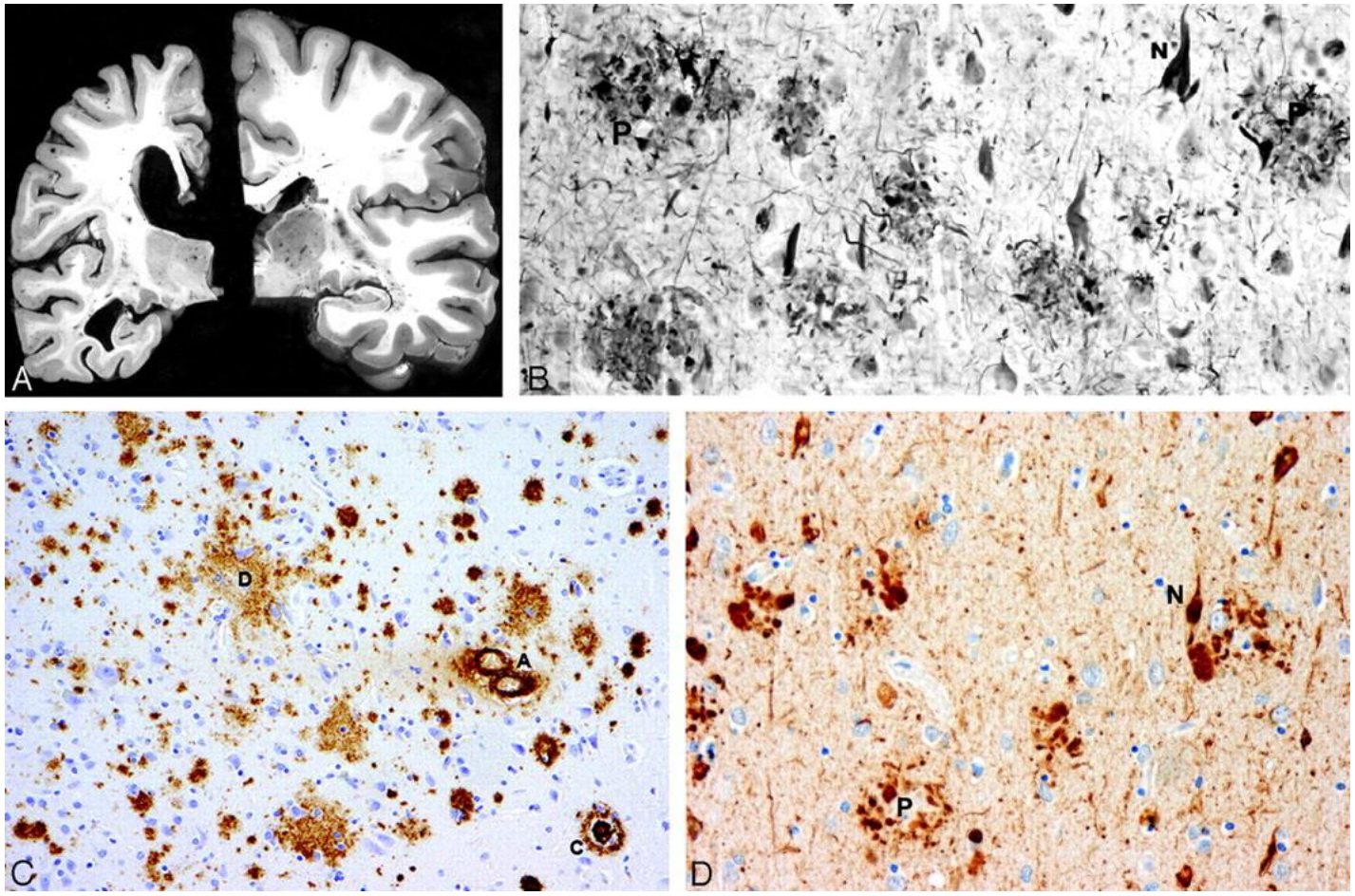
Ana C. Pereira, M.D.
Icahn School of Medicine at Mount Sinai



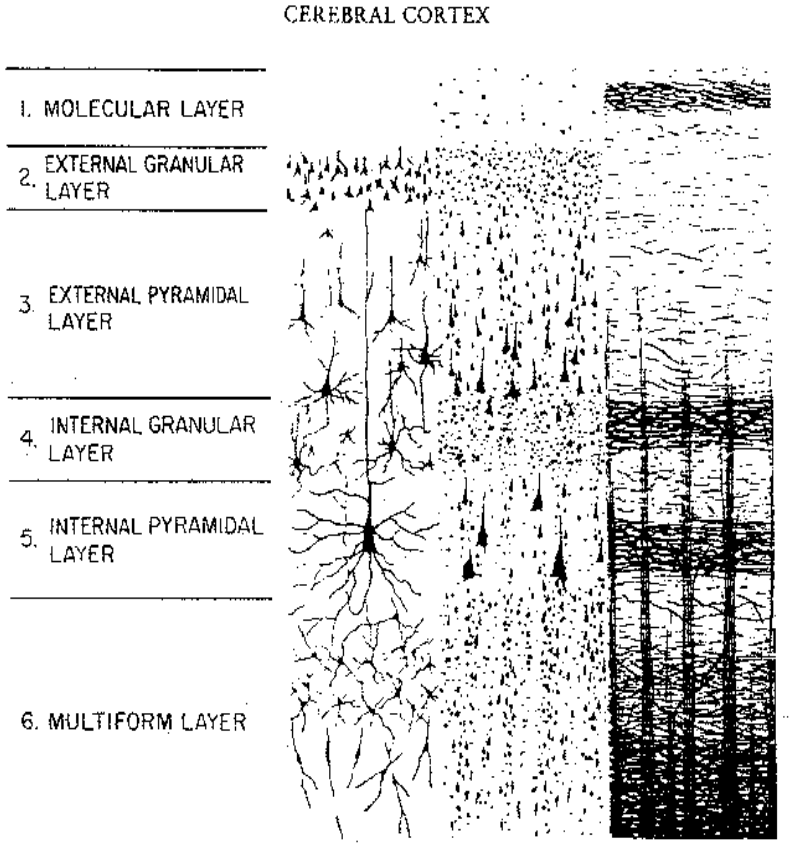
Source: Alzheimer’s Association

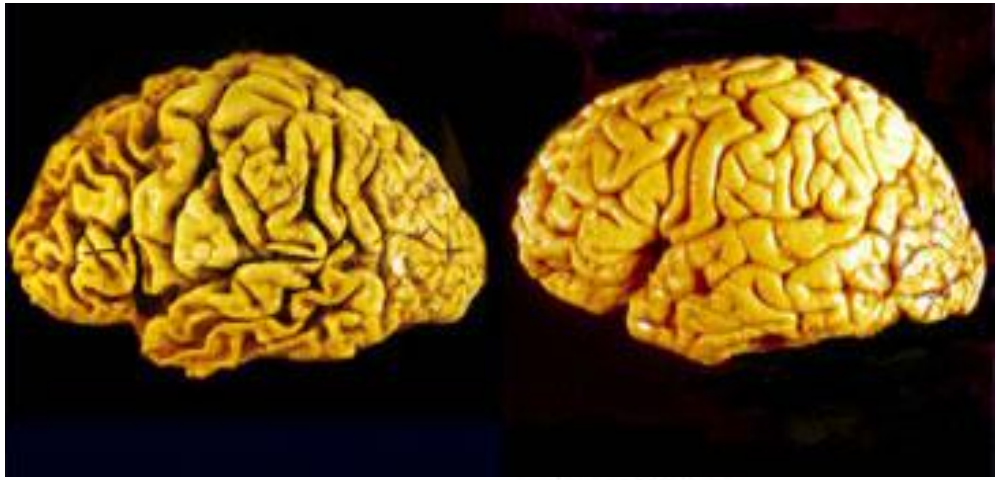

1967
1996
1996
1996
1997
1998
1999
2000
William Utermohlen

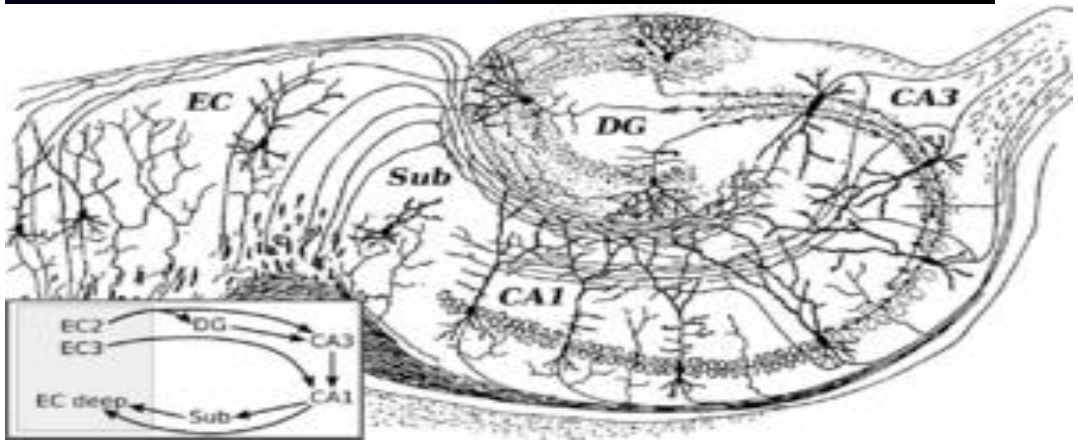
Neuropathological hallmarks: Amyloid plaques (β-Amyloid) and neurofibrillary tangles (hyperphosphorylated tau)



Similar neural circuits in age-related cognitive decline and Alzheimer's disease: glutamatergic pyramidal neurons that furnish corticocortical connections between the association cortices as well as the excitatory hippocampal connections that subserve memory and cognition





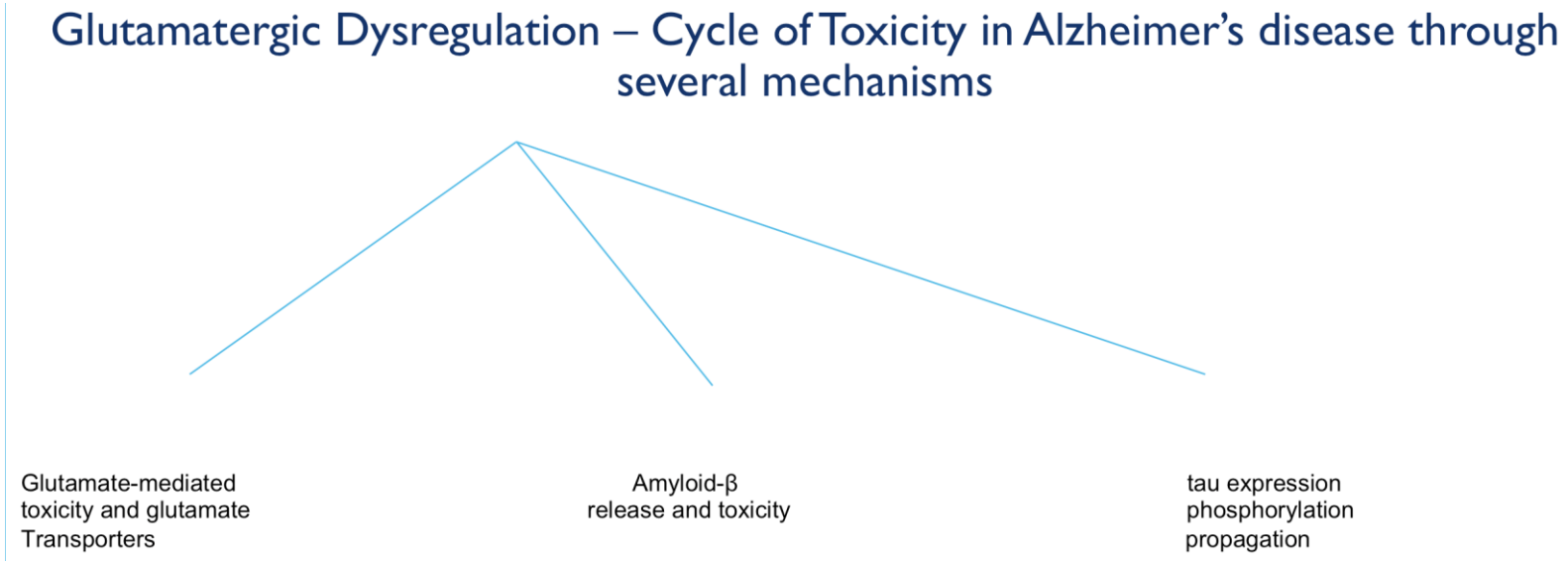


- Aging: Synaptic changes with minimal neuronal loss in excitatory neurons

- Alzheimer's disease: Extensive neuronal loss in glutamatergic neurons

- Neurofibrillary tangles (NFT) accumulate in excitatory pyramidal neurons of the hippocampus and association neocortex, while excitatory neurons in primary cortices and inhibitory interneurons do not form NFT and are resistant to degeneration

- **Interest in studying the susceptibility of these glutamatergic neural circuits to aging and Alzheimer's disease in order to develop effective therapeutic interventions**



Amyloid-β oligomers and glutamate

Neuron
Article

Soluble Oligomers of Amyloid β Protein Facilitate Hippocampal Long-Term Depression by Disrupting Neuronal Glutamate Uptake

Shaomin Li,[1] Soyon Hong,[1] Nina E. Shepardson,[1] Dominic M. Walsh,[2] Ganesh M. Shankar,[1] and Dennis Selkoe[1,*]
[1]Center for Neurologic Diseases, Brigham and Women's Hospital, Harvard Medical School, Boston, MA 02115, USA
[2]Laboratory for Neurodegenerative Research, University College Dublin, Dublin 4, Ireland
*Correspondence: dselkoe@rics.bwh.harvard.edu
DOI 10.1016/j.neuron.2009.05.012

The Journal of Neuroscience, May 4, 2011 • 31(18):6627– 6638 • 6627

Neurobiology of Disease

Soluble Aβ Oligomers Inhibit Long-Term Potentiation through a Mechanism Involving Excessive Activation of Extrasynaptic NR2B-Containing NMDA Receptors

Shaomin Li,* Ming Jin,* Thomas Koeglsperger, Nina E. Shepardson, Ganesh M. Shankar, and Dennis J. Selkoe
Center for Neurologic Diseases, Brigham and Women's Hospital and Harvard Medical School, Boston, Massachusetts 02115

Amyloid-β release and neuronal activity

Neuron

Volume 37, Issue 6, 27 March 2003, Pages 925–937

Article

APP Processing and Synaptic Function

Flavio Kamenetz[1, 2], Taisuke Tomita[4], Helen Hsieh[1, 3], Guy Seabrook[5], David Borchelt[6], Takeshi Iwatsubo[4], Sangram Sisodia[7], Roberto Malinow[1, 2, 3]

Neuronal activity regulates the regional vulnerability to amyloid-β deposition

Adam W Bero[1–4], Ping Yan[1,3,4], Jee Hoon Roh[1–4], John R Cirrito[1,3,4], Floy R Stewart[1–4], Marcus E Raichle[1,5–7], Jin-Moo Lee[1,3,4] & David M Holtzman[1–4]

nature neuroscience

Tau release and neuronal activity

Neuronal activity regulates extracellular tau in vivo

Kaoru Yamada,[1] Jerrah K. Holth,[1] Fan Liao,[1] Floy R. Stewart,[1] Thomas E. Mahan,[1] Hong Jiang,[1] John R. Cirrito,[1] Tirth K. Patel,[1] Katja Hochgräfe,[2,3] Eva-Maria Mandelkow,[2,3] and David M. Holtzman[1]

[1]Department of Neurology, Hope Center for Neurological Disorders, Knight Alzheimer's Disease Research Center, Washington University School of Medicine, St. Louis, MO 63110
[2]DZNE (German Center for Neurodegenerative Diseases), 53175 Bonn, Germany
[3]CAESAR Research Center, 53175 Bonn, Germany

JEM, 2014

EMBO Rep. 2013 Apr;14(4):389-94. doi: 10.1038/embor.2013.15. Epub 2013 Feb 15.

Physiological release of endogenous tau is stimulated by neuronal activity.

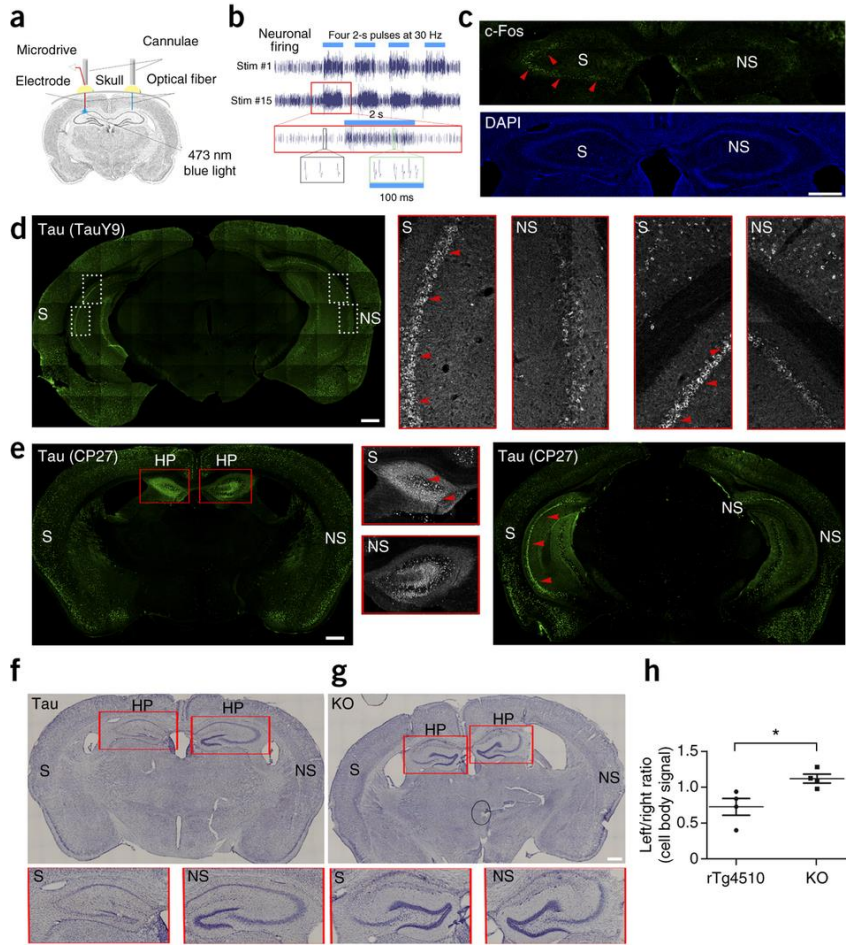
Pooler AM[1], Phillips EC, Lau DH, Noble W, Hanger DP.

Author information

[1]Department of Neuroscience (PO37), King's College London, Institute of Psychiatry, London SE5 8AF, UK.

Neuronal activity enhances tau propagation and tau pathology *in vivo*

Jessica W Wu[1], S Abid Hussaini[1,2], Isle M Bastille[1], Gustavo A Rodriguez[1], Ana Mrejeru[3], Kelly Rilett[1], David W Sanders[4], Casey Cook[5], Hongjun Fu[1], Rick A C M Boonen[1], Mathieu Herman[1], Eden Nahmani[1], Sheina Emrani[1], Y Helen Figueroa[1], Marc I Diamond[4], Catherine L Clelland[1], Selina Wray[6] & Karen E Duff[1,2,7]

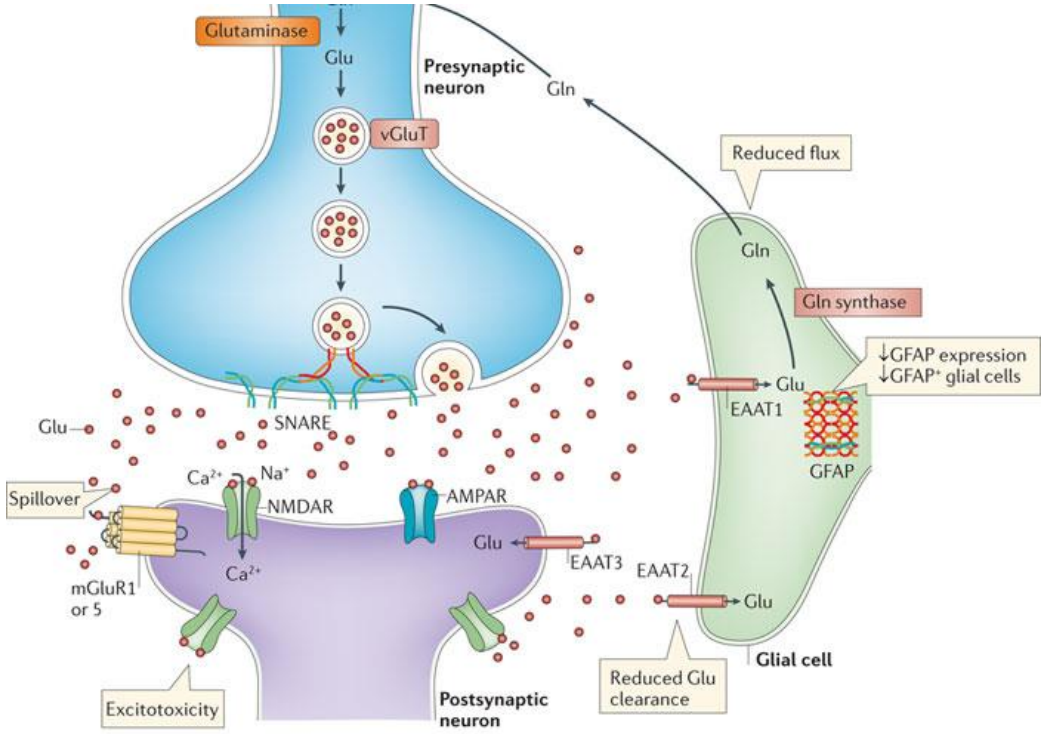


nature neuroscience

The excitatory amino acid transporter 2 (EAAT2 or GLT-1; *Slc1a2*) is the dominant glutamate transporter (responsible for around 95% of the total glutamate uptake)

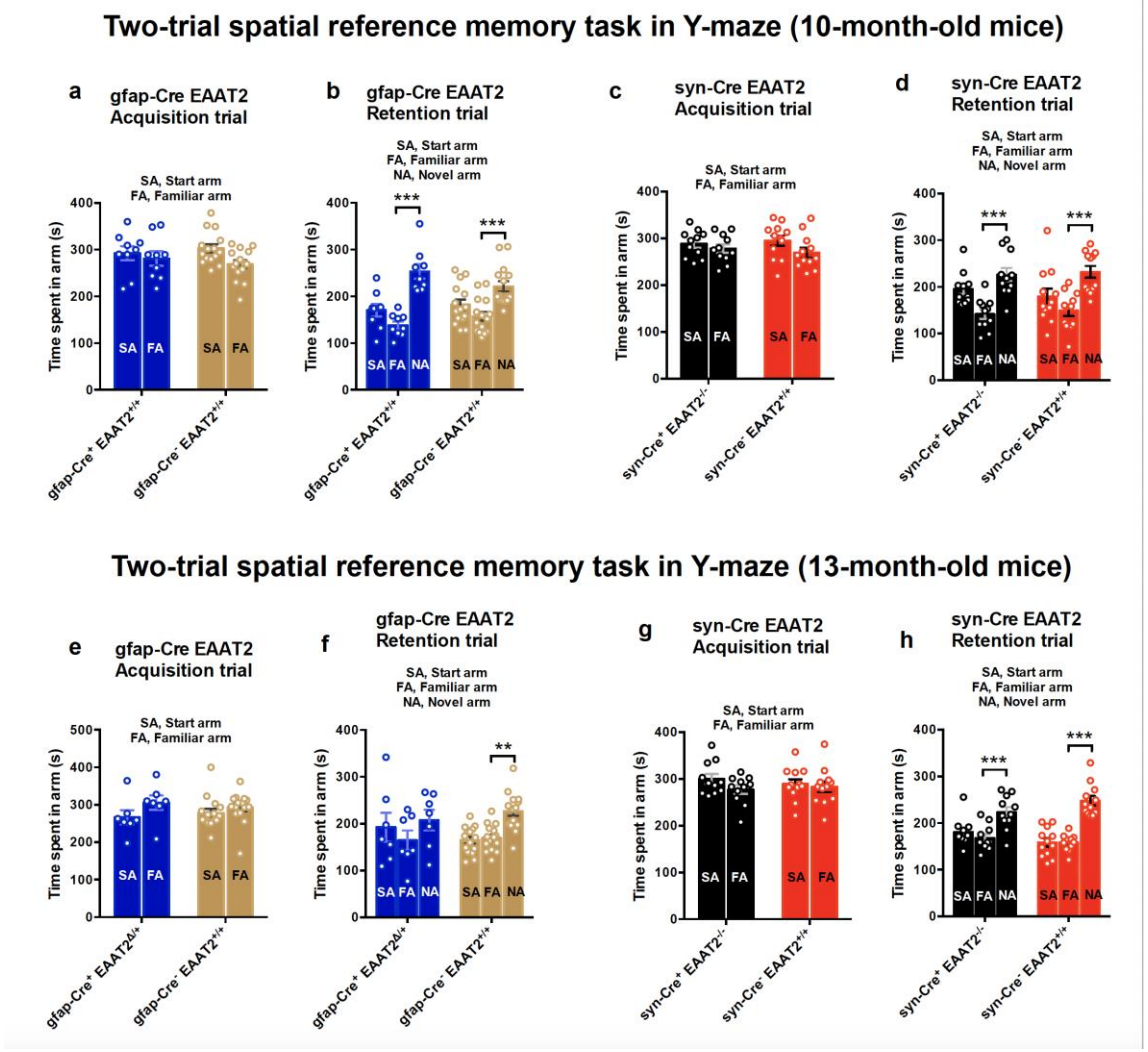
EAAT2 is predominantly expressed in astrocytes but also in neurons and axon terminals

EAAT2 is downregulated in AD



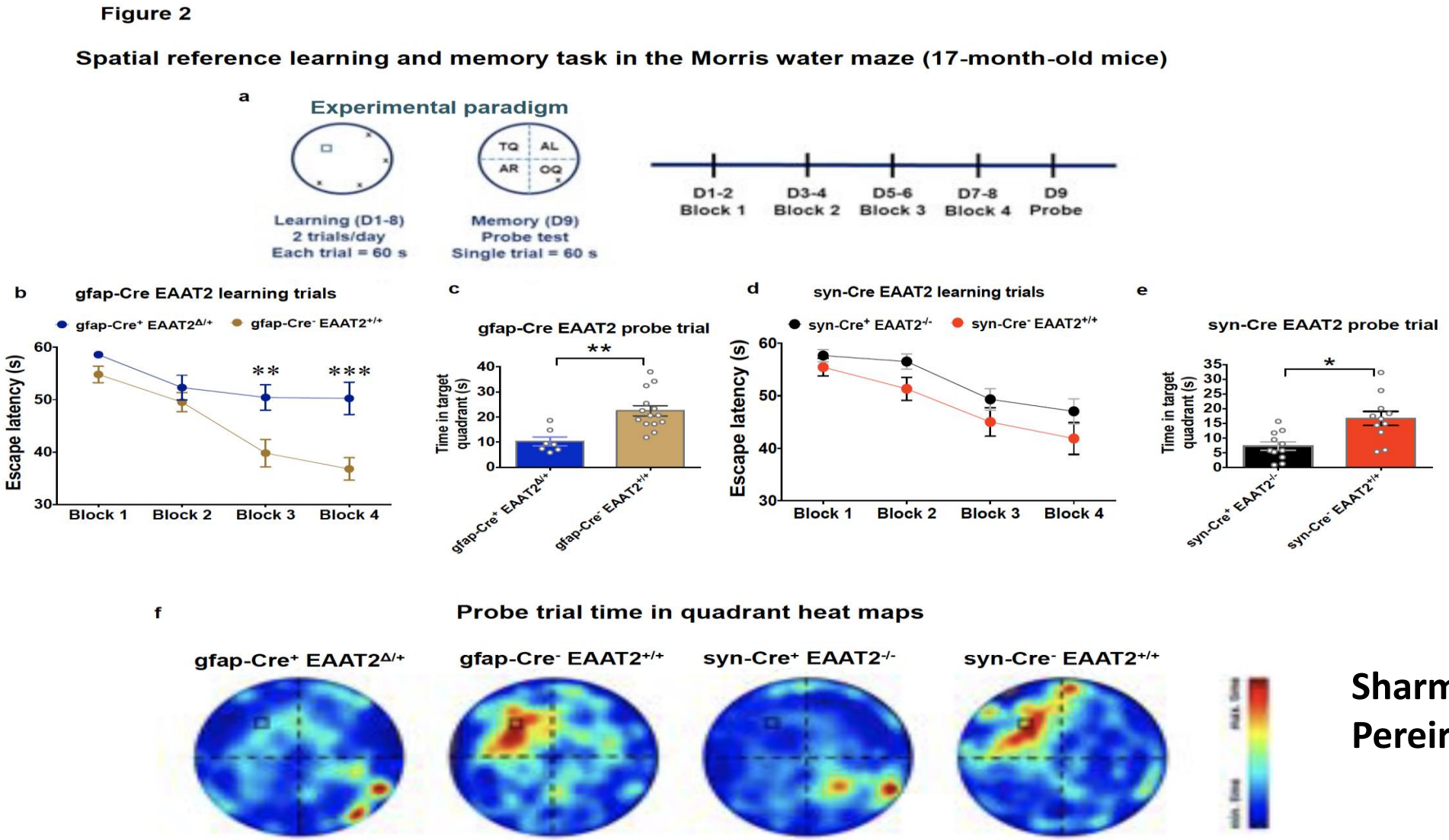
Conditional heterozygous astrocytic GLT-1/EAAT2 knockout mice exhibit accelerated spatial reference memory deficits

Figure 1



Sharma, Kazim…
Pereira, PNAS, 2019

Conditional astrocytic EAAT2 knockout mice exhibit spatial reference learning deficits with accelerated cognitive decline and both astrocytic and neuronal EAAT2 knockout mice exhibit spatial reference memory deficits at 17 months

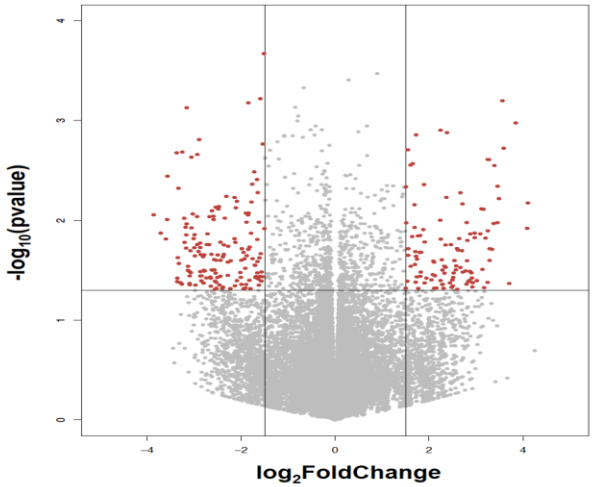


Sharma, Kazim…
Pereira PNAS 2019

Conditional heterozygous astrocytic EAAT2 knockout mice have dysregulated immune signaling and constitutive neuronal EAAT2 knockout mice dysregulated tryptophan metabolism in the hippocampus

Figure 3

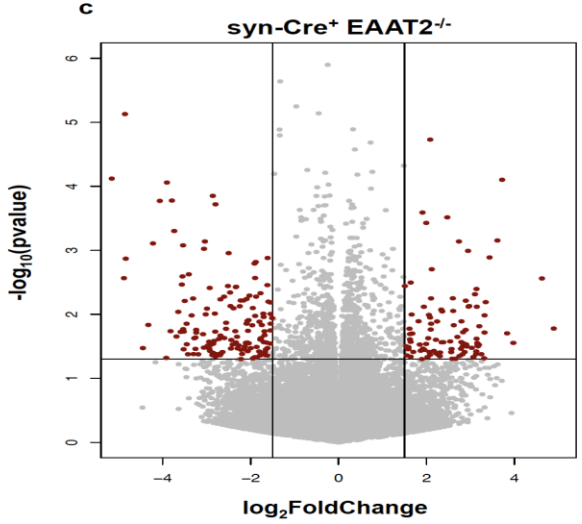
a



b

gfap-Cre+ EAAT2Δ/+ Top Pathways (IPA)	-log(p.value)
Type I Diabetes Mellitus Signaling	3.47
Th1 Pathway	2.22
OX40 Signaling Pathway	2.07
Calcium-induced T Lymphocyte Apoptosis	1.83
Th1 and Th2 Activation Pathway	1.82
Neuroinflammation Signaling Pathway	1.79
IL-17 Signaling	1.54
IL-4 Signaling	1.53
Role of JAK1, JAK2 and TYK2 in Interferon Signaling	1.25
Inflammasome pathway	1.22

c



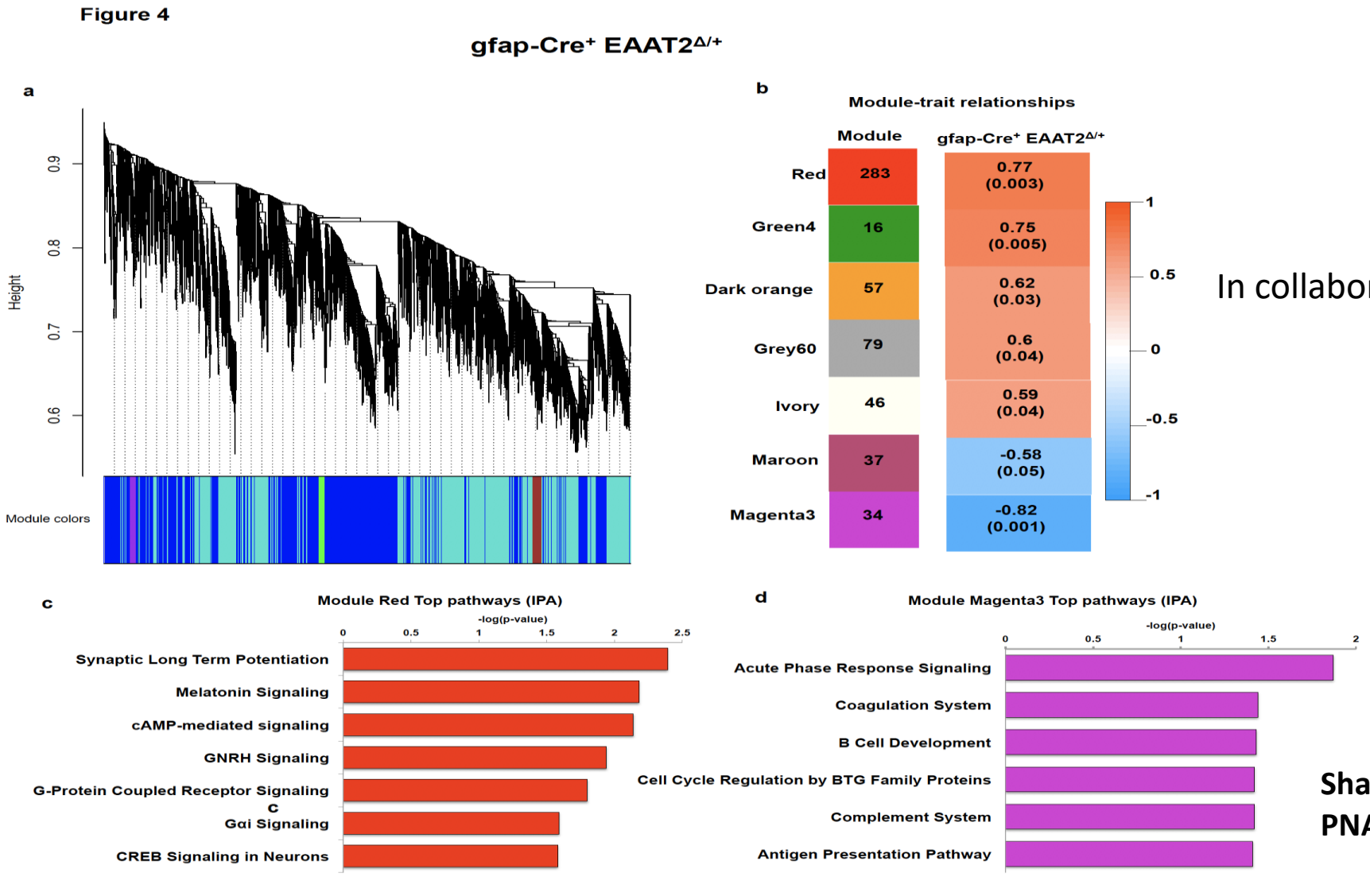
d

syn-Cre+ EAAT2-/- Top Pathways (IPA)	-log(p.value)
Tryptophan Degradation to 2-amino-3-carboxymuconate Semialdehyde	2.02
Transcriptional Regulatory Network in Embryonic Stem Cells	1.92
Serotonin Receptor Signaling	1.81
Serotonin and Melatonin Biosynthesis	1.63
Thrombopoietin Signaling	1.52
NAD biosynthesis II (from tryptophan)	1.48
IL-15 Signaling	1.44

In collaboration with Li Shen

Sharma, Kazim…
Pereira PNAS 2019

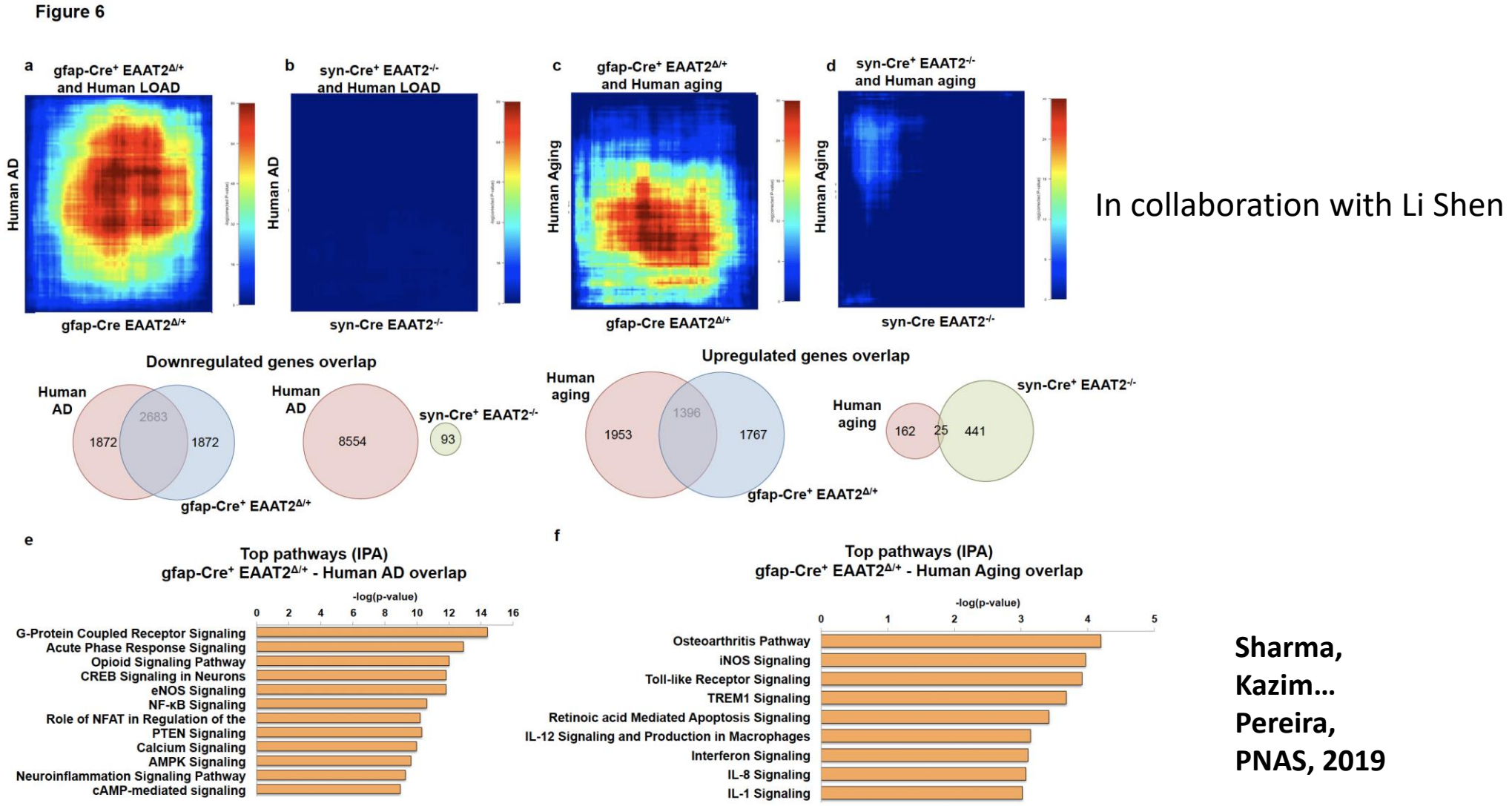
Dysregulation of inflammatory pathways in the hippocampus negatively correlate with cognitive dysfunction in astrocytic EAAT2 knockout mice by weighted gene co-expression network analysis



In collaboration with Li Shen

Sharma, Kazim… Pereira PNAS 2019

Transcriptome from conditional loss of astrocytic not neuronal EAAT2 overlaps with human AD and aging datasets in Rank-Rank hypergeometric overlap (RRHO) comparisons



In collaboration with Li Shen

Sharma, Kazim… Pereira, PNAS, 2019

Summary:

- Conditional heterozygous astrocytic EAAT2 knockout mice present accelerated cognitive decline (and both astrocytic and neuronal EAAT2 KO present spatial reference memory deficit at older age -17mo)

- Astrocytic EAAT2 deficiency mice have dysregulated immune signaling that correlate with cognitive dysfunction

- Transcriptome from conditional loss of astrocytic not neuronal EAAT2 overlaps with human AD and aging datasets in Rank-Rank hypergeometric overlap (RRHO) comparisons

- **These data highlight EAAT2 is a potential therapeutic target for AD**

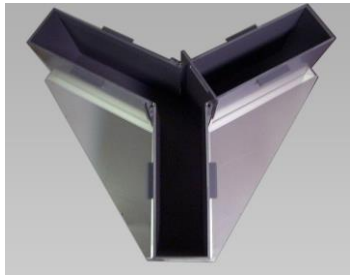
Future Directions:

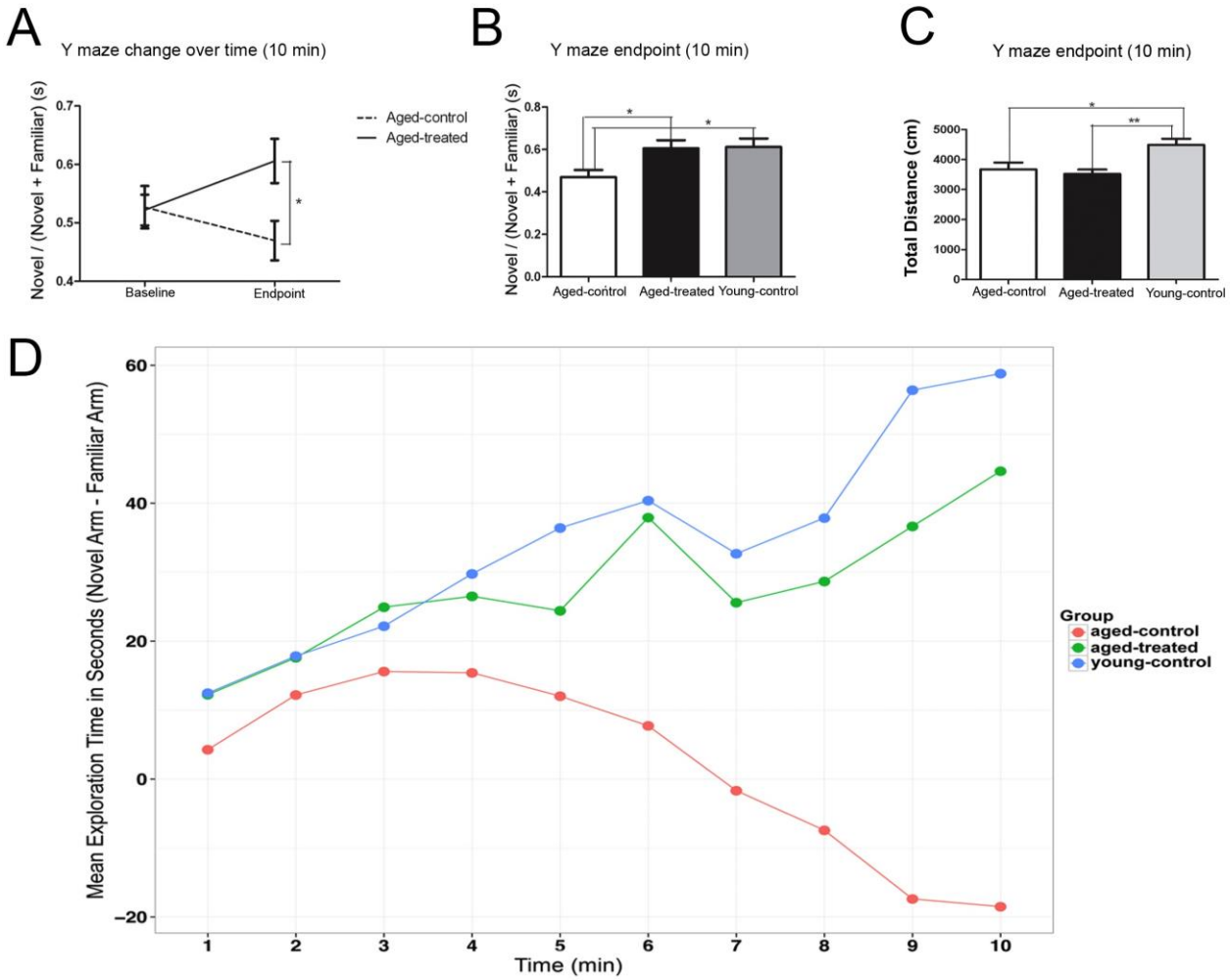
Work funded by **R01 AG063819**

1. **To uncover regulators of excitatory neuronal susceptibility to NFT accumulation and degeneration.** *Hypothesis: Excitatory neurons in the hippocampus and neocortex, when compared to, excitatory neurons in primary motor and visual cortices, and inhibitory interneurons are characterized by unique pathways that make them vulnerable to age-related tau accumulation and neurodegeneration*. Brain tissue from vulnerable regions- Entorhinal cortex and Pre-frontal cortex and resilient regions- Primary motor cortex and Visual cortex will be resected from fresh human post-mortem AD and age-matched brain tissue. Single cell neuronal suspension will be prepared from these brain regions and used for single cell RNA sequencing. We will also isolate polysomal mRNA from vulnerable and resilient brain regions using the novel viral TRAP (vTRAP) technique from cell type specific cre lines: excitatory neurons (Slc17a7-IRES-Cre) and interneurons (Gad2-IRES-Cre) in mouse models of aging and tau (EC-tau). Expression profiles of excitatory neurons from vulnerable brain regions in humans and mice will be compared to excitatory neurons of resilient brain regions and interneurons. Global gene co-expression networks for excitatory and inhibitory neuron populations will be constructed through Weighted Interaction Network Analysis (WINA) and Multiscale Embedded Gene co-Expression Network Analysis (MEGENA). The top key drivers of the modules most associated with AD will be used as candidate targets for experimental validation.
2. **Role of EAAT2 in tau spread**
3. **Molecular targets for EAAT2 TDI**

TRI-INSTITUTIONAL
THERAPEUTICS DISCOVERY INSTITUTE

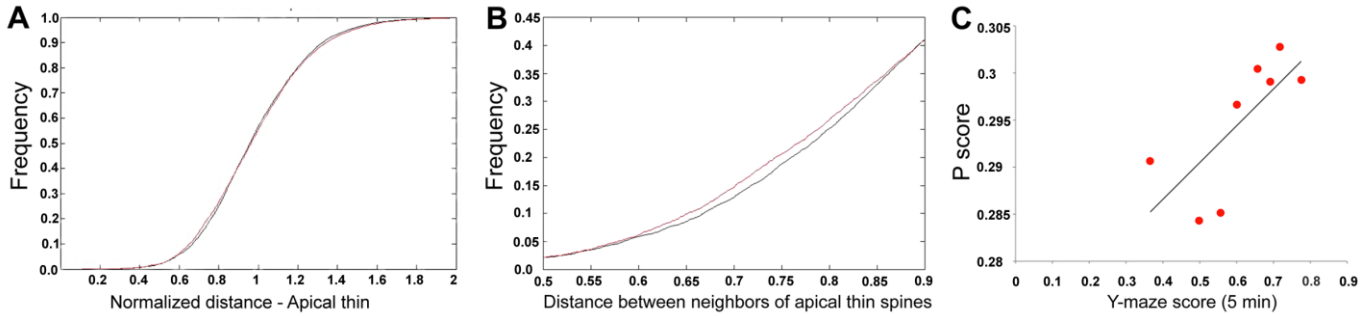
Glutamate modulator Riluzole prevented hippocampal dependent age-related cognitive decline

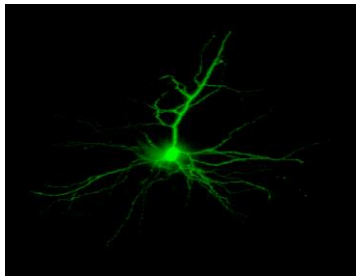


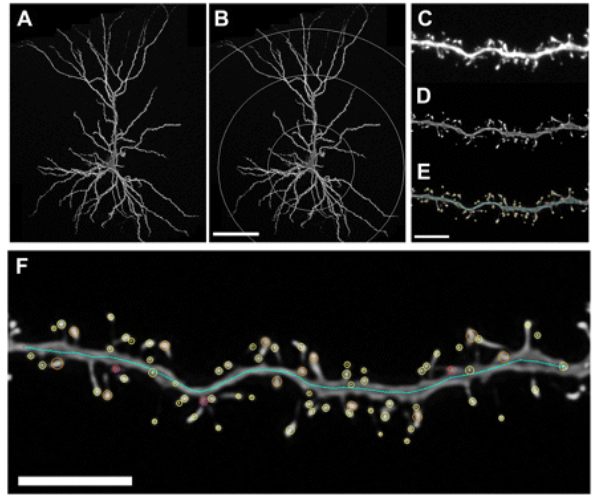


Pereira et al., 2014. PNAS

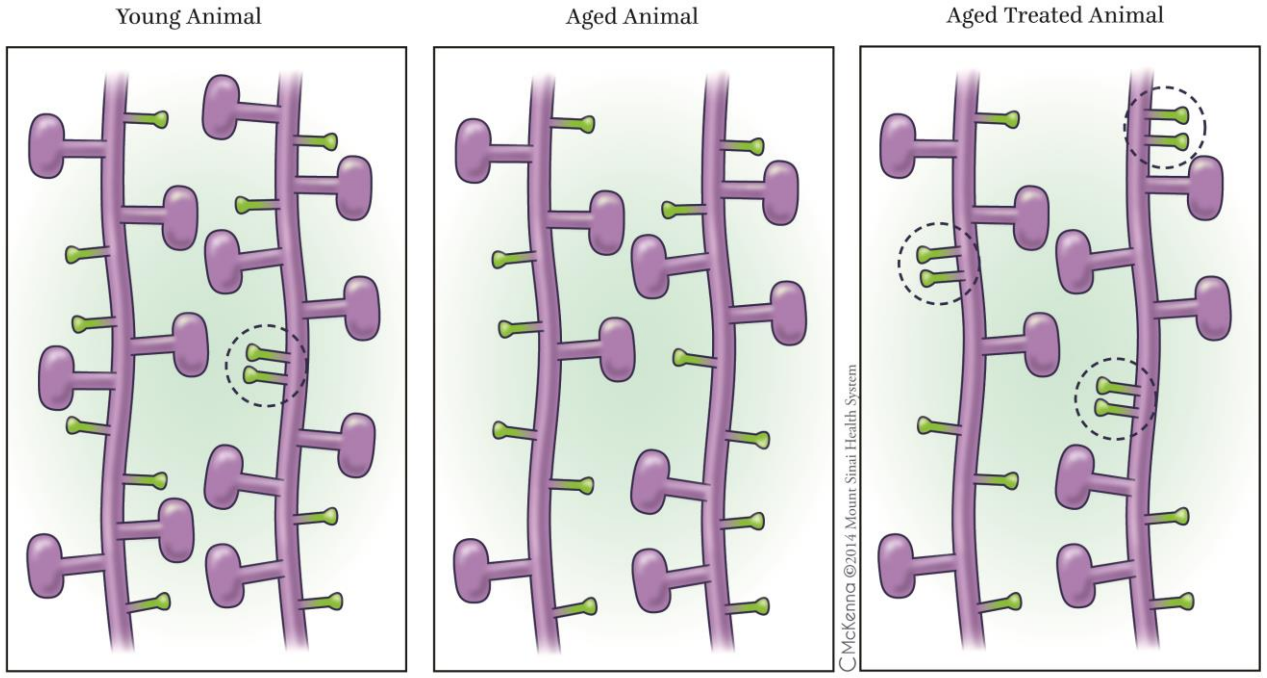
Increased Dendritic Spine Clustering that Correlates with Behavioral Performance







Dendritic spines form the post-synaptic component of most excitatory synapses in the cerebral cortex and are capable of rapid formation, expansion, contraction and elimination (critical for neuroplasticity)



Pereira et al., 2014, PNAS

Bruce McEwen

John Morrison

Patrick Hof

Molecular Psychiatry (2016), 1–10

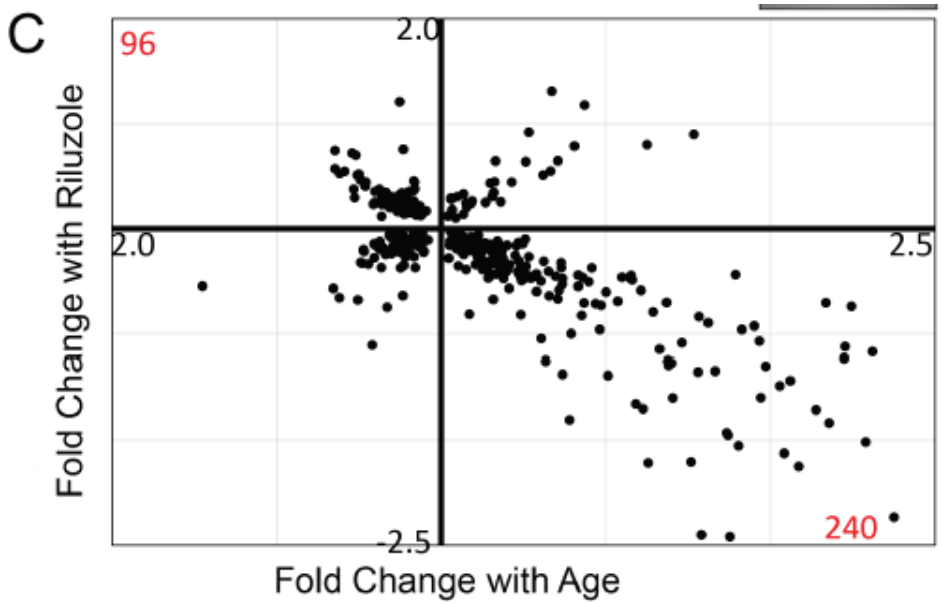
www.nature.com/mp

Molecular Psychiatry

ORIGINAL ARTICLE

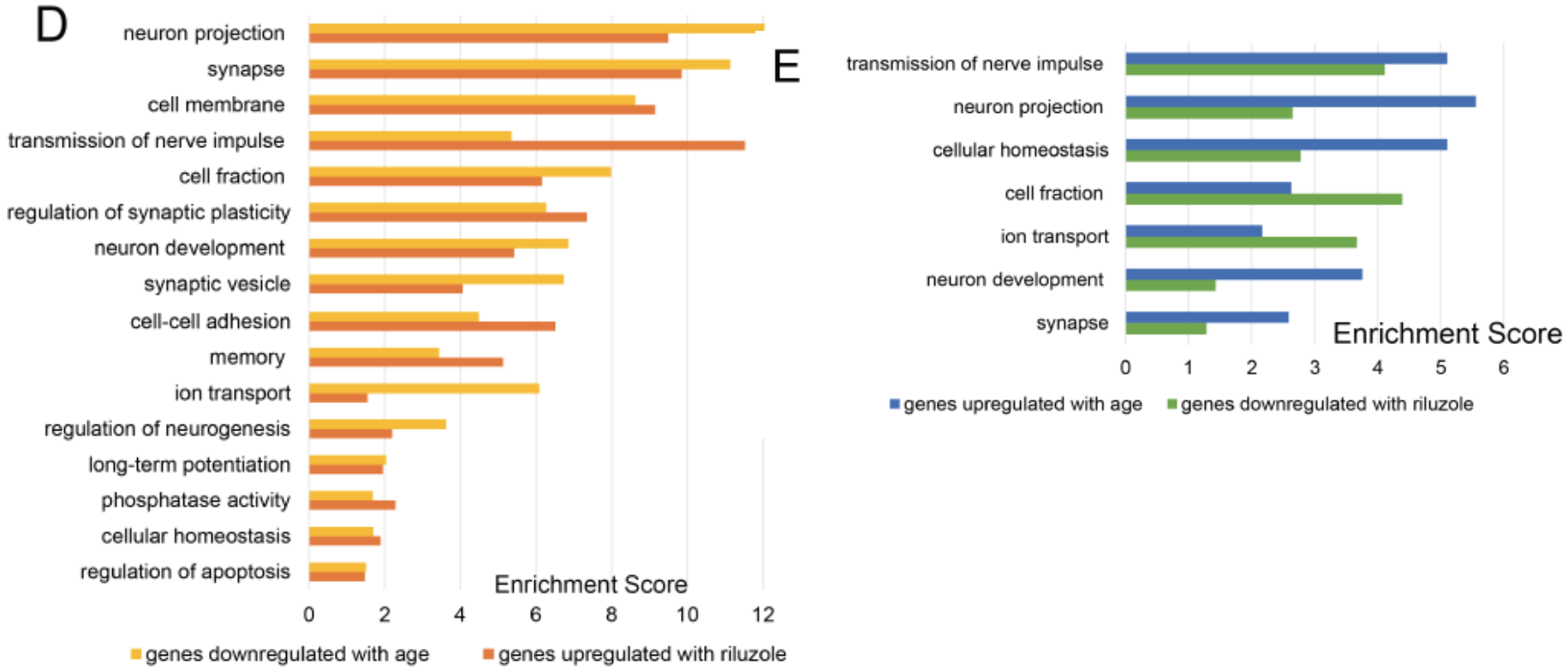
Age and Alzheimer's disease gene expression profiles reversed by the glutamate modulator riluzole

AC Pereira[1,4], JD Gray[1,4], JF Kogan[1], RL Davidson[1], TG Rubin[1], M Okamoto[1,2], JH Morrison[3] and BS McEwen[1]



Significantly Enriched Pathways based on genes differentially expressed by aging and riluzole

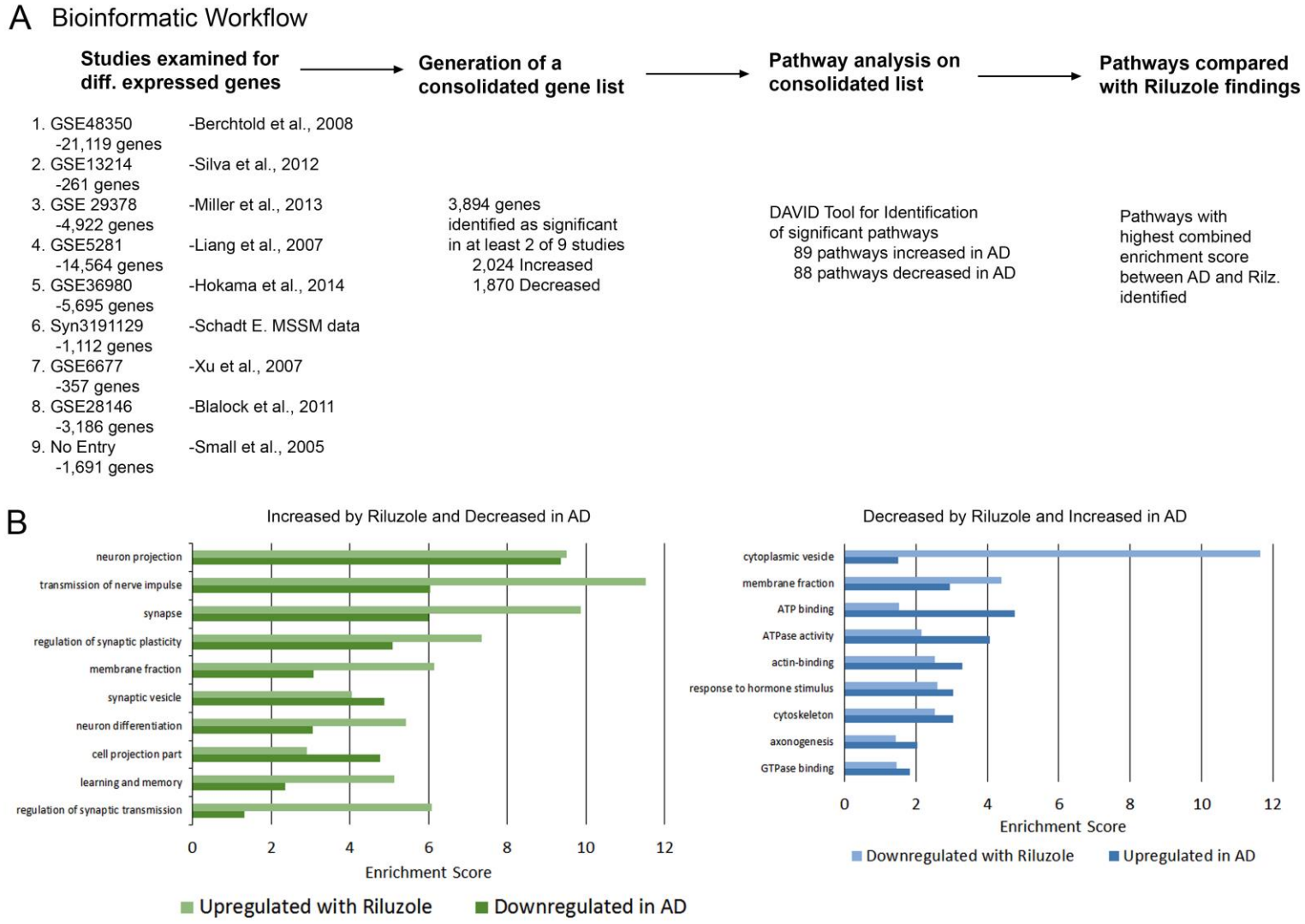
Top Pathways related to neurotransmission and synaptic plasticity

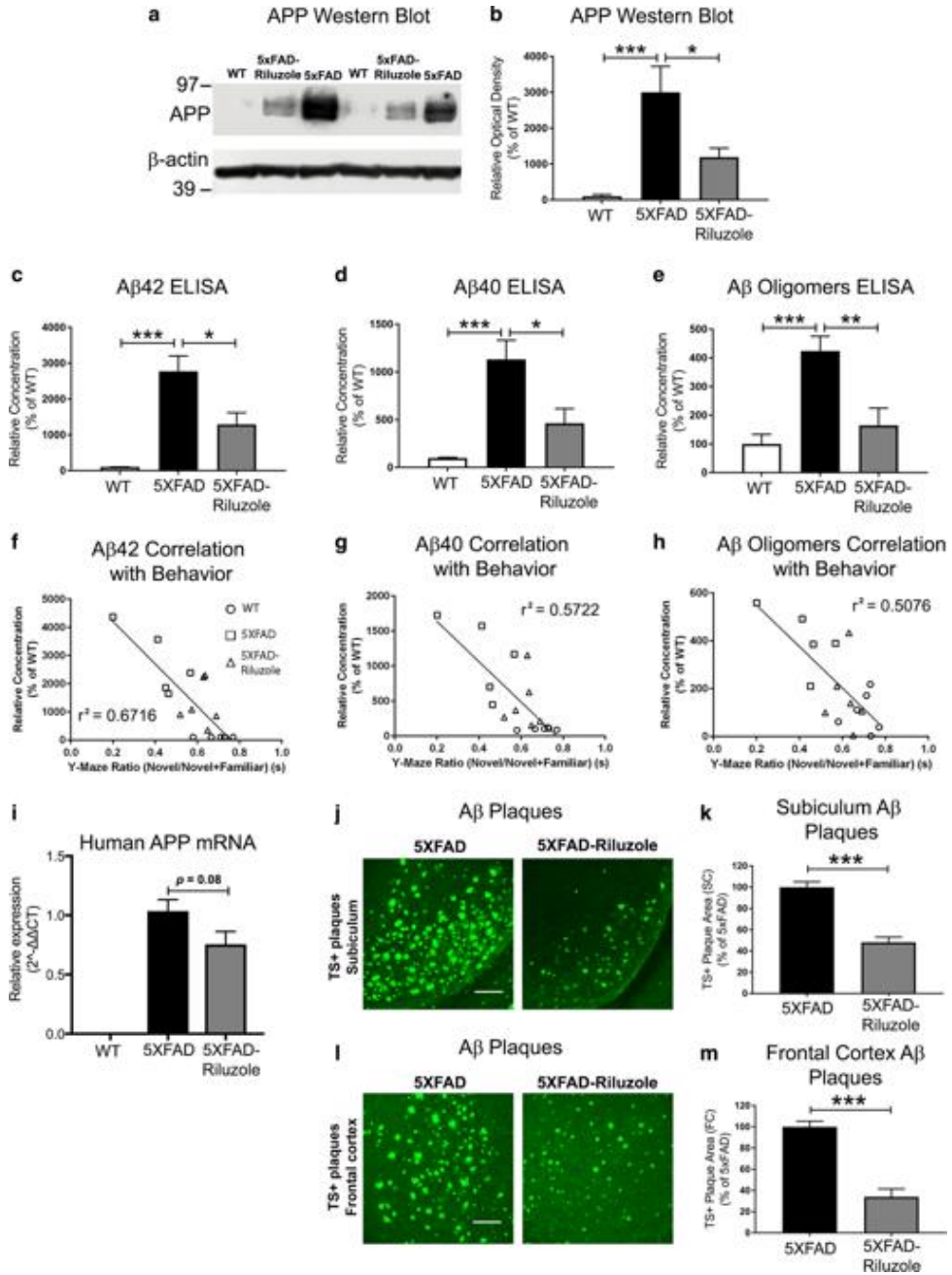


Pereira AC et al, 2016 – Molecular Psychiatry

Enrichment score>1.3 reflects p<0.05

Gene pathways changed by riluzole in aged rats are similar and in the opposite direction to those in post-mortem AD brains

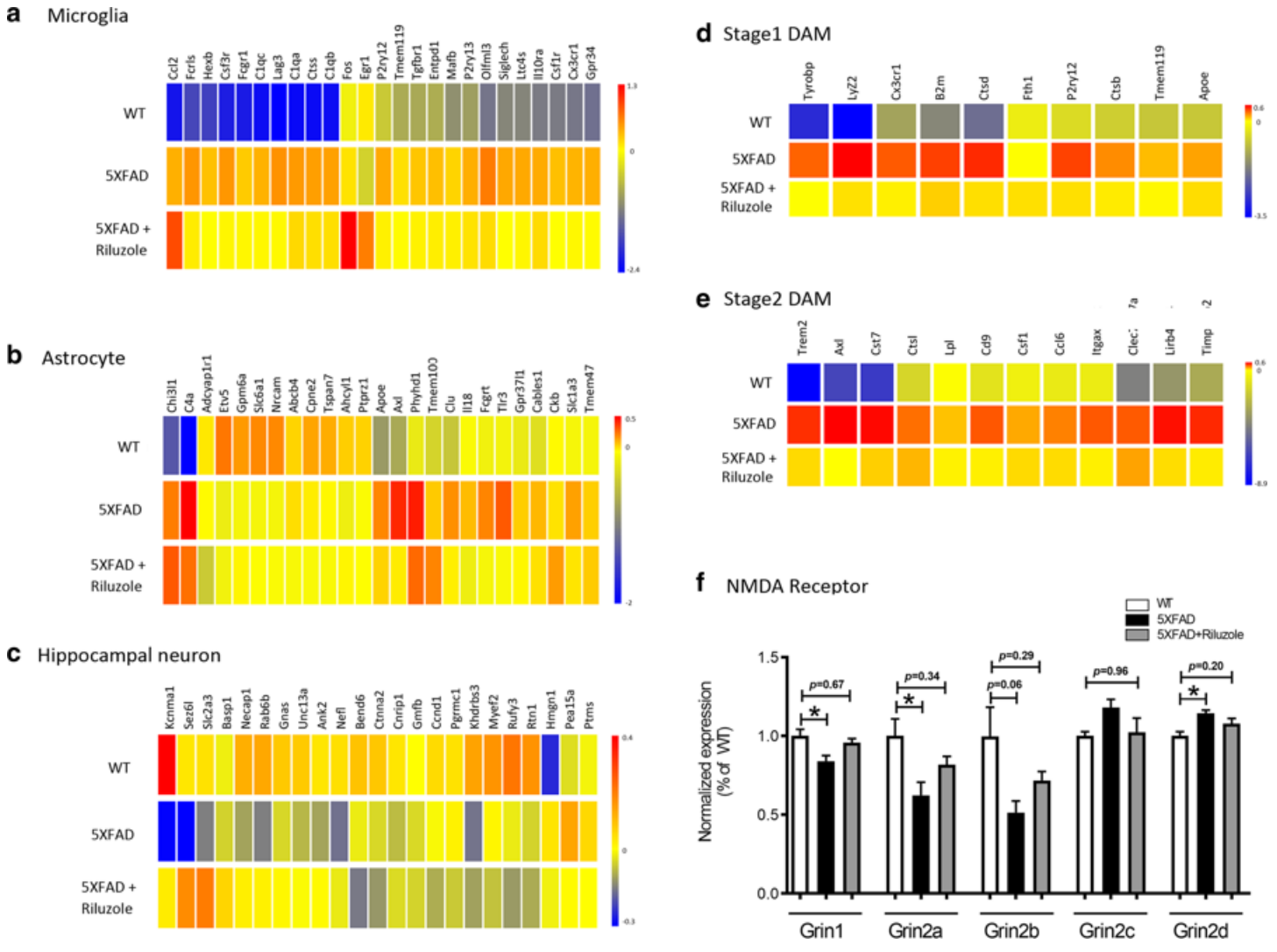




Riluzole treatment reduces Aβ pathology which inversely correlates with memory performance in 5XFAD mice.

Pereira Lab and collaborators Trans Psych 2018

Expression changes in cell-type specific markers and hippocampal NMDA receptor subunits are reversed by riluzole treatment in 5XFAD



Pereira Lab and collaborators Trans Psych 2018

Double-Blind, Randomized, Placebo-Controlled Pilot Trial in Mild Alzheimer's disease
Riluzole 50mg BID x Placebo

Neuropsychological tests	Neuropsychological tests	Neuropsychological tests
Imaging (FDG-PET+MRS)	Imaging (MRS)	Imaging (FDG-PET+MRS)
Baseline	3 months	6 months

Pilot study: 42 subjects

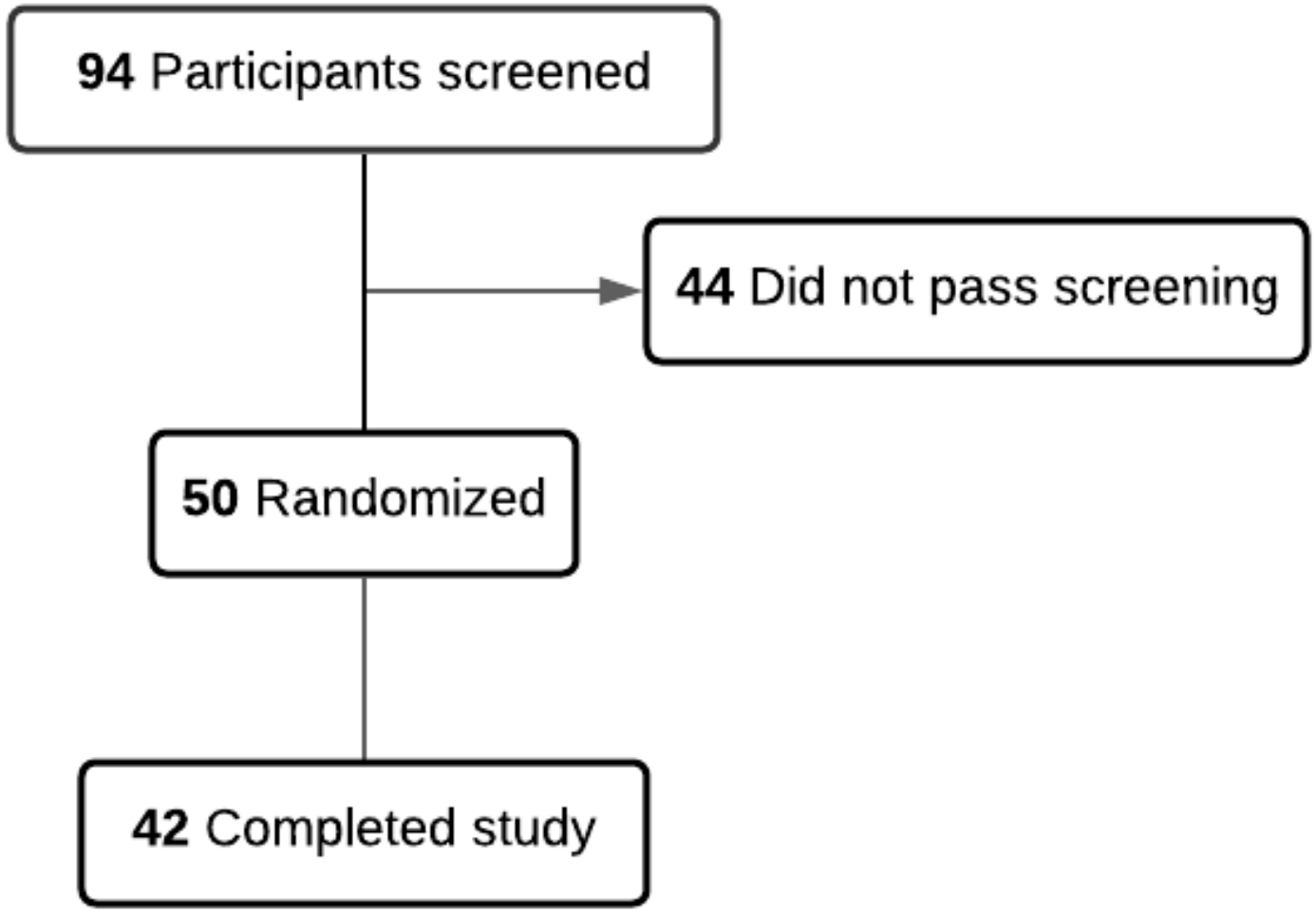
Age cohorts: Age matched cohorts 50-74 yo and 75-95yo (50-95 yo) /actual age range recruited: 58-88 yo

Primary outcome measures: FDG-PET (brain metabolism in pre-specified brain regions) and MRS (N-acetyl aspartate -NAA)

Secondary outcome measures: Neuropsychological testing (to correlate with neuroimaging biomarkers), glutamate (MRS)

Disclosure:

Patent applications 41054762 and 63087610.



Rockefeller University Hospital and Mount Sinai

Table 2. Demographic and Baseline Clinical Characteristics

Characteristic	Placebo (n = 20)	Riluzole (n = 22)	P-Value
Age (years), mean ± SD	74.6 ± 7.7	75.3 ± 5.8	0.73
Sex, no. (%)			0.30
Female	14 (70.0%)	12 (54.5%)	
Male	6 (30.0%)	10 (45.5%)	
Race/ethnicity, no. (%)			1.00
Black or African American	0 (0%)	1 (4.5%)	
Black/non-Hispanic	1 (5.0%)	0 (0%)	
Latino/Hispanic	0 (0%)	1 (4.5%)	
White/non-Hispanic	19 (95.0%)	20 (90.9%)	
Education (years), mean ± SD	15.1 ± 3.1	15.9 ± 3.0	0.39
ApoE4 carrier, no. (%)	8 (40.0%)	15 (68.2%)	0.12
Clinical scales*, mean ± SD			
ADAS-cog	17.9 ± 7.5	22.2 ± 7.9	0.08
ADL total	68.1 ± 9.3	68.4 ± 9.5	0.91
CDR-sum of boxes	3.6 ± 1.8	3.8 ± 1.9	0.73
CDR total	0.6 ± 0.2	0.6 ± 0.2	0.59
MMSE	22.8 ± 2.9	22.5 ± 2.5	0.72
NPI	10.2 ± 11.1	9.6 ± 9.2	0.86

*ADAS-cog = Alzheimer's Disease Assessment Scale, ADL = Activities of Daily Living Inventory scale, CDR = Clinical Dementia Rating scale, MMSE = Mini-Mental State Examination, NPI = Neuropsychiatric Inventory score.

Neuroimaging Biomarkers

FDG-PET

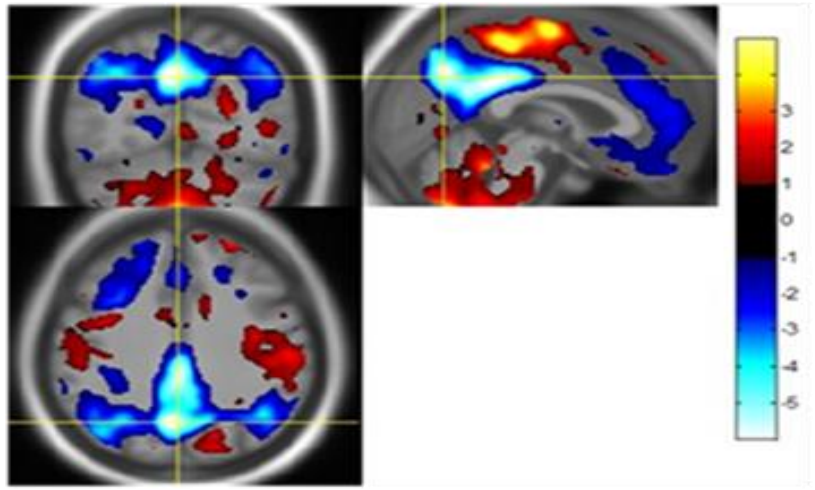


Fig.6: AD signature pattern of hypometabolism; blue areas represent hypometabolism while red shows preserved areas

Magnetic Resonance Spectroscopy (MRS)

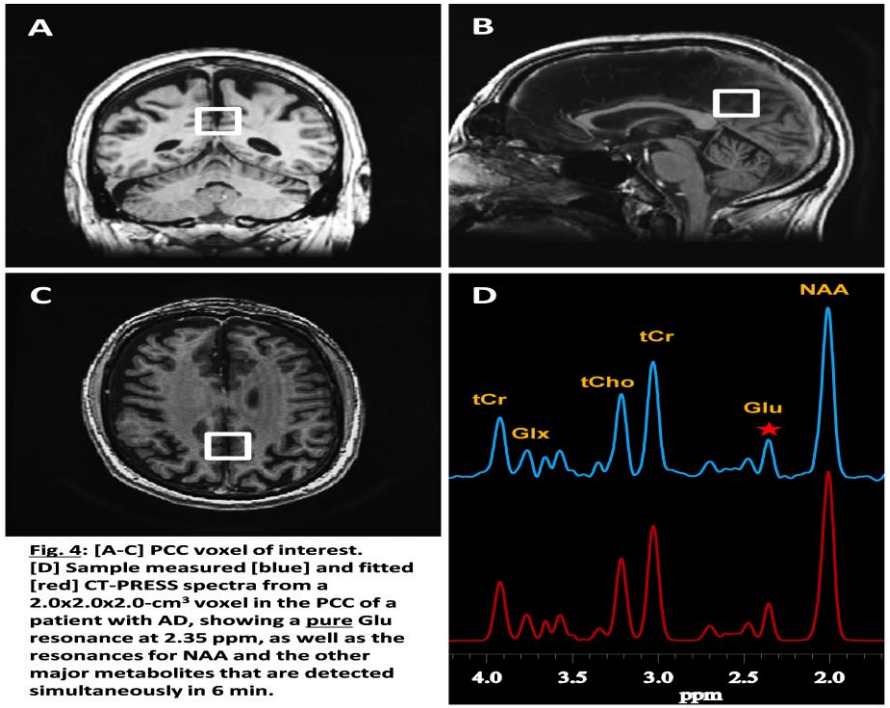


Fig. 4: [A-C] PCC voxel of interest. [D] Sample measured [blue] and fitted [red] CT-PRESS spectra from a 2.0x2.0x2.0-cm³ voxel in the PCC of a patient with AD, showing a pure Glu resonance at 2.35 ppm, as well as the resonances for NAA and the other major metabolites that are detected simultaneously in 6 min.

FDG-PET is a marker of cerebral metabolism, brain function **(FDG-PET correlates with disease progression** -the loss of synaptic activity and neuronal function in AD is closely associated with reduction in glucose metabolism, a primary energy source for the brain). **It's a well established biomarker in AD**

In vivo proton magnetic resonance spectroscopy (^{1}H MRS) allows measurements of glutamatergic compounds - i.e., glutamate (Glu) and neuronal viability marker N-acetyl aspartate (NAA)

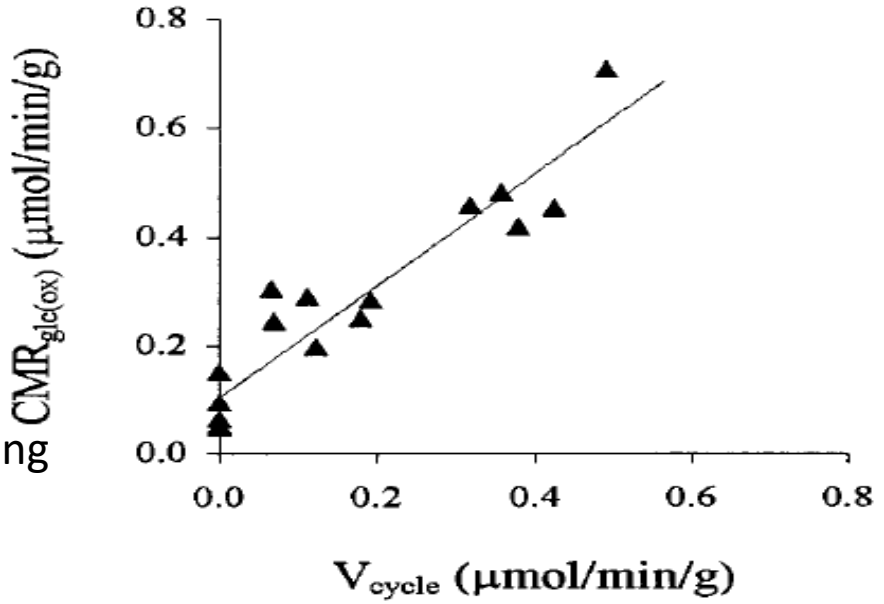
There is a **tight coupling between glutamatergic activity and cerebral glucose metabolism** with stoichiometry close to 1:1.

Proc. Natl. Acad. Sci. USA
Vol. 95, pp. 316–321, January 1998
Neurobiology

Stoichiometric coupling of brain glucose metabolism and glutamatergic neuronal activity

NICOLA R. SIBSON*¶, AJAY DHANKHAR*, GRAEME F. MASON†, DOUGLAS L. ROTHMAN‡, KEVIN L. BEHAR§, AND ROBERT G. SHULMAN*

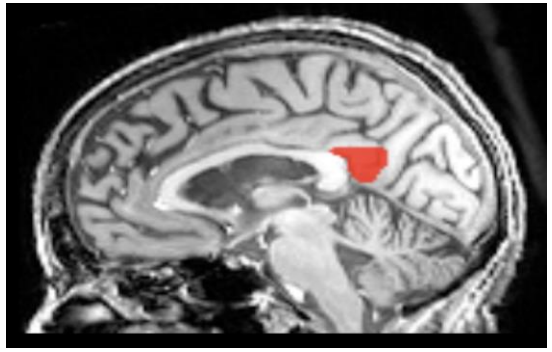
*Departments of Molecular Biophysics and Biochemistry, †Psychiatry, ‡Diagnostic Radiology and §Neurology, Yale University School of Medicine, New Haven, CT 06520



Vc: rate of glutamate neurotransmitter cycling
CMRgc:rate of oxidative glc consumption

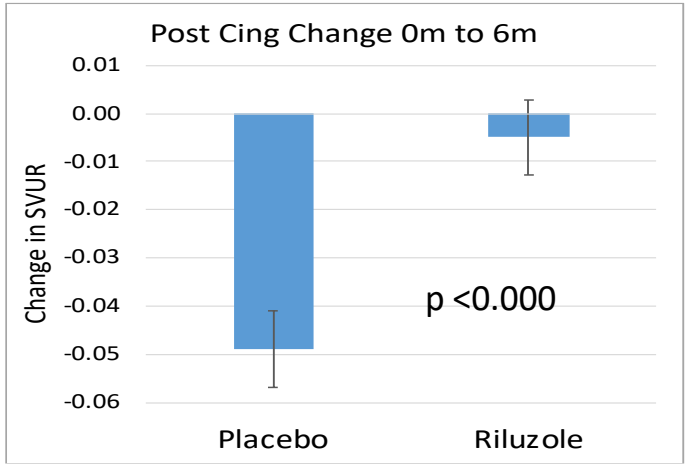
Glutamatergic transmission consumes more than 80% of ATP generated from brain metabolism.

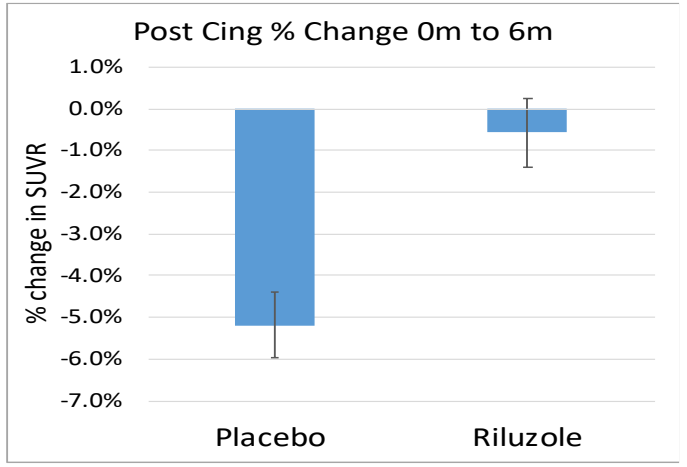
FDG-PET Results: Posterior Cingulate (PC) glucose metabolism is significantly preserved in Riluzole Group in comparison to placebo

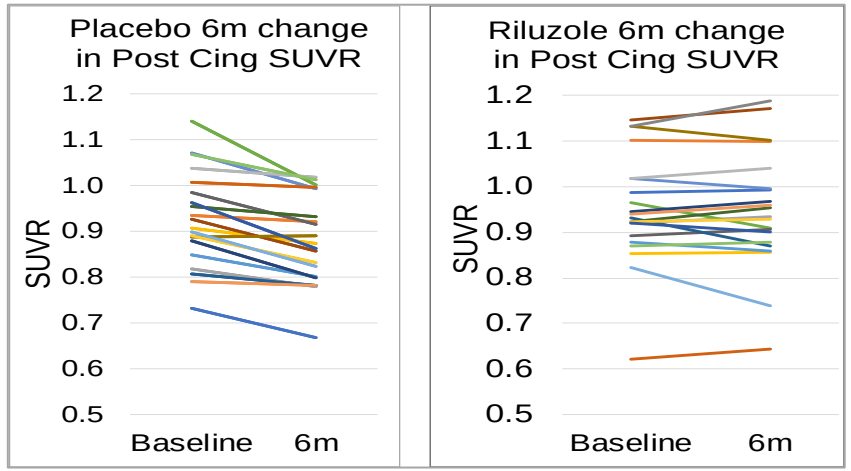


- Bilateral region masked with each participant's gray tissue
- No baseline difference between treatment arms (p<0.55)
- Placebo arm decreased more than riluzole arm (p<0.000)
- Effect is significant across multiple reference regions (paracentral p<0.000, centrum ovale p<0.008, whole brain p<0.016, cerebellar cortex p<0.03)
- Significance stands with and without adjusting for age, gender, education, ApoE carrier status, MMSE baseline and baseline PC value SUVR

Worsen







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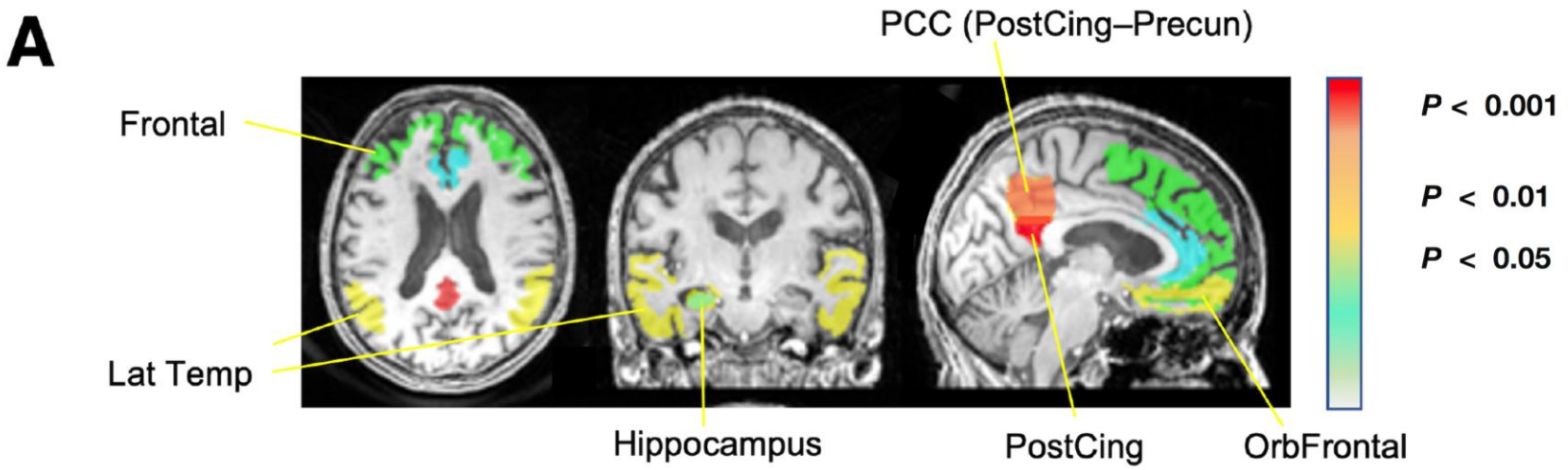
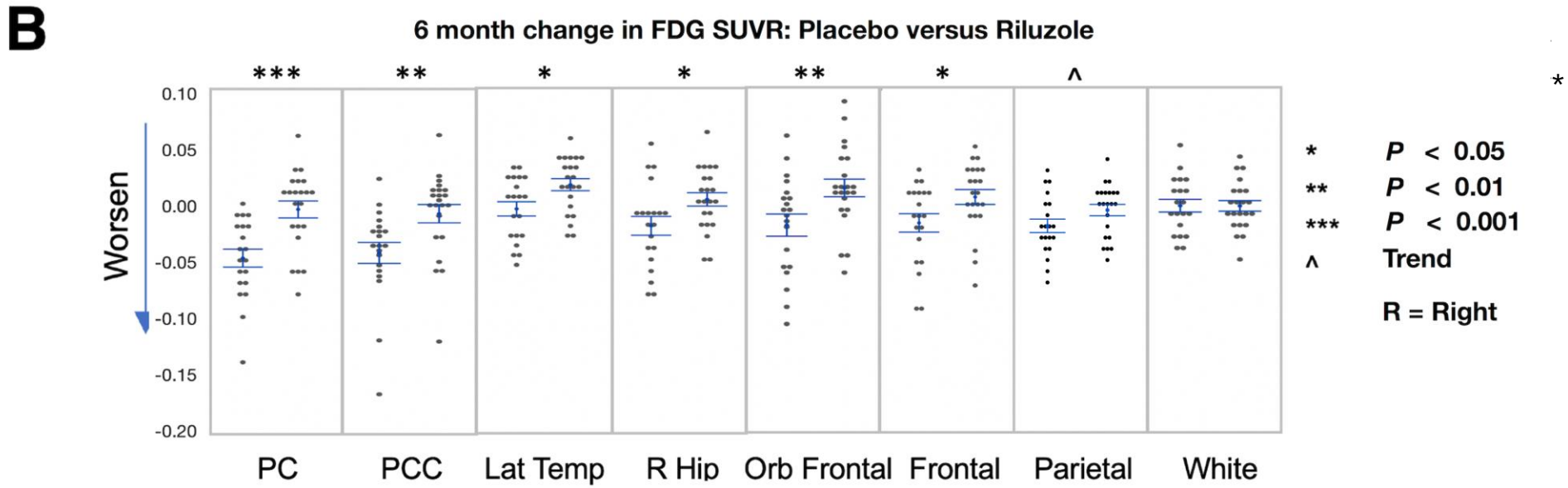
FDG PET: Less metabolic decline in several pre-specified regions of interest in Riluzole group in comparison to placebo





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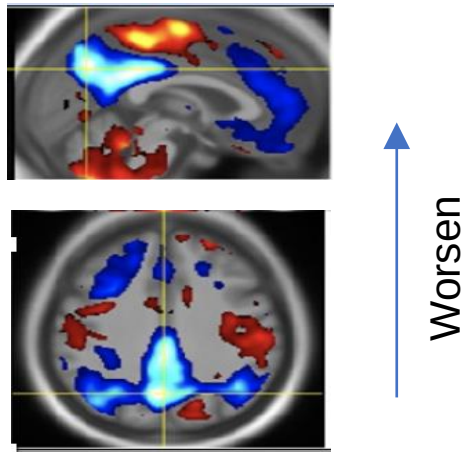
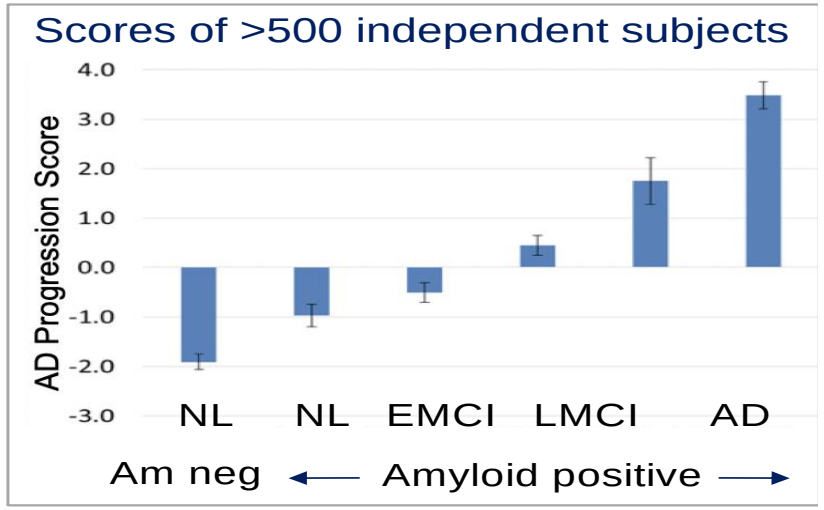
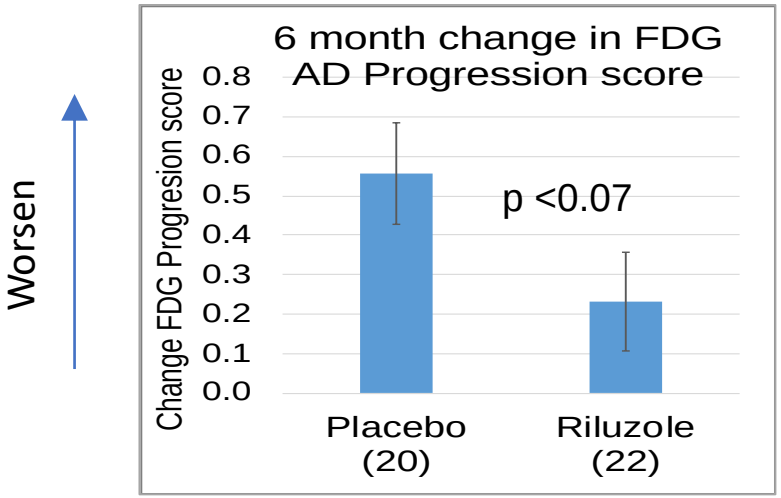
FDG PET AD Progression classifier results

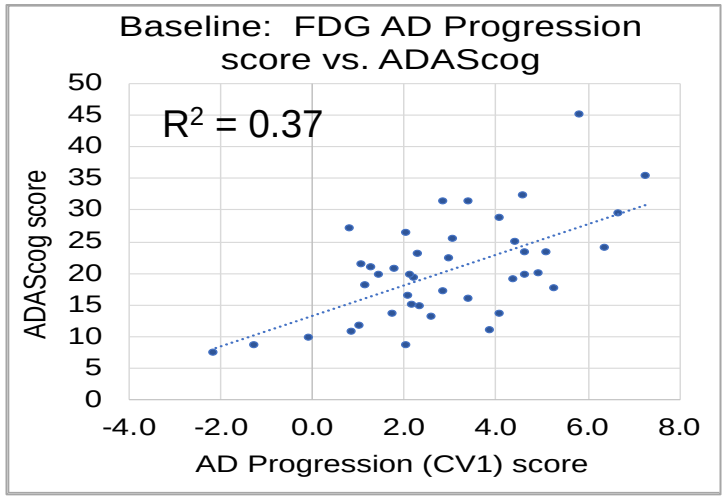
Classifier quantifies pattern of metabolic decline (blue) and preservation (orange) that is increasingly expressed with progression of AD

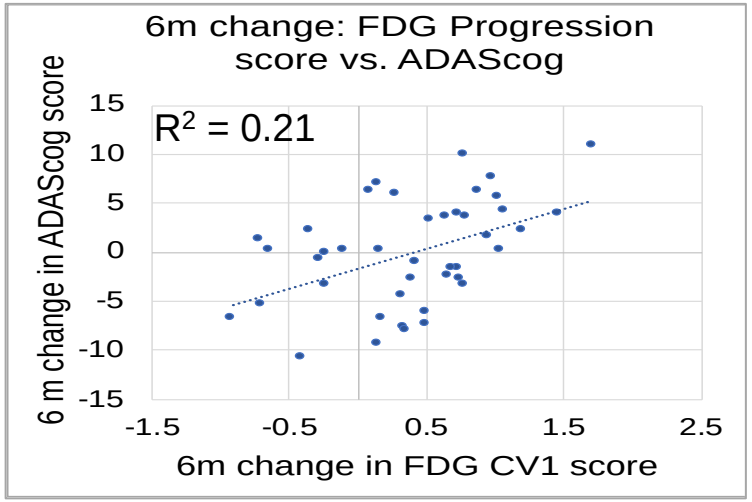
Higher score reflects greater pattern expression











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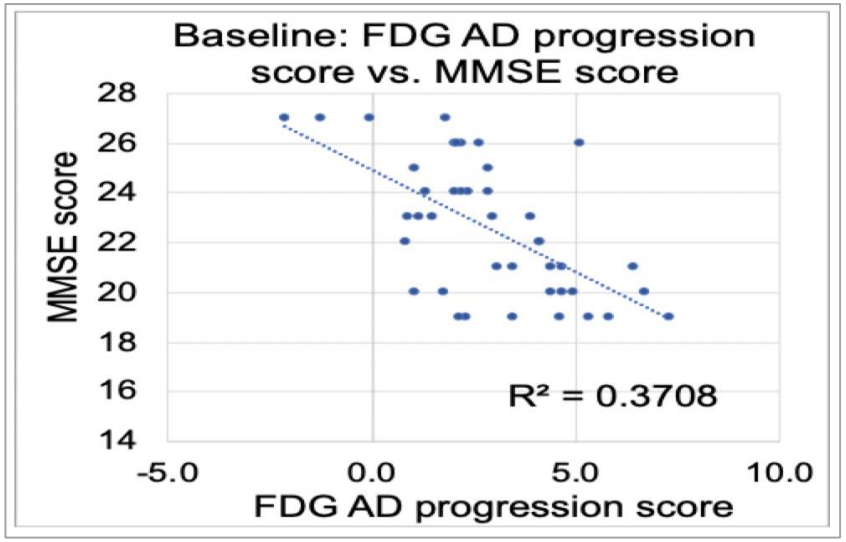
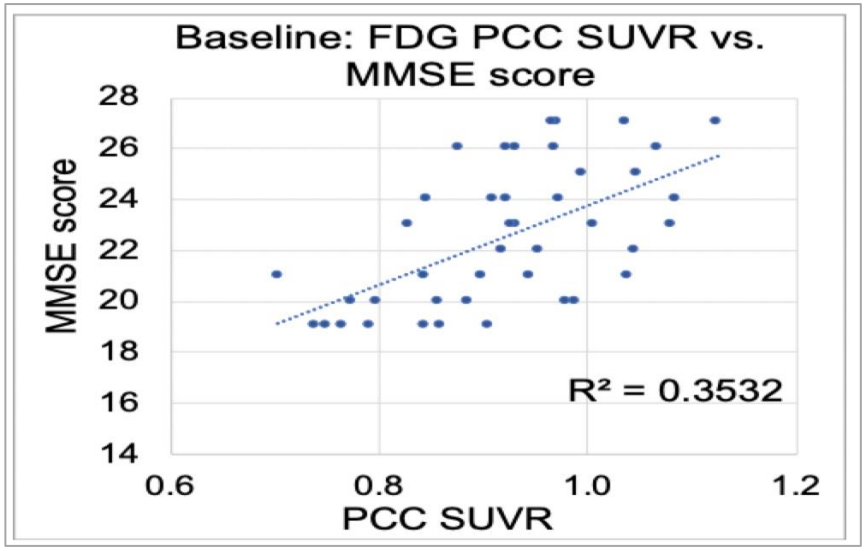
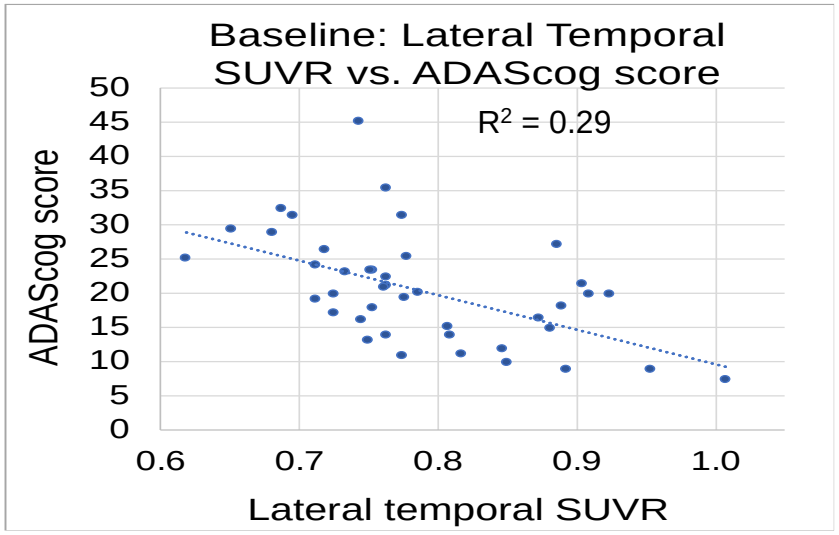
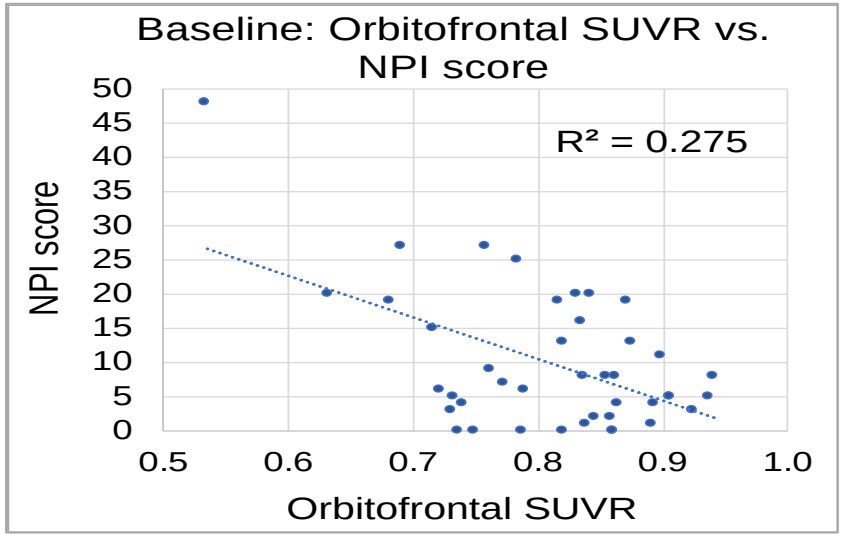
More FDG-PET relationships to cognitive measures









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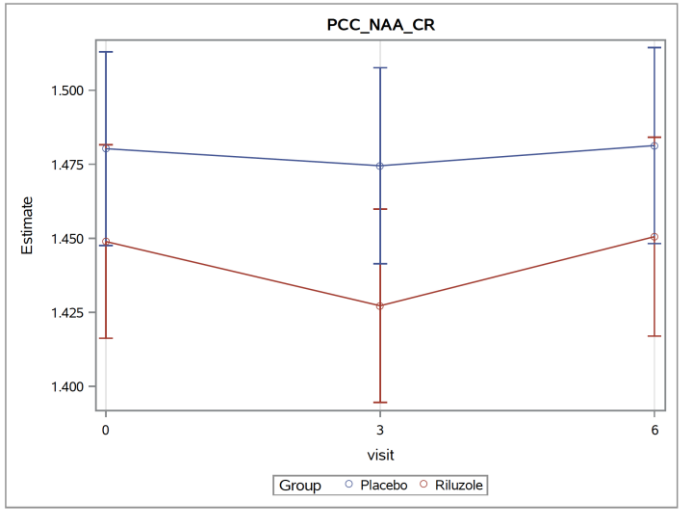
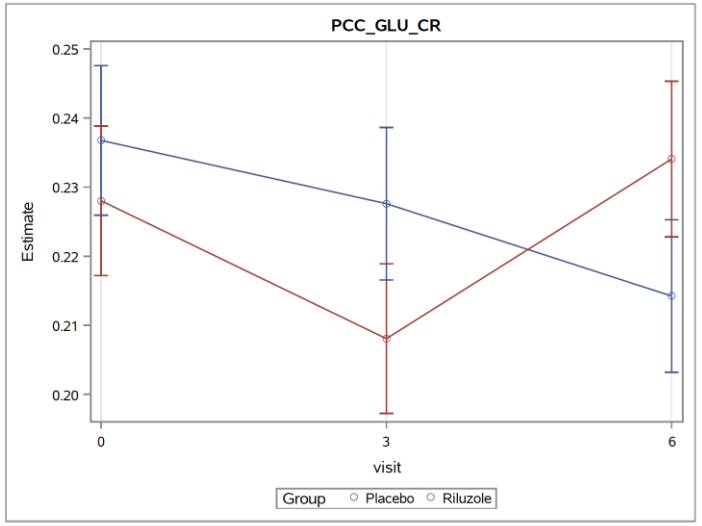
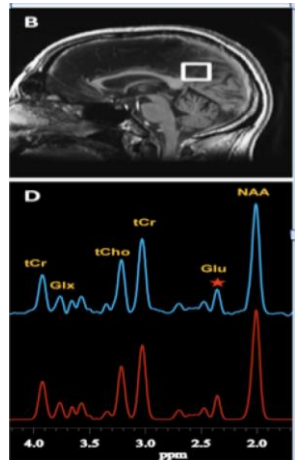
Magnetic Resonance Spectroscopy (MRS):
No significant difference in N-acetyl aspartate NAA/Cr in PC
Glu/Cr has a group by visit significant interaction in PC and higher Glu levels correlate with better cognition

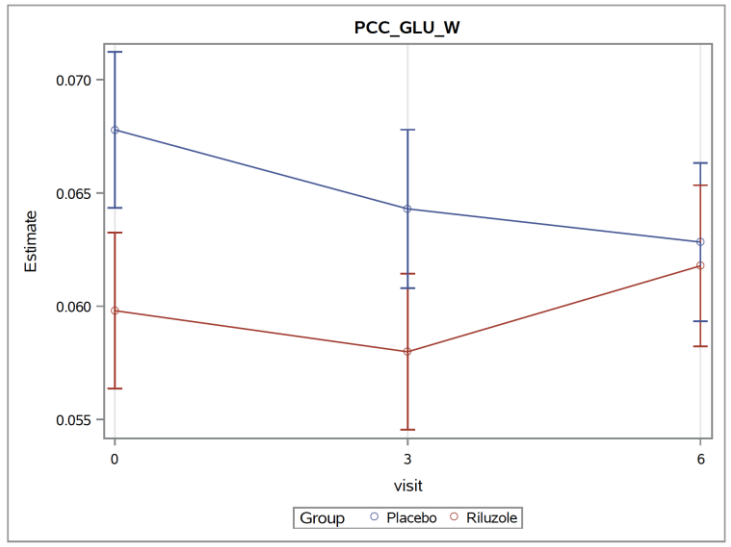


Group: p=0.38
Visit: p=0.70
Group*Visit: p=0.89
6 months p=0.52

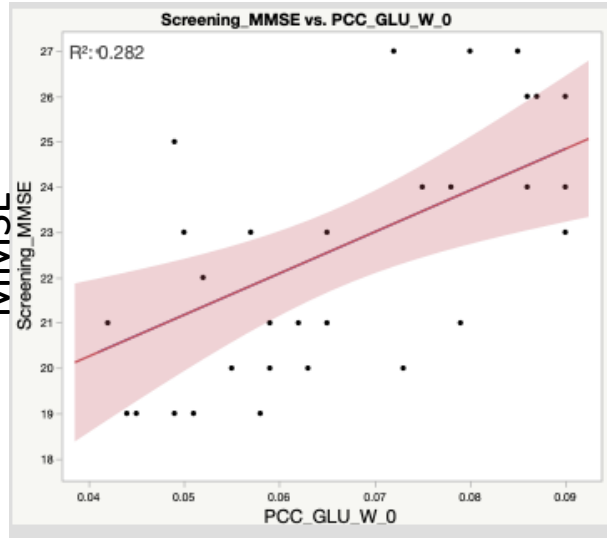




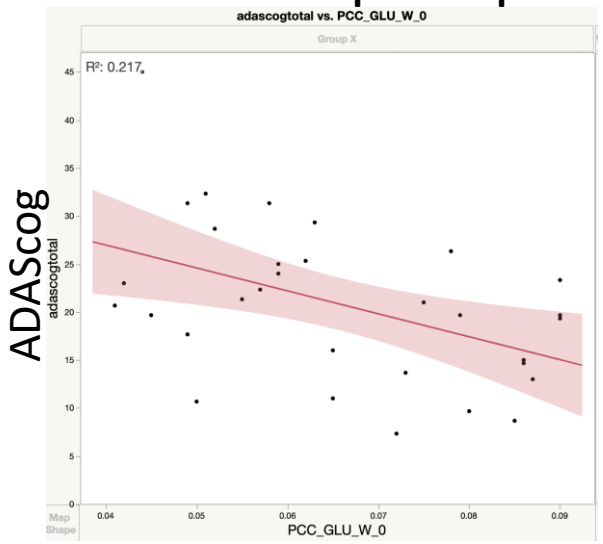
Group: p=0.82
Visit: p=0.19
Group*Visit: p=0.05



MMSE



ADAScog



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In collaboration with Dikoma Shungu, PhD

SUMMARY:

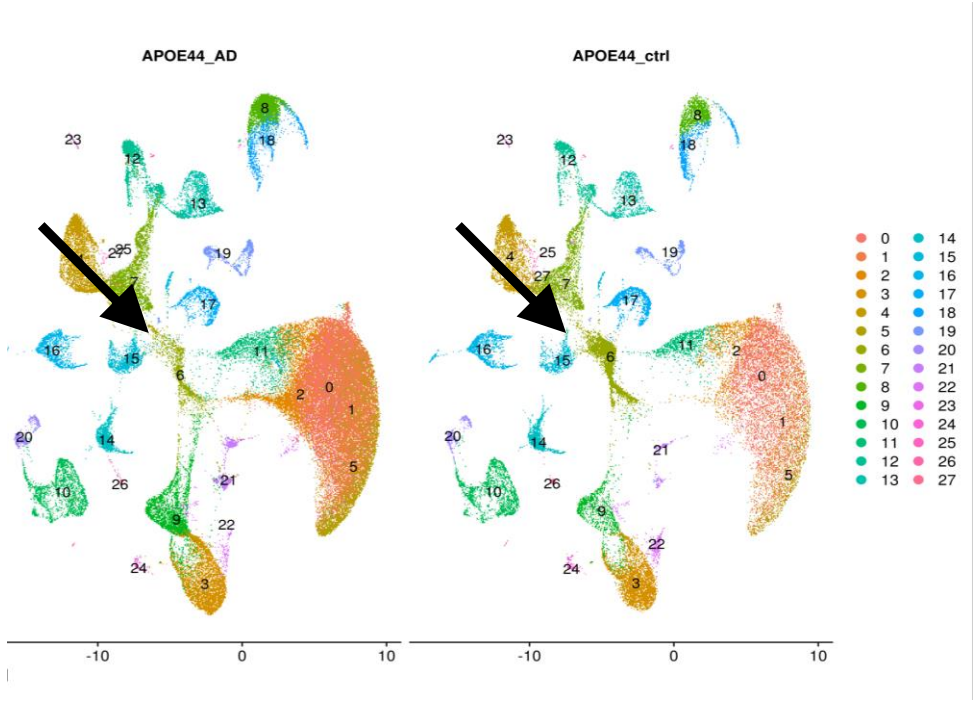
-Pilot randomized placebo controlled trial of riluzole in Alzheimer's disease (AD) **met primary outcome measure of FDG-PET** (well established biomarker in AD and predictor of disease progression) showing significantly less decline in cerebral metabolism in pre-specified regions of interest: **posterior cingulate**, precuneus, lateral temporal, R hippocampus and frontal cortex in riluzole treated subjects in comparison to placebo group. However, no changes were observed in NAA, measured through magnetic resonance spectroscopy (MRS), a second primary outcome measure.

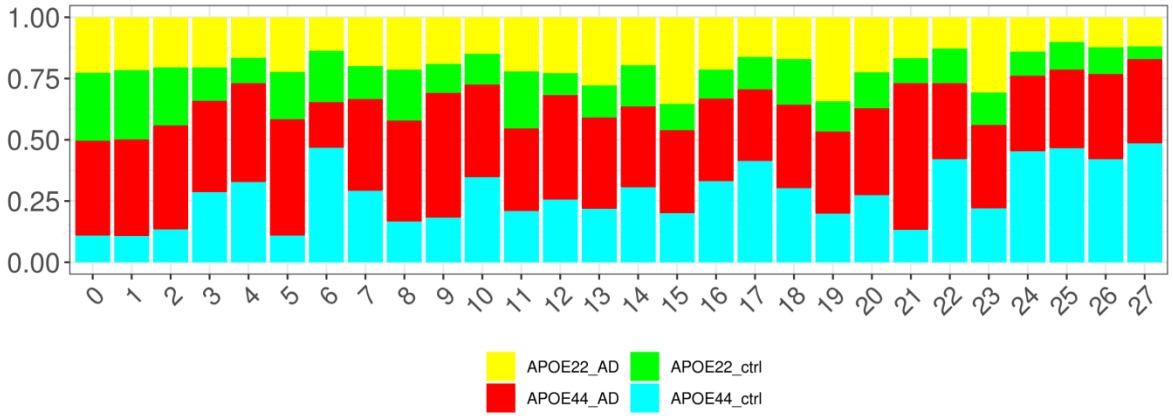
-Strong correlation between cognitive measures and brain metabolism in FDG-PET was observed (met secondary outcome measure)

- Increase in glutamate/Cr levels in PC measured through MRS (glutamate levels in MRS are depleted in AD) – met secondary outcome measure

- These results support performing a clinical trial with riluzole in a large sample size and for a longer duration in AD
- **NOT advised to prescribe riluzole off-label until safety and efficacy are confirmed in a phase 3 trial in AD**

Excitatory neurons are particularly vulnerable in APOE4



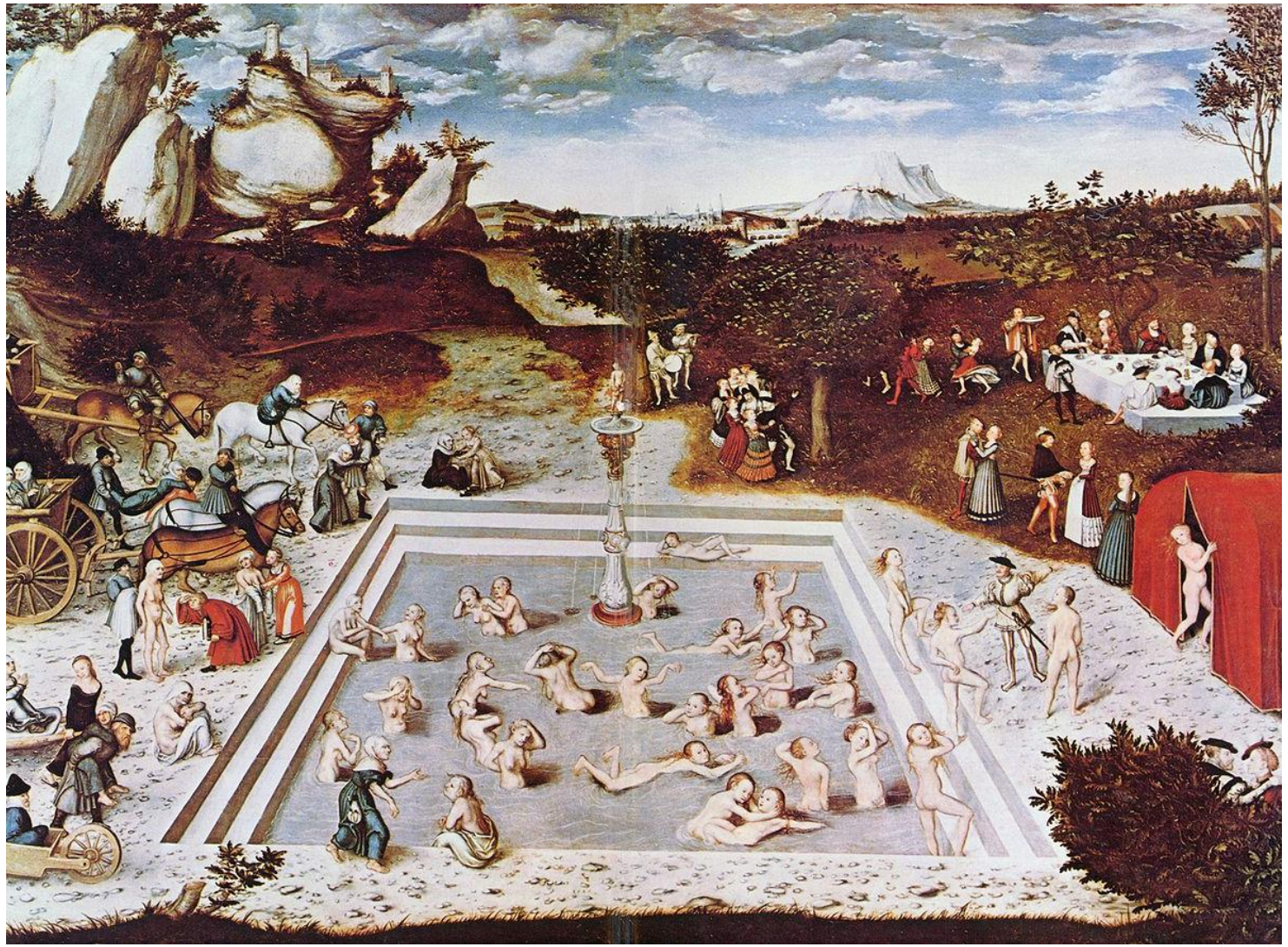


Excitatory
(4,6,7,15,25,27)

Inhibitory
(10,14,15,16,17,20,26)

Astroc
3,9,21,22,24

Oligodendrocytes
(0,1,2,5,11)



The Fountain of Youth by Lucas Cranach the Elder 1546

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