Effects of CV4 on Rodent Model of Alzheimer’s Disease

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Outline

Alzheimer’s Disease (AD) and Glutamate receptors

Cranial Osteopathic Manipulation (COM) on Animal Models of AD

Effect of CV4 Treatment

Spatial Learning and Memory Assay

Aβ Plaque Imaging

Immunoassays

Summary and Closing Remarks
Alzheimer’s Disease

Images courtesy of AD education and referral center National Institute on Aging
NMDA Subtype of Glutamate Receptors

Costa et al., JPET 2010
Rx for Alzheimer's disease (AD):

- **Memantine (Namenda - Allergan)**
  - NMDA receptor blocker
  - For use in moderate to severe AD
  - Oral solid formulations

- Adverse effects
  - Stevens-Johnson syndrome
  - Epidermal necrolysis
  - Suicidal ideation
Etiology of Alzheimer’s Disease

- Age dependent reduction in fluid circulation
- Increased accumulation of metabolic wastes
- Neuroinflammation
- Release of proinflammatory mediators
- Hyperactivation of ion channels (NMDA receptors)

Louveau et al., 2015; Aspelund et al., 2015
Alzheimer’s Disease

An Osteopathic approach for AD

- Cranial Osteopathic Manipulation (COM)
  - In practice for decades
  - Mechanism of action largely unknown
Outline

1. Alzheimer’s Disease (AD) and Glutamate receptors
2. Cranial Osteopathic Manipulation (COM) on Animal Models of AD
   - Spatial Learning and Memory Assay
   - Aβ Plaque Imaging
   - Immunoassays
3. Effect of COM (CV4) Treatment
4. Summary and Closing Remarks
Cranial Osteopathic Manipulation (COM) on Animal Models of AD

**Experimental Design**

**Aim.1**
Assessment of Spatial Learning & Memory by Morris water Maze

**Aim.2**
Identification of 18-month TgF344 or Aged rat model of AD.

**Aim.3**
Demonstration of Aβ clearance in live animals by PET imaging

18 months old rats treated for 7 days

Hope Tobey, DO
Per Gunnar Brolinson, DO
Bradley Klein, PhD
COM improves Spatial Learning and Memory

**Graph: Escape Latency**
- DAY 1 to DAY 8
- UT, YTg, UT, COM
- Escape latency in seconds

**Graph: Training and Probe**
- DAY 1 to DAY 8
- UT, YTg, UT, COM
- Escape latency in seconds compared to training and probe sessions.

**Graph: Total Distance**
- DAY 4
- UT, COM
- Total distance traveled in meters.

**Graph: Entries to NW**
- DAY 4
- UT, COM
- Number of entries to the northeast.

**Graph: Time Spent in NW**
- DAY 4
- UT, COM
- Time spent in the northeast quadrant.

**Graph: Distance in NW**
- DAY 4
- UT, COM
- Distance traveled in the northeast quadrant.
COM improves Spatial Learning and Memory

COM improves spatial learning and memory in naturally aged rat model of AD. Representative video shows the movement of UT (left, swim starts at 13th sec.) and COM (right, swim starts at 13th sec.) treated eighteen months old naturally aged rats. Four quadrants, platform location (circle at northwest quadrant) are marked in the video.
Outline

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Immunoassays
COM Reduces Aβ Protein

**Experiment:**
- Positron Emission Tomography (PET) Imaging
- Florbetapir F\(^{18}\) (AmyVid – Lilly)
- 300-400\(\mu\)Ci – tracer IV injection
- PET scan after 45 min

**Observation:**
- ↓ Florbetapir F\(^{18}\) signal
- Midbrain
- Ventral Tegmental Area (VTA)
- Hippocampus

**Inference:**
- COM increases the clearance of Aβ proteins
**COM Reduces Aβ Protein**

**Experiment:**
- Positron Emission Tomography (PET) Imaging
- Florbetapir F\(^{18}\) (AmyVid - Lilly)
- 300-400µCi tracer IV injection
- PET scan after 45min

**Observation:**
- ↓ Florbetapir F\(^{18}\) signal

**Inference:**
- COM increases the clearance of Aβ proteins
COM Reduces Aβ Protein in Transgenic AD Rats

11-16 month old TgF344-AD Rats
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Summary and Closing Remarks
Amyloid Precursor Protein (APP)

Cleavage sites for α- β- and γ-secretase on APP

Bergström et al., 2016
COM Alters Aβ Levels

Immuno assays

Tyler Lucas
COM Alters Aβ Levels

In association with astrocyte activation and upregulation of aquaporin and lymphatic vessels

Louveau et al., 2017
COM Alters Astrocytes (GFAP) Activation

Increase in expression of GFAP at dentate gyrus in COM group animals

Mike Mykins
COM Alters VEGF-C levels in TgF344-AD rats

11-16 month old TgF344-AD Rats

Immuno assays

VEGF-C

GAPDH

~ 45kDa

~ 35kDa

LYVE1

GAPDH

~ 35kDa

*
COM Numerically Reduces Aβ_{42} in TgF344-AD rats

11-16 month old TgF344-AD Rats

**Aβ_{42}**
- UT: ~4 kDa
- COM: ~35 kDa

**AQP4**
- UT: ~48 kDa
- COM: ~35 kDa
Glutamate and GABA in Synaptic Transmission

Vesicular glutamate transporter-1 (vGlut1)
Glutamate decarboxylase-67 (GAD67)
COM Modulates Synaptic Transmission

Hippocampal CA1 region
COM Modulates Synaptic Transmission

**a.** Images represent the UT and COM treated rat brain sagittal sections immunostained for GAD67 (red), VGluT1 (green) and DAPI (blue).

**b.** Puncta density analysis reveal significant increase in (UT, puncta density 100.0±7.4% vs COM, 279.8±22.4%, p<0.01) PFC region. Scale bar: 20uM. UT, untreated; COM, cranial osteopathic manipulated.

**COM treatment increases the GAD67 expression in the prefrontal cortex (PFC) of aged rats.**
Proteomic Assay

P-value Vs Ion Intensity Ratio

<table>
<thead>
<tr>
<th>SI #</th>
<th>Protein ID</th>
<th>Protein Name</th>
<th>log2 (Treated/Control)</th>
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<tr>
<td>1</td>
<td>D4A435</td>
<td>Intercellular adhesion molecule 5</td>
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<td>Q63564</td>
<td>Synaptic vesicle glycoprotein 2B</td>
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<tr>
<td>5</td>
<td>P21707</td>
<td>Synaptotagmin-1 (Synaptotagmin 1) (SytI) (p65)</td>
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<tr>
<td>6</td>
<td>P97686-2</td>
<td>Neuronal cell adhesion molecule (Nr-CAM)</td>
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<tr>
<td>7</td>
<td>P09606</td>
<td>Glutamine synthetase</td>
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<tr>
<td>8</td>
<td>P07936</td>
<td>Neuromodulin (Axonal membrane protein GAP-43)</td>
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<tr>
<td>9</td>
<td>P02688</td>
<td>Myelin basic protein (MBP)</td>
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<tr>
<td>10</td>
<td>P19527</td>
<td>Neurofilament light polypeptide (NF-L)</td>
<td>-1.65</td>
</tr>
</tbody>
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Immuno assays

Edward Via College of Osteopathic Medicine
Summary

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Possible Mechanism of Action

Clinical Application
Future Directions

• Increase the sample size and repeat the assays

• Quantitative COM

• Ultrasound in clinical trials

Ben-Tzvi et al., 2016
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