Inhibiting Microglial NF-κB Reduces Synaptic Pruning in the Cerebellum

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Background
• Synaptic pruning is an important developmental phenomenon where excess synapses are eliminated by microglia1.
• The Cvetanovic lab studies Spinocerebellar Ataxia Type 1 (SCA1), a genetically inherited neurodegenerative disease that affects the cerebellum2, by using mouse models of the disease3.
• Synaptic pruning deficits are observed in certain mouse models of SCA11.
• Microglia, the resident immune cells of the brain, play a crucial role in neurodegenerative disease as well as synaptic pruning, and are activated in SCA11.
• In order to study the effects of microglial activation on neurological pathology in SCA1, we have created a transgenic mouse line with an inhibition of microglial NF-κB-mediated pro-inflammatory cytokine production4,5 (Figures 1 & 2).

Figure 1: LysM-Cre IKKβ-Flox depletes IKKβ in the microglia only

- Cre Recombinase
- IKK Coding Region (looped)

IKKα and IKKβ

LysM-Cre Recombinase

Gene for IKKβ is excised in the microglia, and is not expressed

Figure 2: Depleting IKKβ inhibits NF-κB mediated cytokine production

Classical IKKβ-mediated NFκB activation:

1) IKKβ is sequestered by IκB
2) IKKβ phosphorylates IκB, freeing NFκB
3) NFκB initiates transcription of proinflammatory cytokines

In LysM-Cre IKKβ-Flox mice, IKKβ is depleted:

1) NFκB is sequestered by IκB
2) No IKKβ is present
3) NFκB remains sequestered and no proinflammatory cytokines are produced

- Since microglia mediates synaptic pruning1, we wanted to see if inhibiting NF-κB affected synaptic pruning. I used immunohistochemical analysis of cerebellar tissue sections from these mice to determine this.
- Aberrant synaptic pruning has a distinct phenotype (Figures 3 & 4)

Results

- Synapses per Cell Body

<table>
<thead>
<tr>
<th>Condition</th>
<th>Antigen retrieval</th>
<th>Primary antibody incubation time</th>
<th>Triton concentration in buffer</th>
<th>Result</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>None</td>
<td>24 hours</td>
<td>1%</td>
<td>Fail</td>
</tr>
<tr>
<td>2</td>
<td>None</td>
<td>48 hours</td>
<td>3%</td>
<td>Fail</td>
</tr>
<tr>
<td>3</td>
<td>Sodium citrate</td>
<td>48 hours</td>
<td>1%</td>
<td>Success</td>
</tr>
<tr>
<td>4</td>
<td>Sodium citrate</td>
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<td>3%</td>
<td>Fail</td>
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<tr>
<td>5</td>
<td>Sodium citrate</td>
<td>48 hours</td>
<td>1%</td>
<td>Success</td>
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<tr>
<td>6</td>
<td>Using anti-Vesicular Glutamine Transporter 2 (VGLUT2) antibody to reveal climbing fiber/Purkinje neuron synapses in the brain</td>
<td></td>
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<tr>
<td>7</td>
<td>Using calbindin to visualize the Purkinje Neurons4</td>
<td></td>
<td></td>
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<tr>
<td>8</td>
<td>Protocoll Optimization</td>
<td></td>
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<td>9</td>
<td>Determined Sodium Citrate antigen retrieval, 24 hour incubation time, and 1% Triton concentration in buffer provided optimal staining</td>
<td></td>
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</tbody>
</table>

Table 1: Protocol Optimization

Discussion
• Although no statistically significant difference can be reported due to small sample size, NF-κB inhibition appears to alter synaptic pruning in mice.
• I have optimized a staining protocol for VGLUT2 and demonstrated that it successfully stains cerebella for synaptic count analysis.
• Future experiments with larger sample sizes are needed to clarify if NF-κB inhibition truly interferes with synaptic pruning.
• Dye injection of inferior olives to stain climbing fibers could determine if multiple climbing fibers are innervating the Purkinje neurons

Acknowledgements

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References