Animal Models of Neuroplasticity: or is it Cognitive Reserve?

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Student Definitions

• “Cognitive reserve is the concept used to describe patients who exhibit AD pathology without the associated cognitive defects.”
• “I will define brain cognitive reserve as functional compensation for AD pathology induced by enhancement of mental and physical activity.”
Overview:

• Impact of an enriched environment on building reserve in a higher animal model
• Role of BDNF in reserve – enables learning and recall
• Molecular reserve in MCI – reserve in action?
The aged canine as a model of human brain aging

• Canines develop learning and memory deficits beginning in middle age.

• Like humans, with age, canines:
  – show increased individual variability in cognition.
  – naturally accumulate beta-amyloid.
  – Accumulate oxidative damage (proteins, lipids) and mitochondrial dysfunction

• Represents an animal model of MCI
Can Behavioral Enrichment/Exercise, and/or Diet, reduce the Development of Age-Dependent Cognitive Dysfunction in Canines?
### Longitudinal Study

<table>
<thead>
<tr>
<th></th>
<th>Aox Diet (-)</th>
<th>Aox Diet (+)</th>
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<tbody>
<tr>
<td><strong>Behavioral Enrichment (-)</strong></td>
<td>N=12 Old</td>
<td>N=12 Old</td>
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<tr>
<td><strong>Behavioral Enrichment (+)</strong></td>
<td>N=12 Old</td>
<td>N=12 Old</td>
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<tr>
<td></td>
<td>N=8 Young</td>
<td>N=9 Young</td>
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Old beagles – 8 to 12 years at start  
Young beagles – 2 to 5 years at start  
Treatment duration – 2.8 years
Canine Antioxidant Diet

Antioxidants
- dl-alpha tocopherol acetate-1050 ppm (20 mg/kg - 800 IU/day)
- Stay-C (ascorbyl monophosphate)-100 ppm or ~100 mg/day
- Spinach, carrot granules, tomato pomace, citrus pulp, grape pomace: 1% each in exchange for corn (Increased ORAC by 50%, equivalent to 4-5 servings of fruits and vegetables/day)

Mitochondrial cofactors
- dl-Lipoic acid: 135 ppm (2.7 mg/kg)
- l-carnitine: 300 ppm (6 mg/kg)
Enrichment Protocol

- Play toys
- Kennelmate
- 3-4 weekly walks
- Additional cognitive experience/education

Controls
Discrimination learning: shape, color, size
Discrimination Learning is impaired with age

- 10 trials/day
- 40 training sessions
- **Criteria**: 70% of trials correct, 3 successive test sessions

![Bar chart showing errors to criterion for Black/White discrimination between young and old subjects.](chart)
Combined intervention (EE+AOX) prevents cognitive decline with age

EE = behavioral enrichment
AOX = antioxidant diet

Reversal performance:

% of animals failing to reach criteria

Year 1  Year 2  Year 3

Control  AOX  EE  AOX + EE

Task: Object  Size  B/W discrim
Can the interventions “reverse” age-related cognitive dysfunction?
Spatial Memory improved by EE+AOX

P<.05

Year of Study

Errros to Criterion

Year 1  Year 2  Year 3

Control  EE  AOX  EE+AOX

Year of Study
Synapse Markers are increased in the combined treatment group (SNAP25)
Mitochondrial ROS production as a function of age and EE+AOX treatment

Head, Cotman, Sullivan, 2009
Enrichment+AOX reduces age-related oxidative damage: SOD, superoxide to water; GST, detoxifies HNE

(Oppi, Cotman, et al., 2008)
The Interventions reduce age-related Caspase-3 activation

Control (C/C), antioxidant (C/A), enrichment (C/E) combination (E/A)

Immunofluorescent staining for cleaved caspase-3 and cleaved product (fractin) in the canine frontal cortex.
Combined EE+AOX increases BDNF mRNA, and counteracts BDNF decline with age

(Fahnestock, Cotman, et al., 2010)
How do the treatments affect Beta-amyloid?

• Levels decreased?
• Or maybe increased tolerance?
Combined EE+AOX decreases beta-amyloid plaque load.
Change in Amyloid load does not correlate with behavioral improvement

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<tr>
<th></th>
<th>Pearson correlation</th>
<th>p value</th>
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<tr>
<td><strong>Spatial Memory Performance</strong></td>
<td>0.198</td>
<td>n.s</td>
</tr>
<tr>
<td><strong>Black/white discrimination errors</strong></td>
<td>-0.096</td>
<td>n.s</td>
</tr>
<tr>
<td><strong>Black/white reversal errors</strong></td>
<td>-0.134</td>
<td>n.s</td>
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Enrichment, Aox treatment: minimally reduce AB42
Hypothesis for synergistic effectiveness of the combined EE + AOX treatment

• **AOX intervention** improves mitochondrial function:
  – may “**enable**” enrichment stimuli to better engage plasticity and protective mechanisms

• **Combined EE+AOX** results in greater learning improvements, pathology reduction, and BDNF induction than either EE or AOX alone

• **Brain tolerates β-amyloid**
Role of BDNF/Exercise on Enabling Cognitive Performance

• Can BDNF/exercise enable learning of normally sub-threshold events?
  – Test with Object Location Memory Task, an example of hippocampal-dependent “incidental” learning

• How critical is exercise-induction of BDNF in hippocampus for enabling learning?
Exercise mice 3 wks, expose to sub-threshold training for object location memory and determine if mice now learn and retain experience 24hrs.

**Sub-threshold training (3 min):** animal cannot discriminate between familiar and novel object location.

**24h Retention Test Performance**

(Stefanko et al. 2009)
Exercise enables sub-threshold learning. The effect is equivalent to Sodium Butyrate (NaB) – a histone deacetylase inhibitor.

Sub-threshold OLM performance (long term memory)
BDNF is required for Exercise to enable sub-threshold learning

Blocking exercise induction of BDNF with siRNA prevents discrimination of the novel location
Does MCI engage Molecular Reserve?

- Is MCI mild AD?
- Are compensatory molecular mechanisms engaged in the MCI brain?
- Evaluate with microarray analyses
Microarray study: MCI, AD, normal aged

• Well powered microarray study (63 cases, see below)
• 4 brain regions
  – EC, HC, Superior Frontal Gyrus (SFG)
  – Somatosensory gyrus (PCG) – “control region”
• Affymetrix (HgU133 plus 2.0 chips)

<table>
<thead>
<tr>
<th></th>
<th>cases</th>
<th>age (yrs)</th>
<th>brain regions</th>
<th># arrays</th>
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<tbody>
<tr>
<td>Aged Control</td>
<td>24</td>
<td>85 ± 6.8</td>
<td>EC, HC, SFG, PCG</td>
<td>57</td>
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<tr>
<td>MCI</td>
<td>12</td>
<td>87 ± 4.4</td>
<td>EC, HC, SFG, PCG</td>
<td>40</td>
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<tr>
<td>AD</td>
<td>27</td>
<td>85 ± 6.2</td>
<td>EC, HC, SFG, PCG</td>
<td>78</td>
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MCI cases cluster closely

Entorhinal Cx  Hippocampus

Prefrontal Cx  Somatosensory Cx

Black: MCI
Red: AD
Green: Aged Control
Electron transport genes: extensive upregulation across brain in MCI

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<tr>
<th></th>
<th>EC</th>
<th></th>
<th>HC</th>
<th></th>
<th>PCG</th>
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<th>SFG</th>
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<tr>
<td>COMPLEX:</td>
<td>I</td>
<td>II</td>
<td>III</td>
<td>IV</td>
<td>V</td>
<td>I</td>
<td>II</td>
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<td>AGING</td>
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<td>MCI vs Aged</td>
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<td>AD vs Aged</td>
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Red = downregulated
Green = upregulated
Energy and synaptic genes:
Downregulated in AD, but Upregulated in MCI

[Bar charts showing comparisons between MCI vs aged Con, AD vs aged Con, and MCI vs AD for energy and synaptic genes.]
MCI brain mobilizes mechanisms to increase anabolic + metabolic function

• Upregulation of Protein biosynthesis/trafficking/turnover
• Synaptic genes mobilized
  – Neurotransmitter release machinery (SNAREs)
  – Neurotransmitter receptors (Glu, GABA, Ach)
  – Synaptic structure and stabilization genes
• Upregulation of Mitochondrial energy generation
• Molecular mobilization in MCI brain, likely serving to support cognitive reserve (ultimately fails with decline to AD)
Summary

- Brain mobilizes many diverse mechanisms to maintain function in the wake of age and pathology build up.
- Environmental enrichment, exercise, and cognitive training enhance molecular counteractive strategies.
- Even in MCI the molecular machinery is engaged to counteract decline.
- Cognitive reserve may be integrated brain plasticity.
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MCI: Gene expression is not intermediate between Aged and AD profiles