Influences on an anti-inflammatory drug, Ibuprofen, on spatial memory and N-Methyl D-Aspartate receptor expression during aging

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Memory defines us
Memory decline with aging

- One of the first cognitive dysfunctions to arise with aging (Albert et al. 1992)

- 40% of individuals within the fifth decade and 85% of elderly over the age of 80 suffer from AAMI (Larrabee et al. 1994)
Figure 1. Total fertility rate and life expectancy at birth: world, 1950-2050

(UN World Statistics, 2002)
Biologically, memory can be defined as:

A reinforced neuronal pathway
Analogous to our daily pathways

Facilitating a neuronal pathway builds the strength of a memory
↑ Neurotransmitter Release - through increased production and storage
↑ Formation of additional synaptic connections
Long-term Potentiation

A mechanism underlying memory formation

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N-Methyl D-Aspartate Receptors

One subtype of glutamate receptor that provide regulatory roles in neurotransmission associated with the performance of memory tasks
NMDA receptors are made up of a combination of 3 major groups of subunit families

1. GluN1
   - Has 8 different splice variants
   - Aging particularly affects the GluN1-1 and GluN1-3 variants

2. GluN2A-D
   - Aging particularly affects the GluN2B subunit

3. GluN3A-B
   - It is not known how aging affects these subunits
Inflammation may account for aging changes in the NMDA receptor

- Lim. et al., 2000 study: transgenic mouse model for Alzheimer showed chronic oral ibuprofen treatment was capable of suppressing plaque pathology including amyloid deposition.

- The Mesches et al., 2004 study: an anti-inflammatory drug, sulindac, improved spatial short-term memory and reverse the effects of aging on protein expression of NMDA receptor subunits in old mice.
Purpose of this study

- It is not known whether previously seen effects are at the level of mRNA and/or protein and whether it would improve both long and short-term spatial memory and NMDA receptor expression at younger ages.

- In the present study we analyze the effects of an anti-inflammatory drug, ibuprofen, on spatial working (short-term) and reference (long-term) memory and NMDA receptor at the mRNA level.

- Targeting the NMDA complex may play a role in developing treatment options to prevent or improve age-related changes in memory.
Hypothesis

- If inflammation is playing a role during aging on NMDA receptors, we would expect to see:

1) Ibuprofen enhance memory compared to control groups

2) Improve mRNA expression of GluN2B and all GluN1 subunits as a whole and the GluN1-1 and GluN1-3 splice variants, but not affect GluN1-2 splice variants

3) Ibuprofen would reduce cytokine levels compared to control groups, especially at older ages
Materials & Methods
This research required 48 male, C57BL/6 mice of four different age groups:

- 5 months of age: 6
- 14 months of age: 6
- 20 months of age: 6
- 26 months of age: 6

### Treatment 1

<table>
<thead>
<tr>
<th>Number of Mice per Age Group</th>
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### Treatment 2

<table>
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Testing Techniques:

1. Cognitive memory
   → Morris Water Maze

2. mRNA densities of NMDA receptor subunits
   → In Situ Hybridization

3. Cytokine levels in brain and spleen
   → Reverse Transcriptase-Polymerase Chain Reaction
1. Behavioral Cognitive Testing

The Morris Water Maze
Memory Assessment

Memory is measured as cumulative and average proximities to the platform.

Early place trial showing wandering path to target (black dot).

Later trial showing a more direct path to target (black dot).
Morris Water Maze - spatial memory tasks
4 Different Memory Tasks

1. Spatial Long Term Memory
   - Place trials
   - Probe trials

2. Cognitive Flexibility
   - Reversal trials

3. Spatial Short-term memory
   - Working memory trials: Naïve and Delay

4. Cued control trials
2. **In Situ** Hybridization

1. One half was used for sectioning for *in situ* hybridization
2. Using probes for genes of interest- cDNA labeled with 33P-dATP,
3. Brain and standard images were captured autoradiography
4. The different prefrontal and frontal cortex brain regions analyzed for mRNA expression were:
   - deep (cortical layers IV-VI)
   - superficial (cortical layers II-III) layers of ventral orbital cortex, lateral orbital cortex, prefrontal cortex
   - insular cortex
3. Reverse Transcriptase-Polymerase Chain Reaction

1. Tissue samples from brain and spleen collected

2. Interleukin I (IL-1beta) cytokine RNA isolated

3. RNA was reverse transcribed into cDNA for quantitative real time polymerase chain reaction technique used to amplify DNA
Results

Effect of Ibuprofen on:

1. Cognitive memory
2. mRNA densities of NMDA subunit and GluN1 slice variants
3. IL-1beta cytokine levels in the brain and spleen
1. Effect of Ibuprofen on cognitive memory
Long-term memory

Place trials showed effect of age but there was no significant effect of treatment across trial types.

*p < 0.05 for difference from 5 month old mice. Bracket indicates significant differences collapsed across treatments. Mean ± SEM, N = 6.
Oldest ibuprofen treated mice showed significantly better performance in the delayed trial, compared to naive, as did both treatment groups at 14 months of age * p < 0.05 for difference from naive trial of the same age and treatment. Mean ± SEM N = 6.
There was a significant effect of age but no significant effect of treatment. \( *p < 0.05 \) for difference from 5 month old mice. Bracket indicates significant differences collapsed across treatments. Mean \( \pm \) SEM, N = 6.
There were no significant effects of age or treatment on performances in the cued control task, an assessment of sensory/motor skills and motivation. Mean ± SEM. N = 6.
2. Effect of ibuprofen on mRNA densities of NMDA subunit and GluN1 slice variants
mRNA density of GluN1 subunit and splice variants in lateral frontal cortex

Ibuprofen decreased mRNA density for GluN1-pan and GluN1-1-containing subunits, but increased mRNA for GluN1-3-containing subunits across ages. No effect on GluN1-2.

No effect
mRNA densities of GluN2B subunit in lateral frontal cortex

Ibuprofen decreased mRNA density for GluN2B in all but the oldest group

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mRNA densities of NMDA subunits in lateral frontal cortex

mRNA densities for NMDA receptor subunits and splice variants in layer II-III of lateral frontal cortex

- GluN1-pan
- GluN1-1
- GluN1-2
- GluN1-3
- GluN2B

- Expected to increase with ibuprofen
- Actual results
3. Effect of ibuprofen on IL-1beta cytokine levels in the brain and spleen
IL-1B cytokine levels in the brain and spleen

IL-1beta levels, as a fold change from 5-month-old controls, in the brain (a) and spleen (b) from different ages and treatments. There was a significant overall effect of age (p<0.02), but no effect of ibuprofen in IL-1beta levels in the brain or spleen. N = 5-6. Mean ± SEM.
Average subject weight (g) averaged across weighing sessions

- There was a significant overall effect of age ($p<0.01$), but no significant main effect of treatment on individual mouse weights when averaged across age groups and different weighing days ($p=0.12$). $N = 5-6$. Mean ± SEM.
Discussion
Expected Results

• If inflammation is playing a role during aging on NMDA receptors, we would expect to see:

1) Ibuprofen enhance memory compared to control groups ✔

2) Improve the expression of GluN2B and all GluN1 subunits as a whole and the GluN1-1 and GluN1-3 splice variants, but not affect GluN1-2 splice variants ✗

3) Ibuprofen would reduce cytokine levels compared to control groups, especially at older ages ✗

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What study results suggest:

1) The effects of ibuprofen on cognitive behavior and mRNA expression did not appear to be through an anti-inflammatory mechanism.

2) Ibuprofen may be affecting NMDA subunit gene expression through a negative feedback mechanism.
Ibuprofen may not be enough to override epigenetic mechanisms regulating cytokine expression. Rao et al., 2012's study: Chronic treatment of drugs for Alzheimer's and Bipolar Disease acting at the cellular level may provide transient protection correcting neuroinflammatory and synaptic remodeling, but may not provide full recovery by not targeting epigenetic regulation of cyclooxygenase-2 (COX-2) neuroinflammatory markers. Due to the hypomethylated state of the COX-2 CpG promoter region, inflammation, pain, fever, platelet aggregation, thromboxane, arachidonic acid, prostaglandins, and inflammatory signaling are involved.
Limitations of this Study

- It may be possible there were toxicity effects. To rule out this possibility we had to have retained some tissue samples for analysis.

- To determine if ibuprofen was really acting on the brain, it would be necessary to analyze ibuprofen in the brain using mass spectroscopy. This is currently something we are looking into incorporating into this study.
Future of this study

- Currently still ongoing study
- Analyzing the effects of ibuprofen on protein may reveal new information
Acknowledgements

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Thank You