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

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Source: Alzheimer’s Association
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

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

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Neurobiology of Disease

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

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Amyloid-β release and neuronal activity

Neilson

APP Processing and Synaptic Function

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Iwatsubo4, Sangram Sisodia9, Roberto Maloney7,1, 2, 3, smashed

Neuronal activity regulates the regional vulnerability
to amyloid-β deposition

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Jin-Moo Lee1, 3, 4 & David M Holtzman1, 4
Neuronal activity regulates extracellular tau in vivo

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Physiological release of endogenous tau is stimulated by neuronal activity.

Pooler AM1, Phillips EC, Lau DH, Noble W, Hanger DP.

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Neuronal activity enhances tau propagation and tau pathology in vivo

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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

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.

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:

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**

   Work funded by **R01 AG063819**
Glutamate modulator Riluzole prevented hippocampal dependent age-related cognitive decline

Pereira et al., 2014. PNAS
Increased Dendritic Spine Clustering that Correlates with Behavioral Performance

Pereira et al., 2014, PNAS

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

AC Pereira, JD Gray, JF Kogan, RL Davidson, TG Rubin, M Okamoto, JH Morrison and BS McEwen
Significantly Enriched Pathways based on genes differentially expressed by aging and riluzole

Top Pathways related to neurotransmission and synaptic plasticity

Enrichment score > 1.3 reflects p < 0.05

Pereira AC et al, 2016 – Molecular Psychiatry
Gene pathways changed by riluzole in aged rats are similar and in the opposite direction to those in post-mortem AD brains.

**Bioinformatic Workflow**

1. Studies examined for diff. expressed genes
   - GSE48350: 21,119 genes
   - GSE13214: 261 genes
   - GSE 29578: 4,922 genes
   - GSE52841: 14,464 genes
   - GSE36980: 5,449 genes
   - Syn3191129: -8,112 genes
   - GSE6677: -387 genes
   - GSE29146: -3,118 genes
   - No Entry: 1,181 genes

2. Generation of a consolidated gene list
   - 3,894 genes identified as significant in at least 2 of 9 studies
   - 2,024 Increased
   - 1,870 Decreased

3. Pathway analysis on consolidated list
   - DAVID Tool for identification of significant pathways
     - 89 pathways increased in AD
     - 88 pathways decreased in AD

4. Pathways compared with Riluzole findings
   - Pathways with highest combined enrichment score between AD and Ritz identified

**Enrichment Analysis**

- **Increased by Riluzole and Decreased in AD**
  - Cytokine signaling in AD
  - Mitogen-activated protein kinase (MAPK) signaling pathway
  - Adipocyte differentiation

- **Decreased by Riluzole and Increased in AD**
  - Neurotrophin signaling in AD
  - ErbB signaling in AD
  - Calcium signaling in AD

**Gene Expression**

- **Upregulated with Riluzole**
  - Increased in AD

- **Downregulated in AD**
  - Decreased by Riluzole
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
Double-Blind, Randomized, Placebo-Controlled Pilot Trial in Mild Alzheimer’s disease
Riluzole 50mg BID x Placebo

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.
94 Participants screened

44 Did not pass screening

50 Randomized

42 Completed study
Table 2. Demographic and Baseline Clinical Characteristics

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

*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.
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 (\(^1\text{H MRS}\)) 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.

Stoichiometric coupling of brain glucose metabolism and glutamatergic neuronal activity

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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

In Press, Brain
Matthews, D….. Pereira AC, Brain 2021
FDG PET: Less metabolic decline in several pre-specified regions of interest in Riluzole group in comparison to placebo.

In Press, Brain
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

Scores of >500 independent subjects

Baseline: FDG AD Progression score vs. ADAScog

\[ R^2 = 0.37 \]

Matthews, D….. Pereira AC, Brain 2021
More FDG-PET relationships to cognitive measures

Matthews, D….. Pereira AC, Brain 2021
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

Matthews, D..... Pereira AC, Brain 2021
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)

- **Astrocytes**
  - 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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