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 microglia[1].
- The Cvetanovic lab studies Spinocerebellar Ataxia Type 1 (SCA1), a ~~genetically~~ inherited neurodegenerative disease that affects the cerebellum[2], by using mouse models of the disease[3].
- Synaptic pruning deficits are observed in certain mouse models of SCA1[4].
- Microglia, the resident immune cells of the brain, play a crucial role in neurodegenerative disease as well as synaptic pruning, and are activated in SCA1.[5]
- 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 production[6]. (Figures 1 & 2)

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

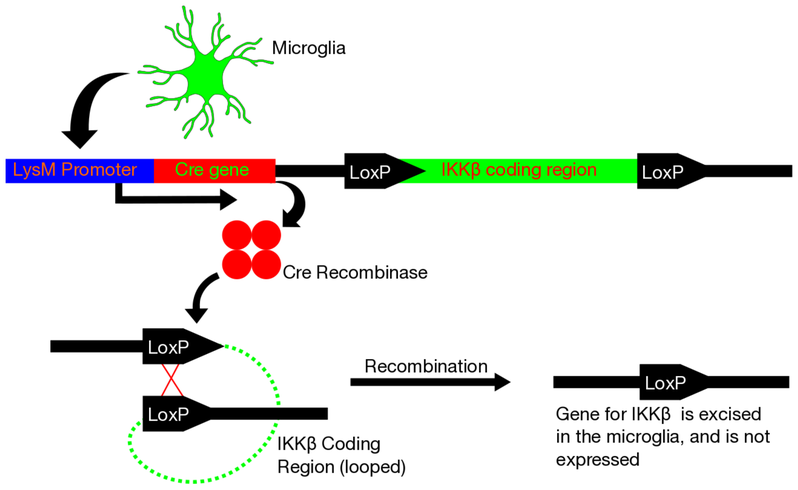
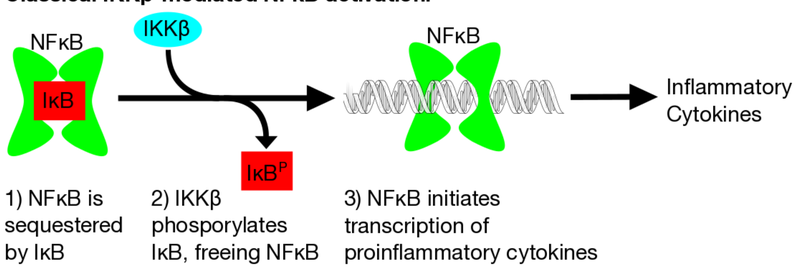
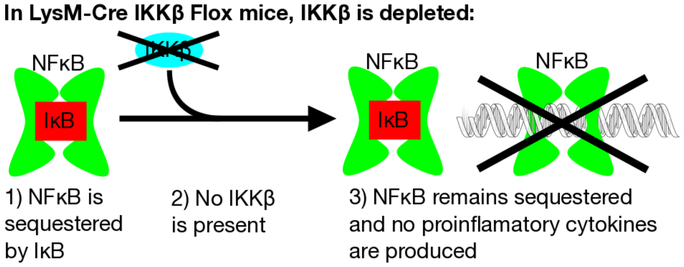


Figure 2: Depleting IKKβ inhibits NF-κB mediated cytokine production

Classical IKKβ-mediated NFκB activation:



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



- Since microglia mediate synaptic pruning[1], 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)[7]

Figure 3: Purkinje Neurons and synapse elimination in the developing cerebellum

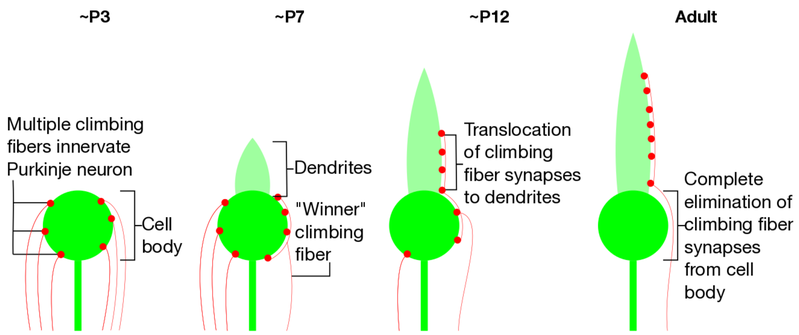
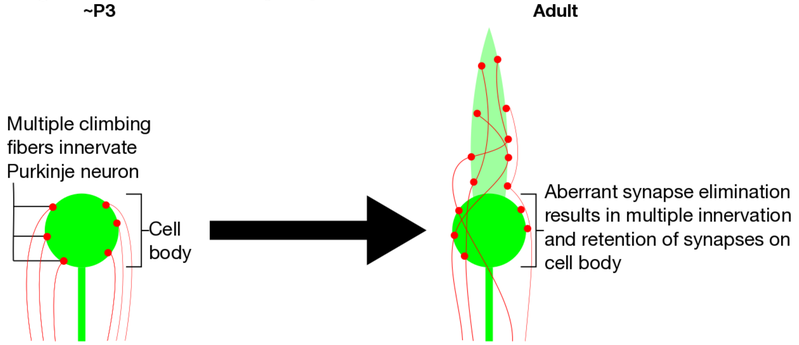


Figure 4: Aberrant synapse elimination in the cerebellum



- If inhibiting microglial NF-κB activity contributes to synaptic pruning, then we would expect to see an increase in synapses innervating the cell body of the Purkinje Neurons of LysM-Cre IKKβ-Flox mice.

Methods

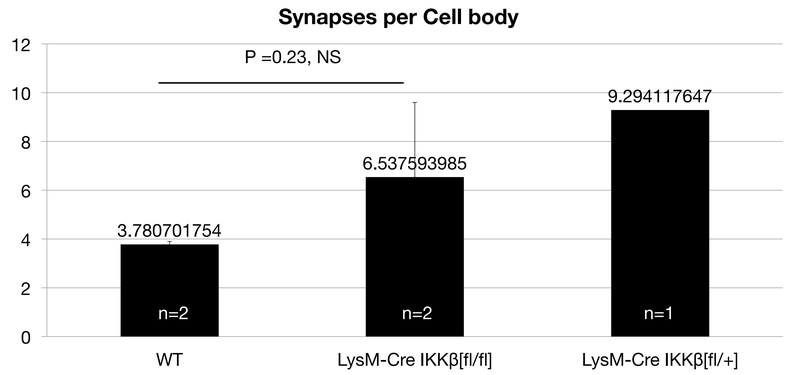
- I used immunohistochemical tissue staining to visualize specific proteins in the cerebellum.
  - I used anti-Vesicular Glutamine Transporter 2 (VGLUT2) antibody to reveal climbing fiber-Purkinje neuron synapses in the brain
  - I used calbindin to visualize the Purkinje Neurons[8]
- Protocol Optimization
  - I tested a number of protocol variations to determine optimal staining conditions for this combination of antibodies (Table 1)
  - It was determined Sodium Citrate antigen retrieval, 24 hour incubation time, and 1% Triton concentration in buffer provided optimal staining

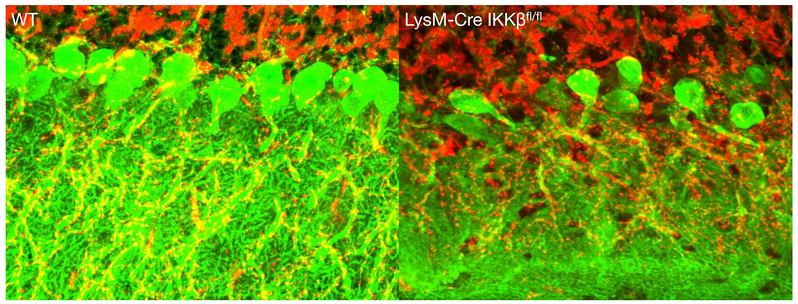
Table 1: Protocol Optimization

Condition	Antigen retrieval	Primary antibody incubation time	Triton concentration in buffer	Result
1	None	24 hours	1%	Fail
2	None	48 hours	1%	Fail
3	None	48 hours	0.3%	Fail
4	Sodium Citrate	24 hours	1%	Success
5	Sodium citrate	48 hours	1%	Success
6	Urea	24 hours	0.3%	Fail

- 7 mouse brains were selected and sectioned for immunohistochemistry
- Mounted sections were imaged using an Olympus FluoView1000 confocal microscope
  - Of the 7 brains, 2 were damaged during tissue processing
- Images were analyzed using IMARIS software
  - VGLUT2 and Calbindin images were superimposed, and synapses revealed by VGLUT2 positive dots present on the Purkinje neuron bodies identified-by Calbindin were counted.
  - An increase in synapses on the cell body indicates a deficit in synaptic pruning.
- Statistical analyses were performed using R Statistics software

Results





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 synapse 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

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