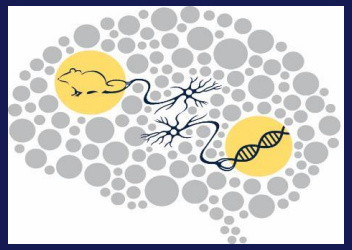

UCIrvine | Department of Neurobiology and Behavior

Microglial Roles in Auditory Brainstem Development

Karina S. Cramer

April 26, 2023

ARO Seminar Series

Outline

- Auditory brainstem circuits: how do they work, and what is special about them?
- What are glial cells and how do they contribute to circuits?
- Microglia in the auditory brainstem
  - What happens when you eliminate them during development?
  - What happens when they come back?
  - How do they work?
- Conclusions and future directions

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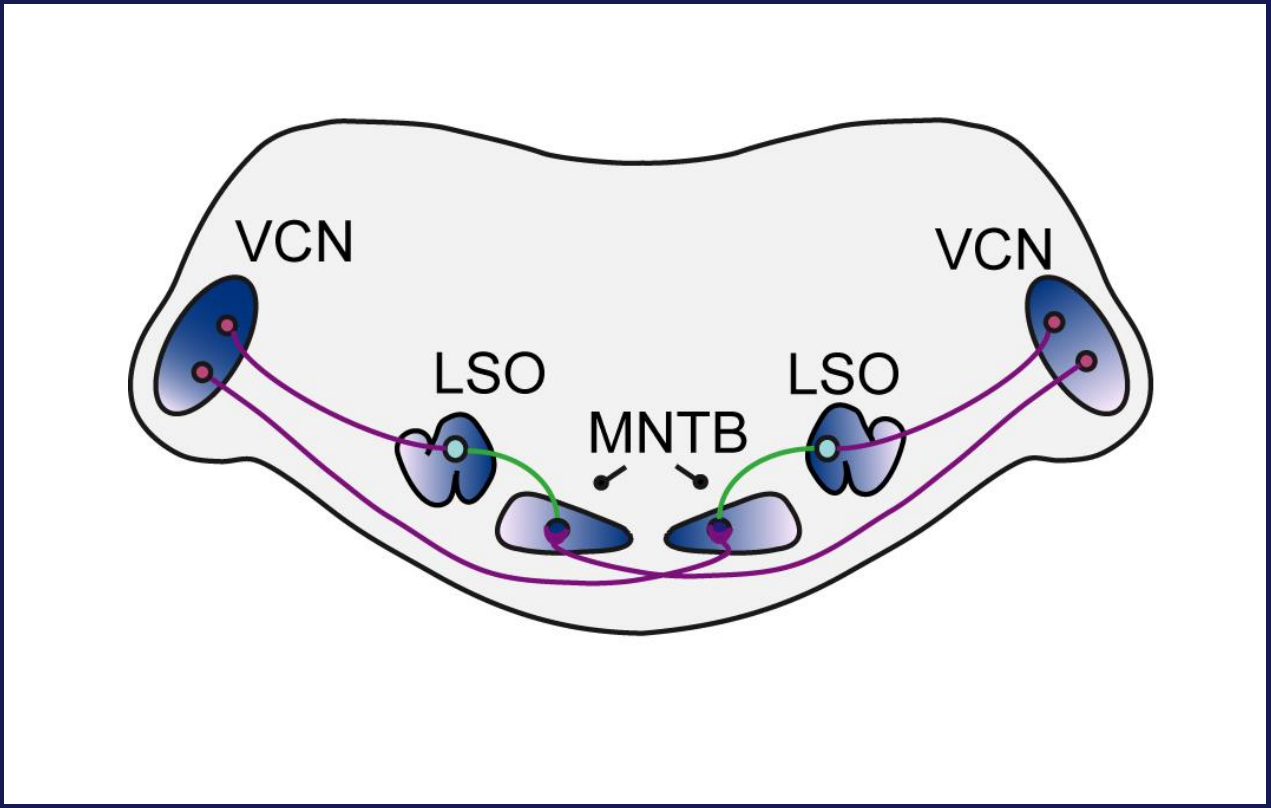
Mammalian auditory brainstem pathways



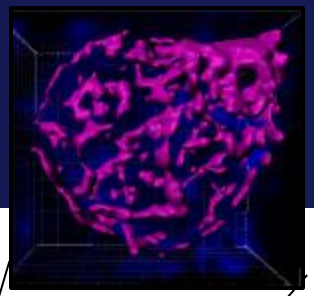
Cramer and Rubel, 2016

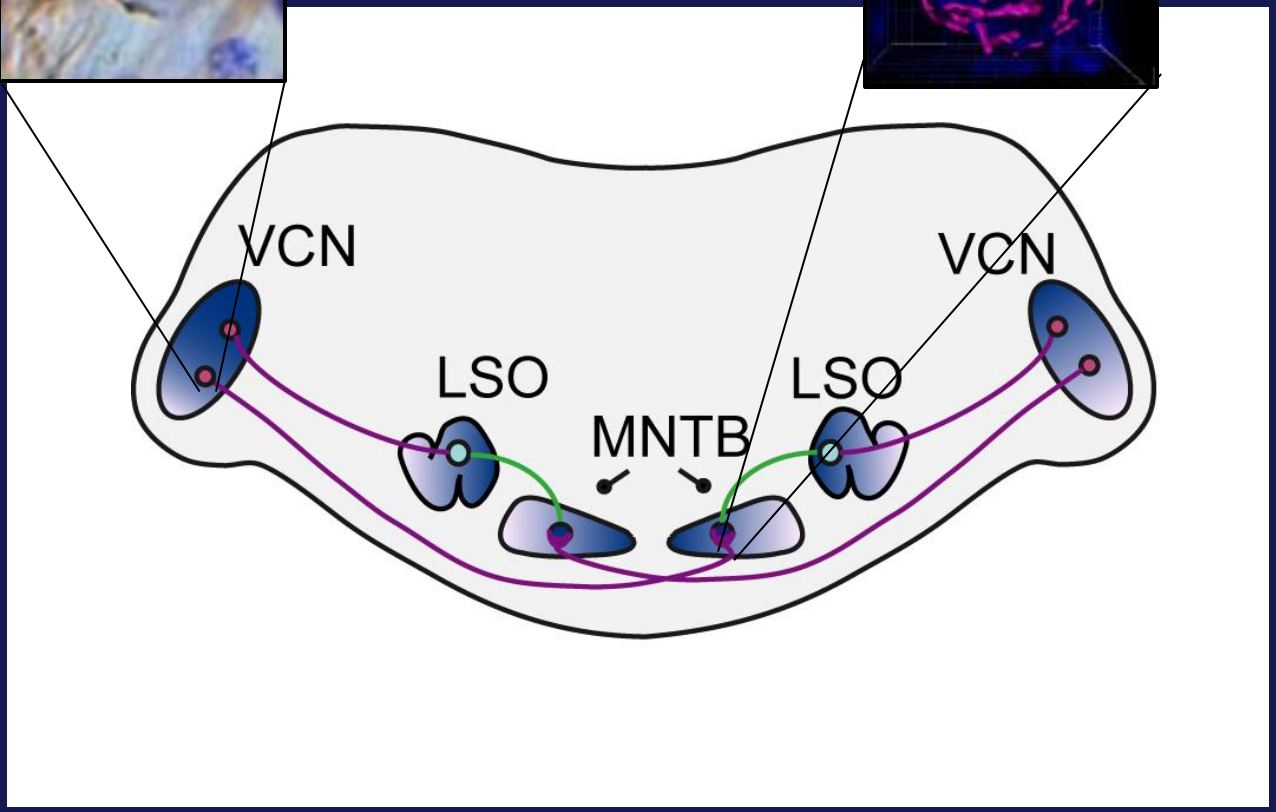
- **Sound localization**
- Formation of specialized synapses
- Synaptic pruning

Specialized synapses in the auditory brainstem

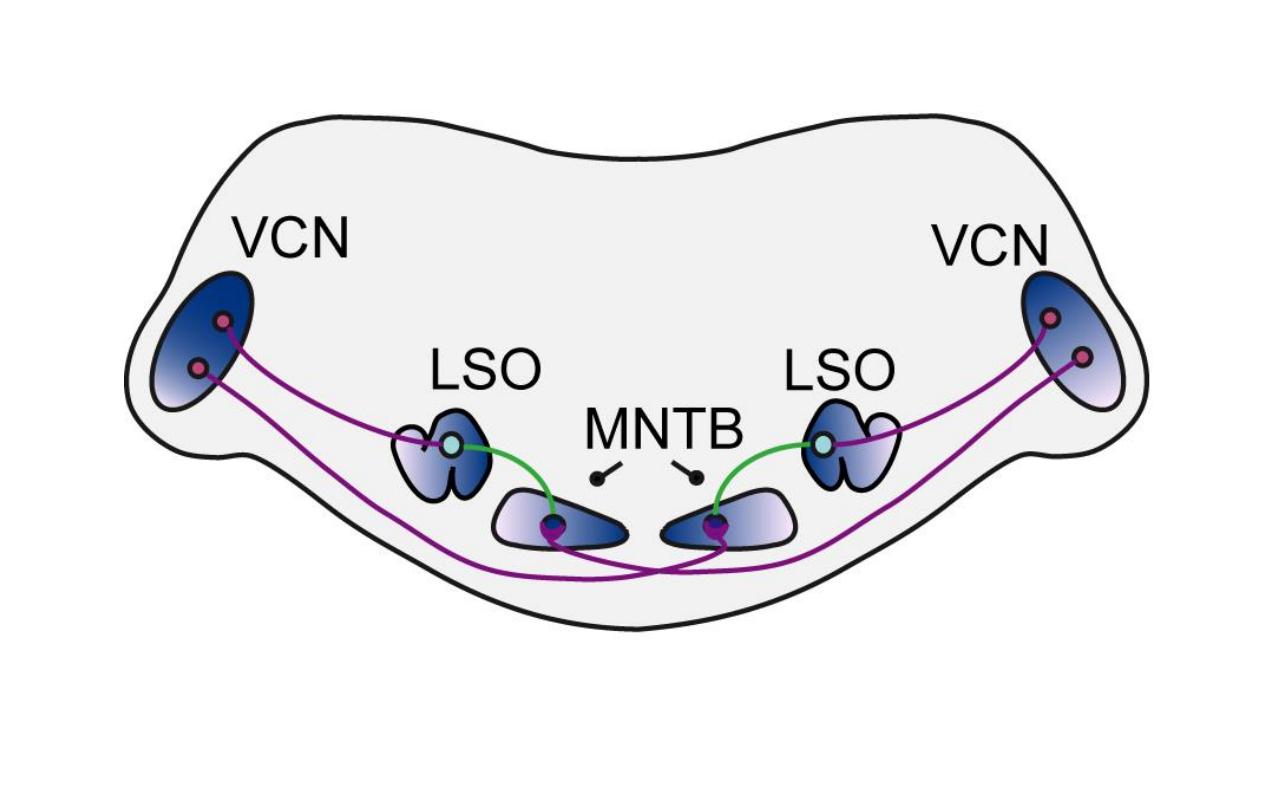
Endbulb of Held from auditory nerve fibers

Calyx of Held and the MNTB principal neuron





Mammalian auditory brainstem pathways



Glial functions

- Maturation of synapses
- Formation of specialized synapses
- Synaptic pruning

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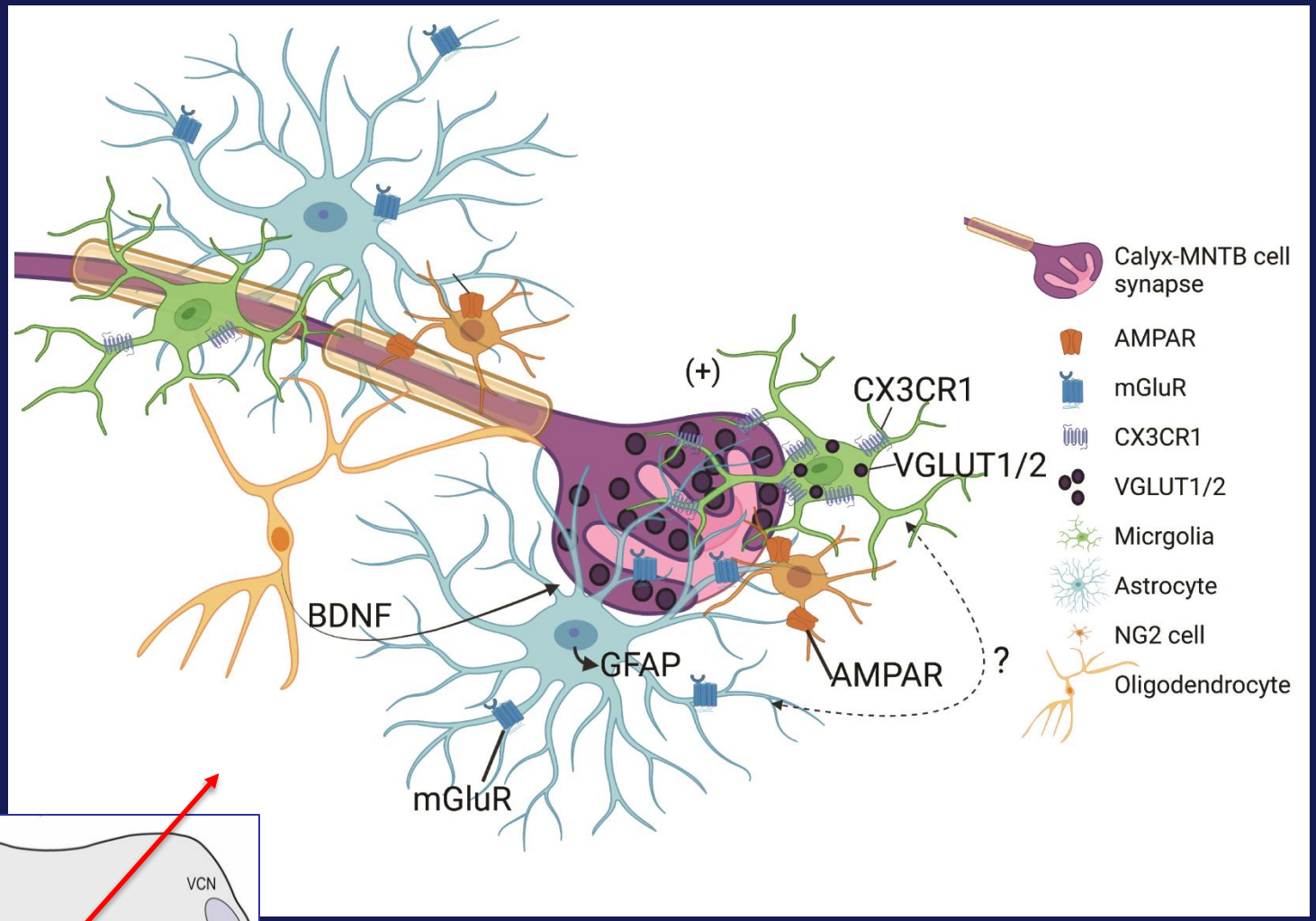
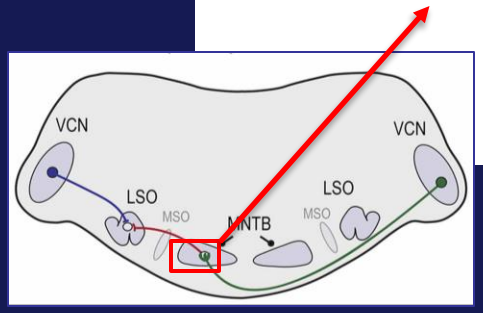
The calyx of Held synapse and its entourage



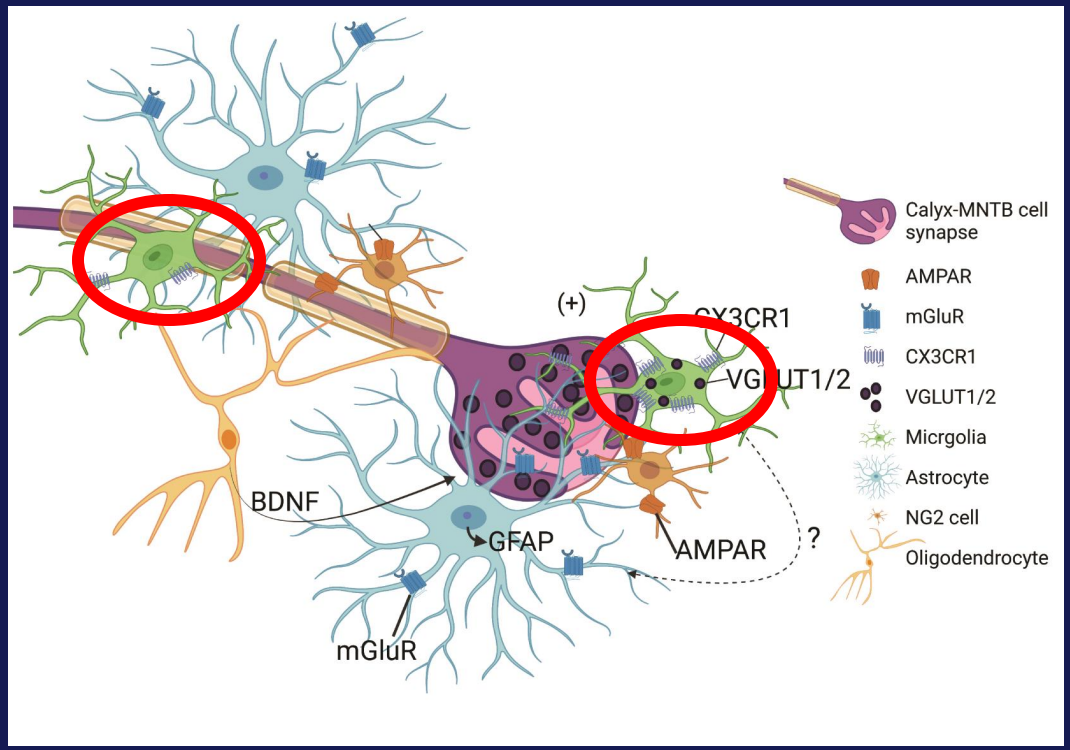


Chokr et al., 2022

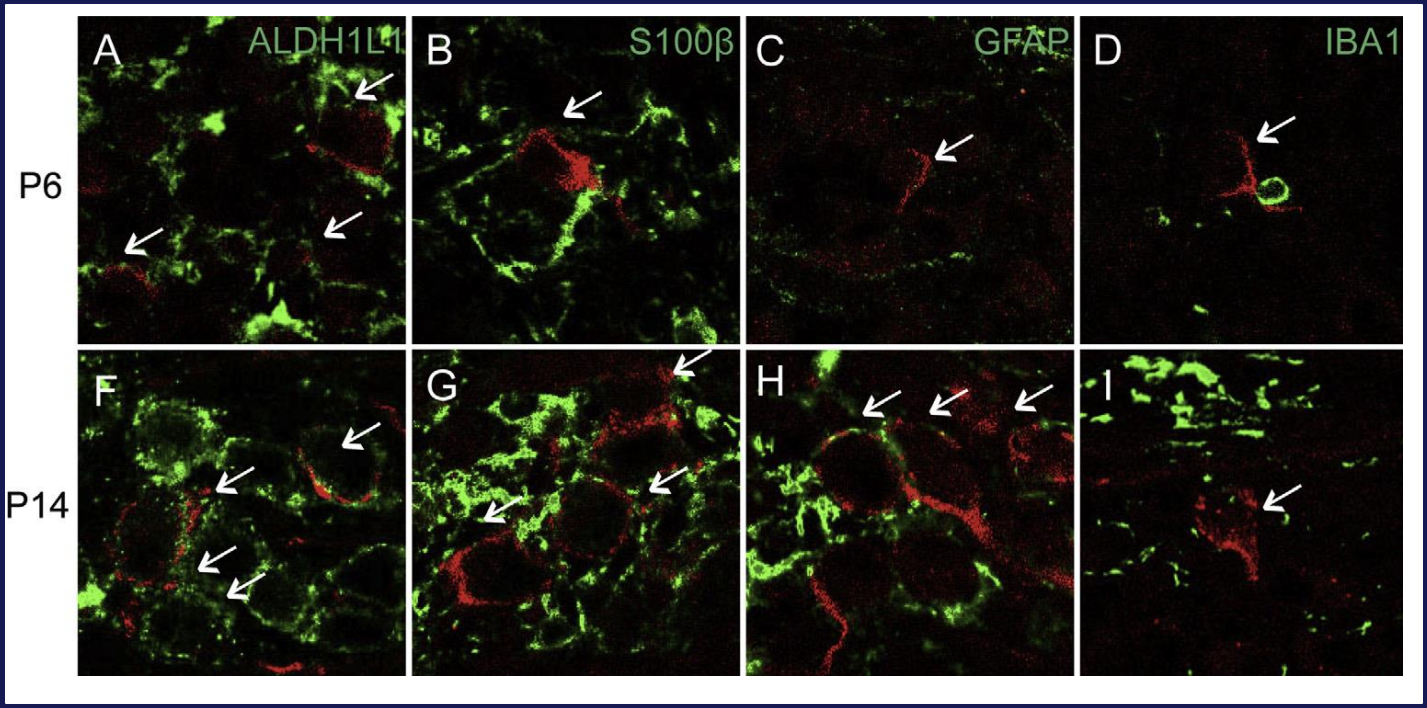
The calyx of Held synapse and its entourage

Microglia

- Immune cells of the CNS
- Populate the auditory brainstem in the early postnatal period
- Enter the brain during embryonic development



The calyx of Held synapse and its entourage



Dinh et al., 2014

- Early markers for astrocytes and microglia are seen in apposition to labeled calyx of Held at P6.
- In addition, markers for microglia and mature astrocytes are seen around calyx at P14.

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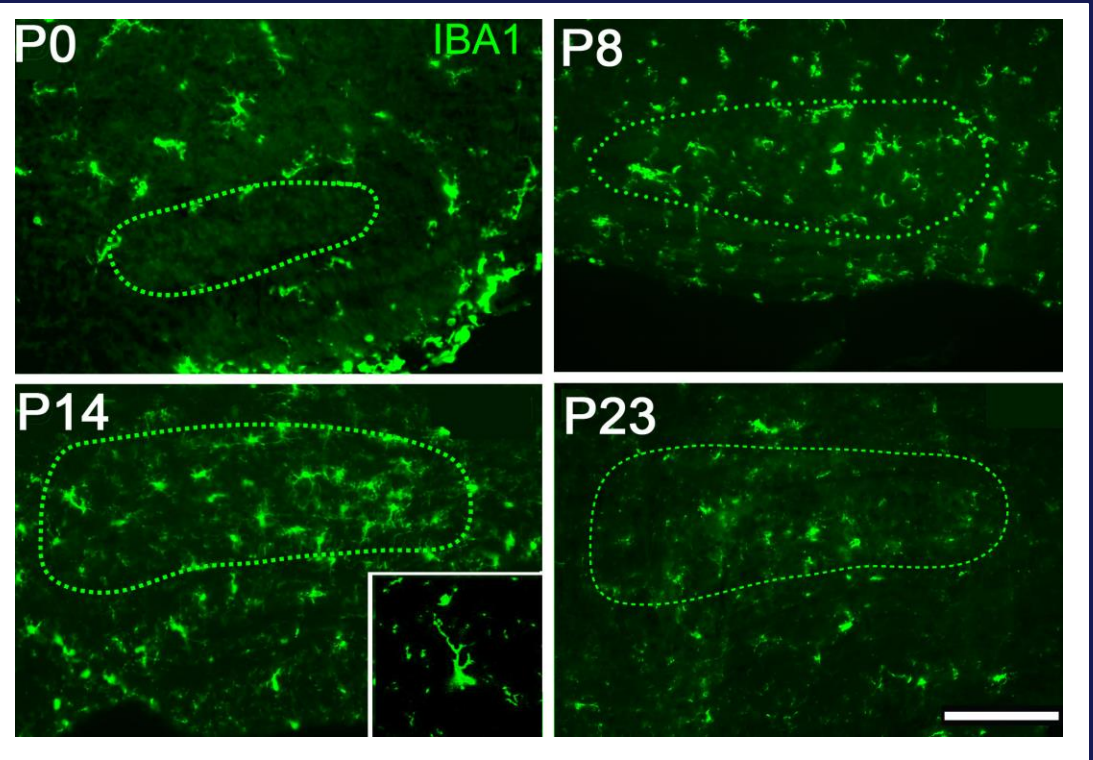
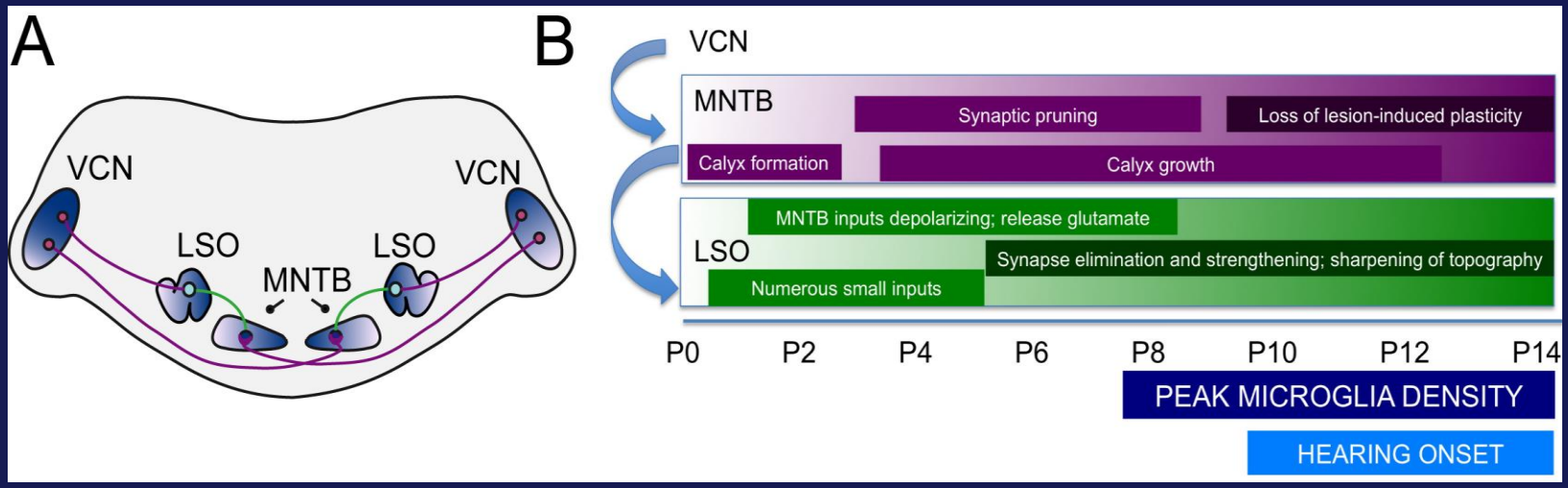
- **Microglia in the auditory brainstem**
  - What happens when you eliminate them during development?
  - What happens when they come back?
  - Which microglial signaling pathways are important?
- Conclusions and future directions

Circuit formation: Role of microglia



- Microglia emerge early in postnatal development
- Microglia become more numerous and more ramified during this period, peaking at P10-14
- Apposed to developing calyx of Held

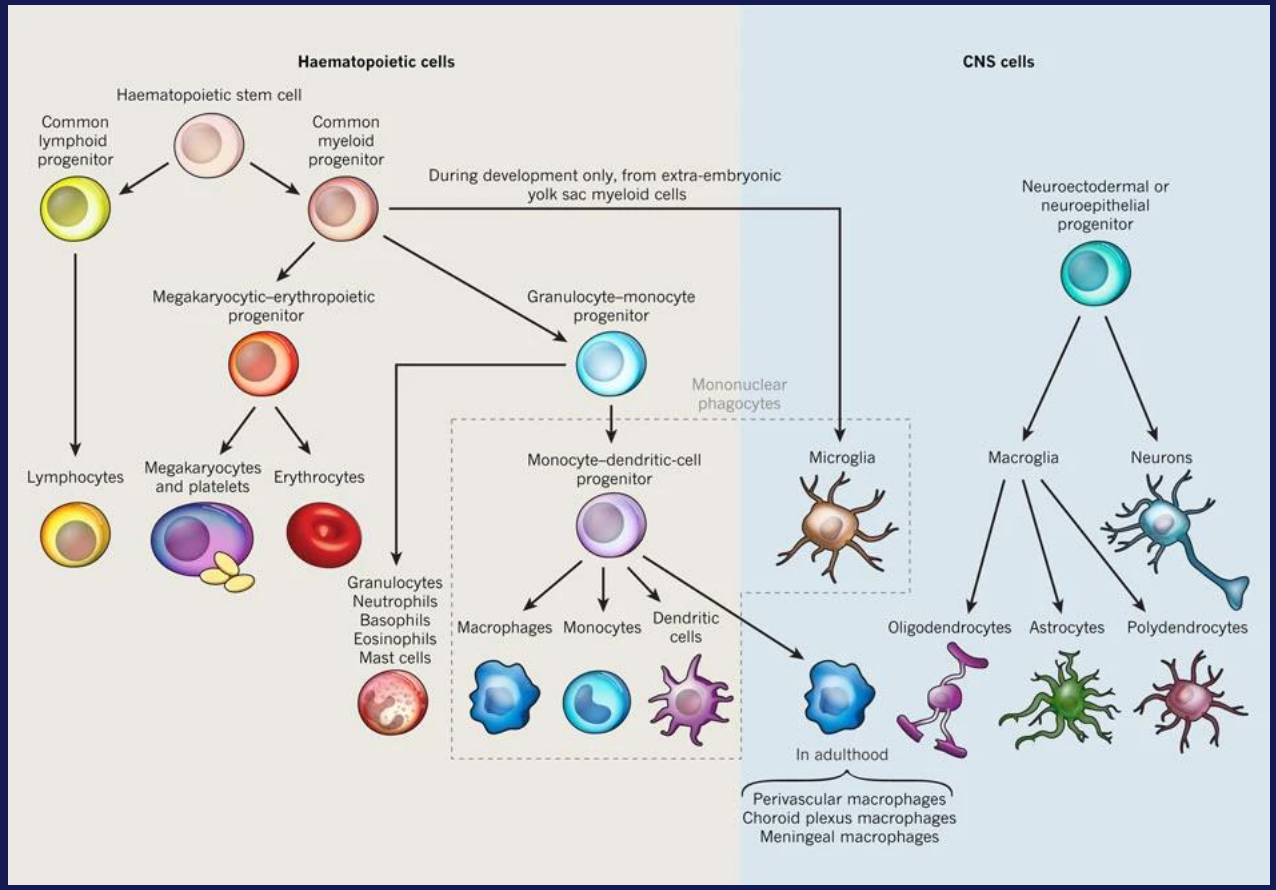
Microglia in auditory system development



Formation of auditory circuits:

- How do microglia contribute to unique aspects of auditory system development?

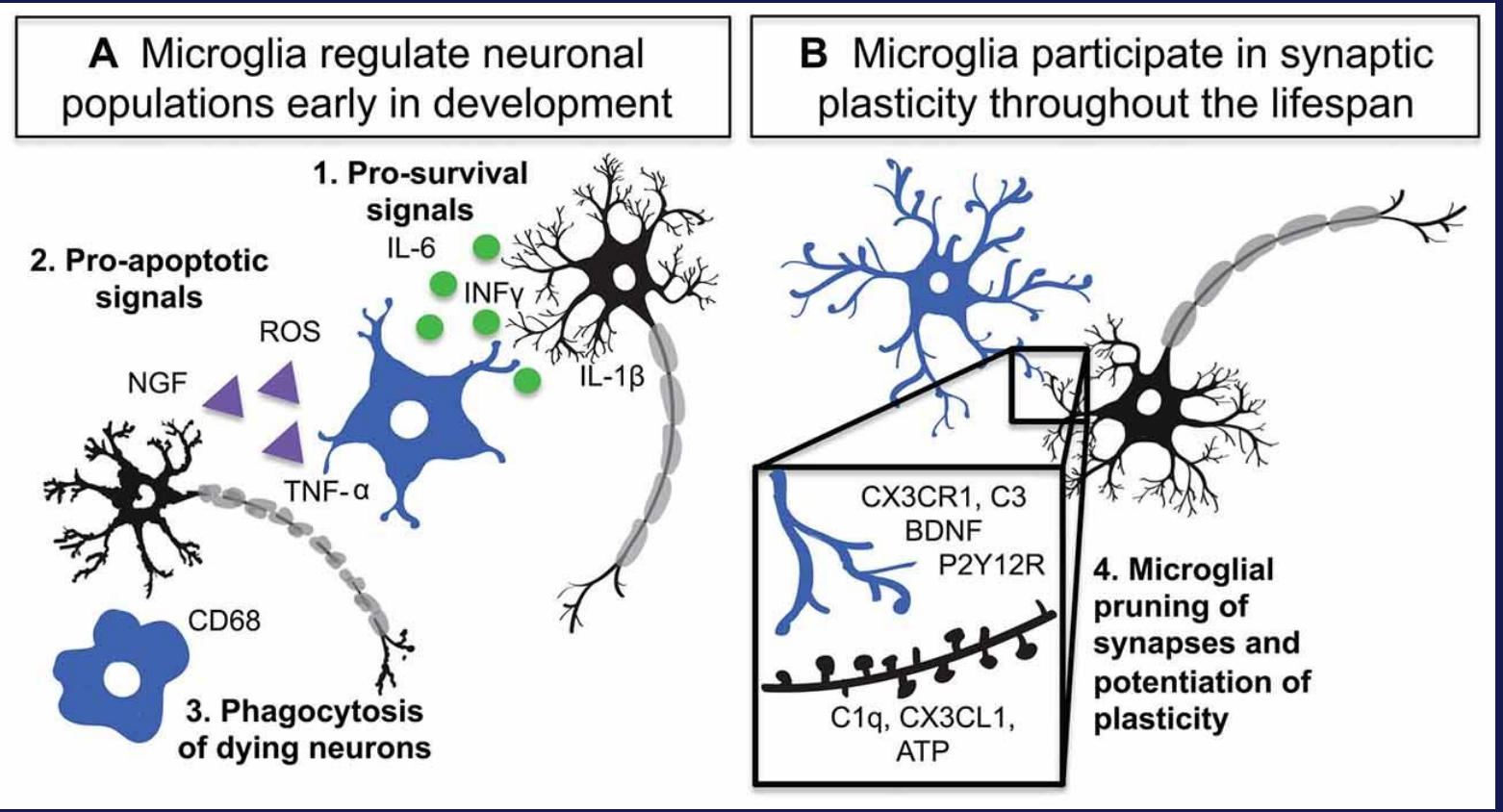
Microglial cell lineages



Ransohoff and Cardona, Nature, November 2010

Microglia arise from an early branch of the hematopoietic lineage and enter the CNS. Contrasts with macroglia, which derive from neural lineage.

Microglia regulate circuit development and maturation

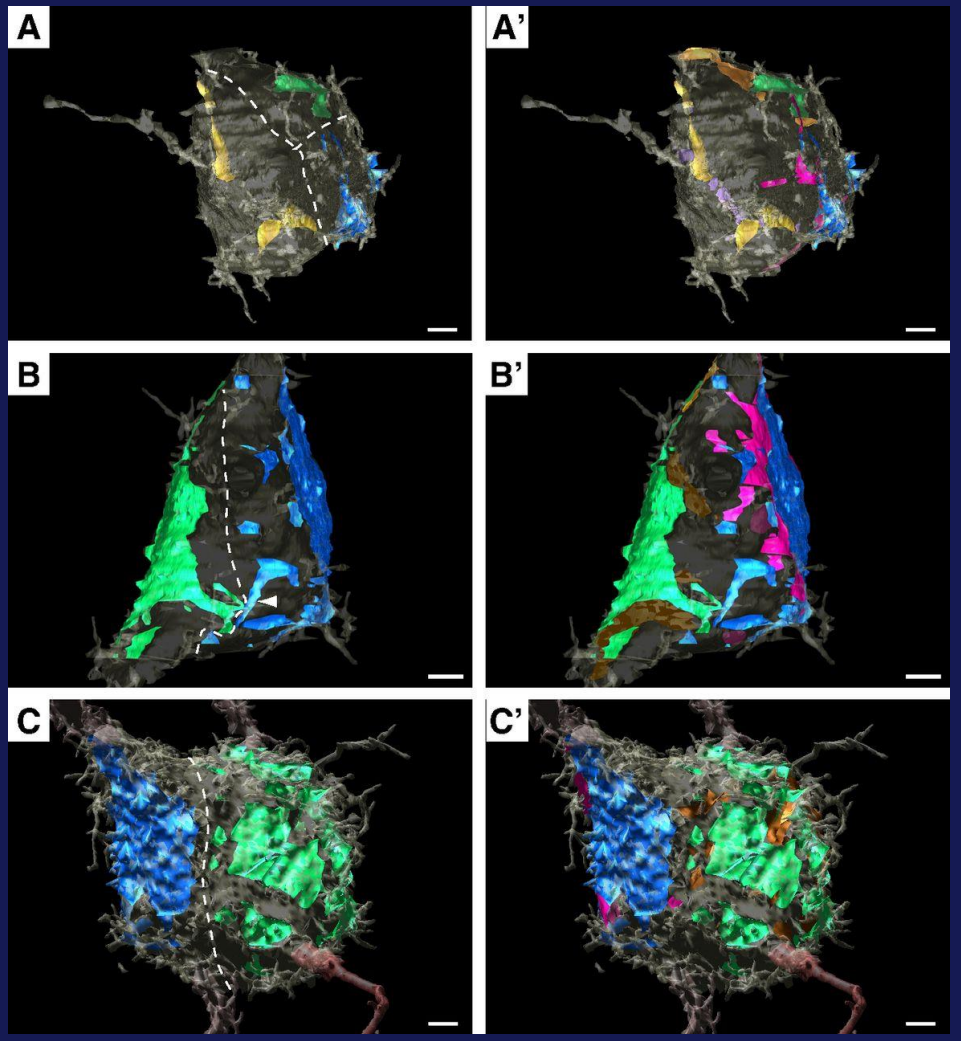


Wong et al., Front. Synaptic Neurosci., 19 June 2017 | https://doi.org/10.3389/fnsyn.2017.00011

Emergence of monoinnervation in MNTB

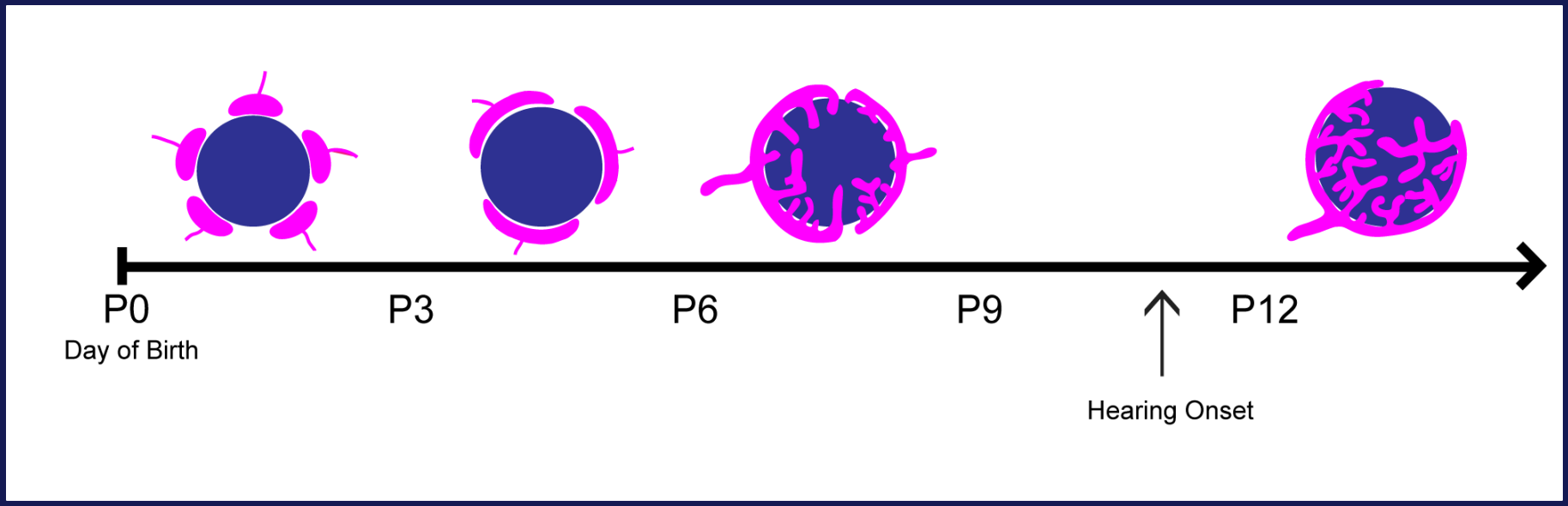
EM tomography studies of developing calyx

- Several small inputs seen at P3.
- Multiple inputs are segregated into territories. Initially similar in size.
- Inputs enlarge and a dominant input is seen by P4.
- Dominant input covers most of MNTB cell by P6.



Holcomb P S et al. J. Neurosci. 2013;33:12954-12969

Development of VCN-MNTB projection



- “Protocalyx” by P2, multiple inputs
- Form calyceal terminations by P3
- **Dominant input by about P6, then monoinnervation emerges**

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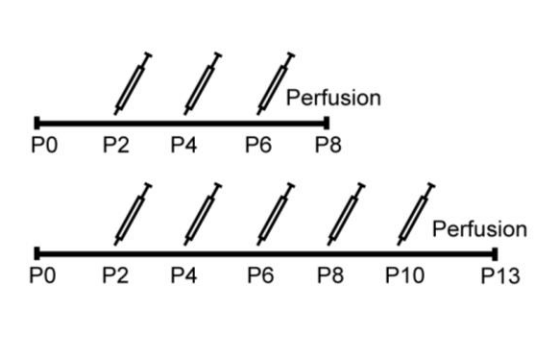
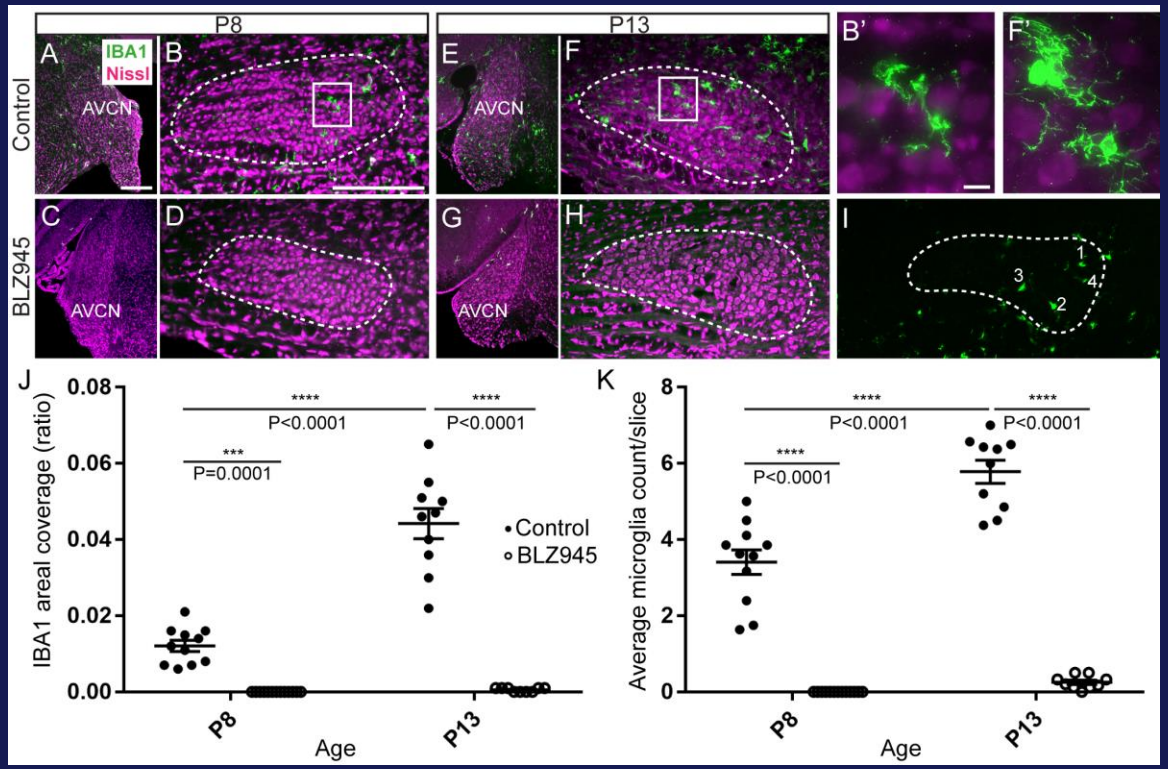
Test the function of microglia in the developing brainstem

- Eliminate microglia during postnatal development
- Test whether microglia depletions results in regulatory changes in astrocyte populations
- Test whether microglia depletion decreases synaptic protein levels in MNTB
- Test whether microglia depletion impairs calyx of Held development and pruning

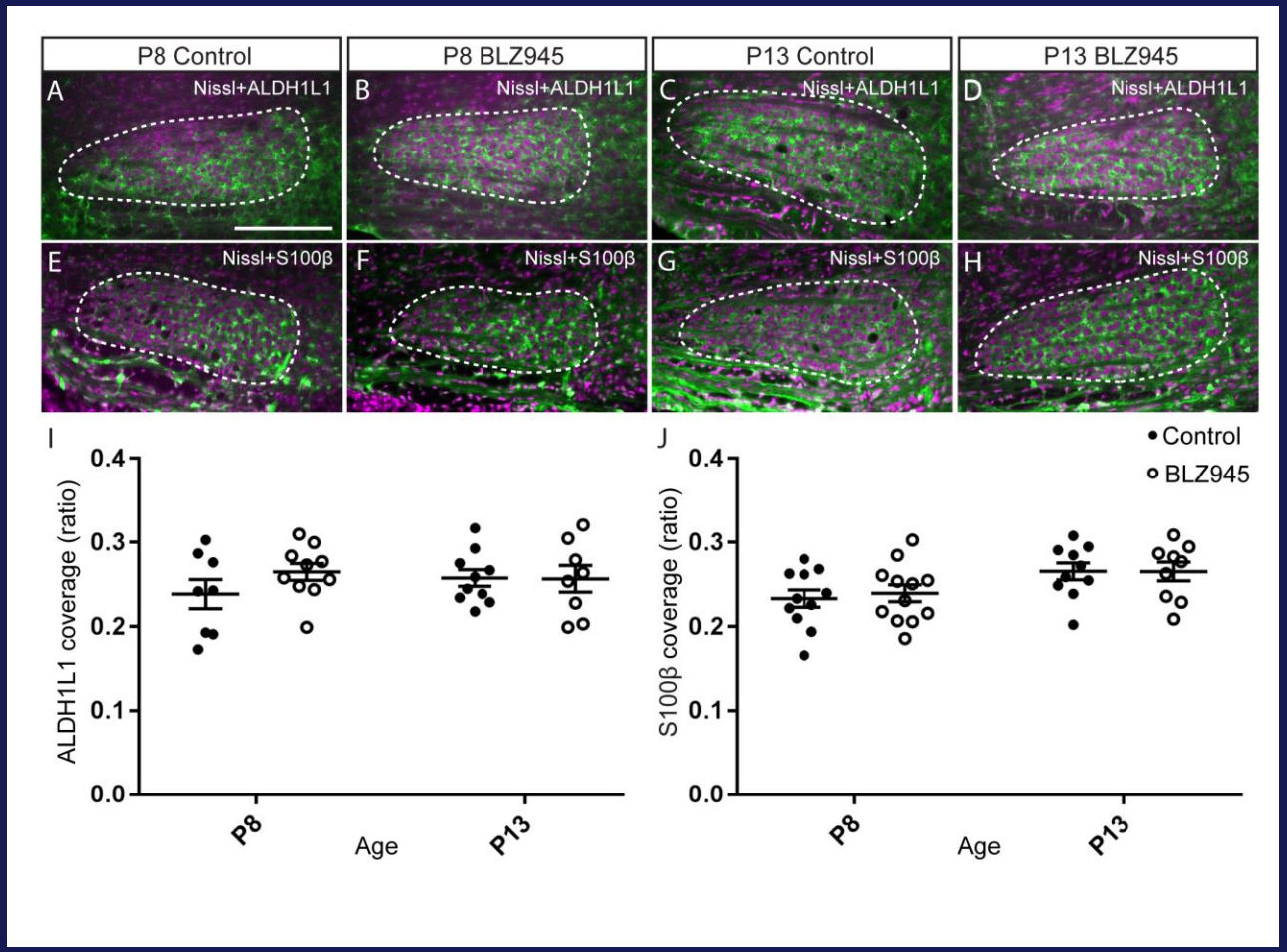
Elimination of microglia in the developing brainstem





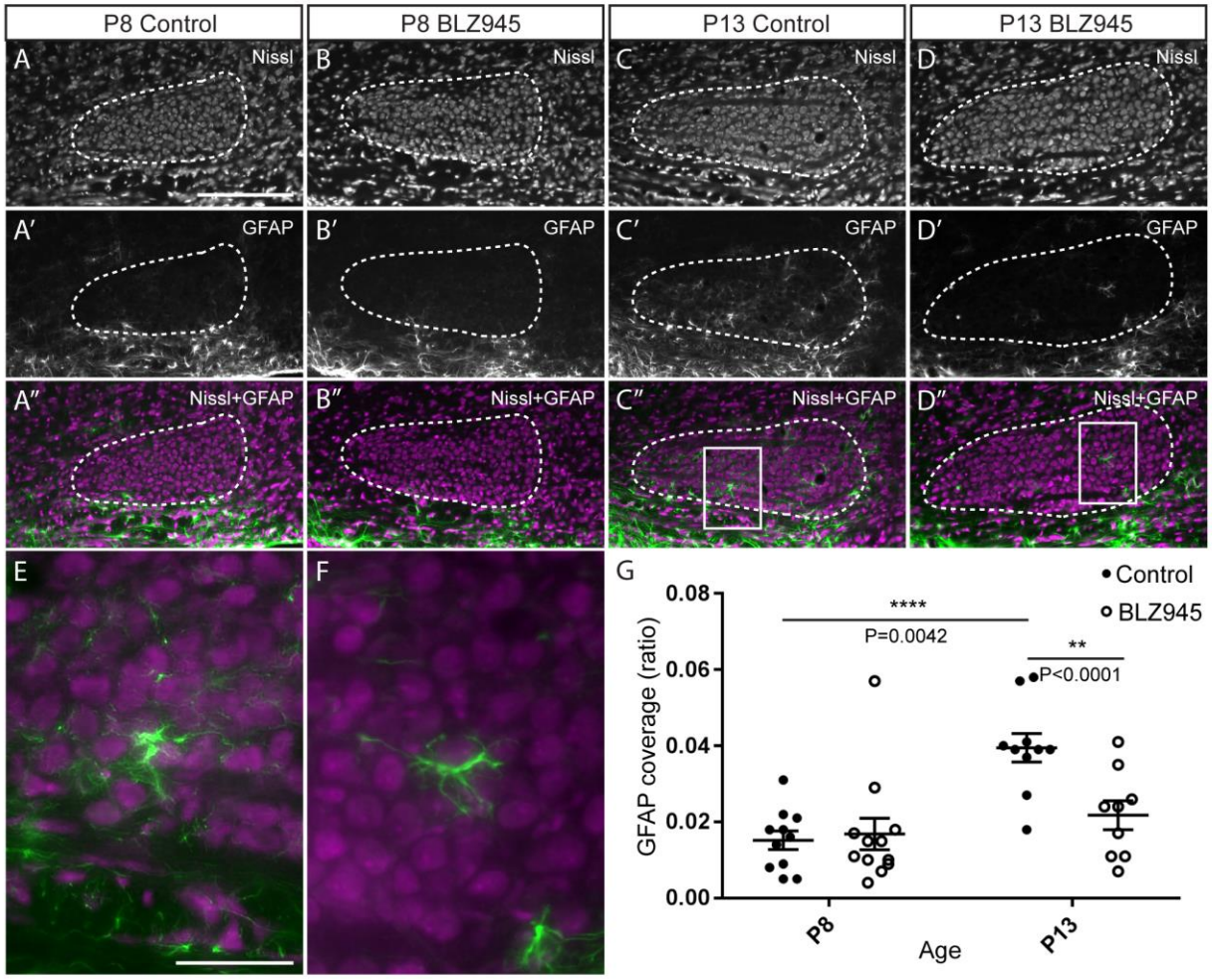
- Microglia depend on CSF1R activity for their survival; chronic inhibition eliminates microglia.
- Subcutaneous administration of BLZ945 eliminates microglia in the developing brain.

Astrocyte markers in microglia depleted MNTB



- The astrocyte markers ALDH1L1 and S100ß are present early in MNTB.
- Their expression levels are not altered by microglia depletion.

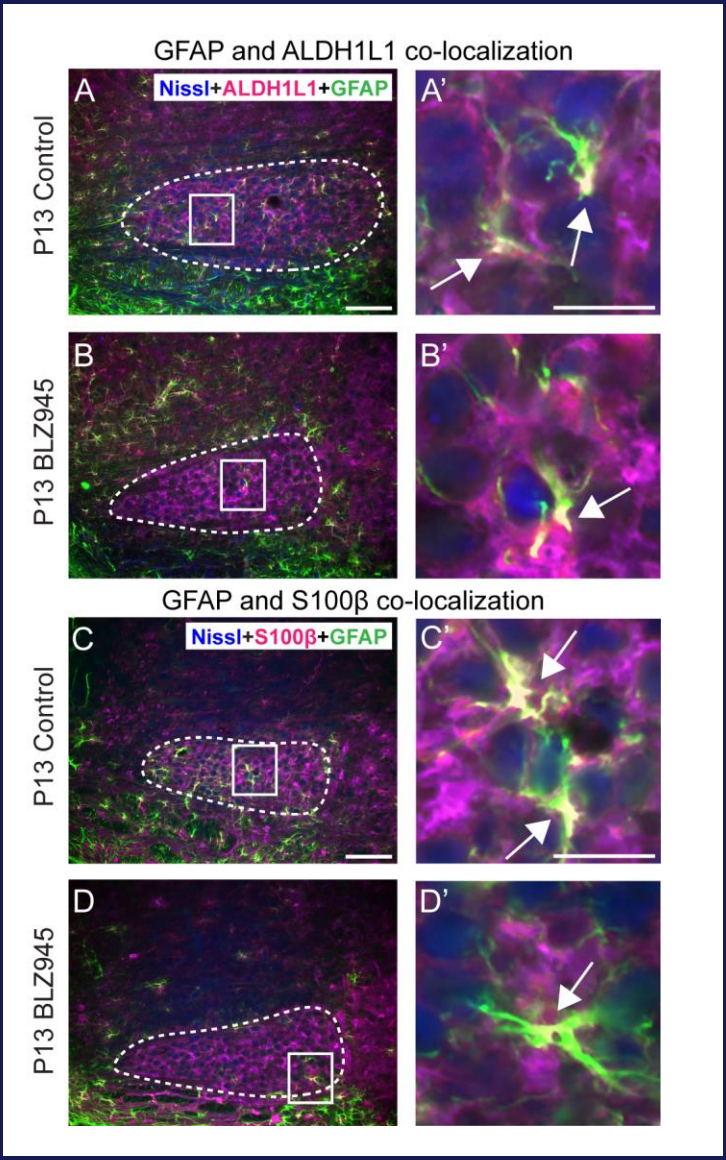
Effects of microglia depletion on astrocytes



The astrocyte marker GFAP is seen late in development in MNTB.

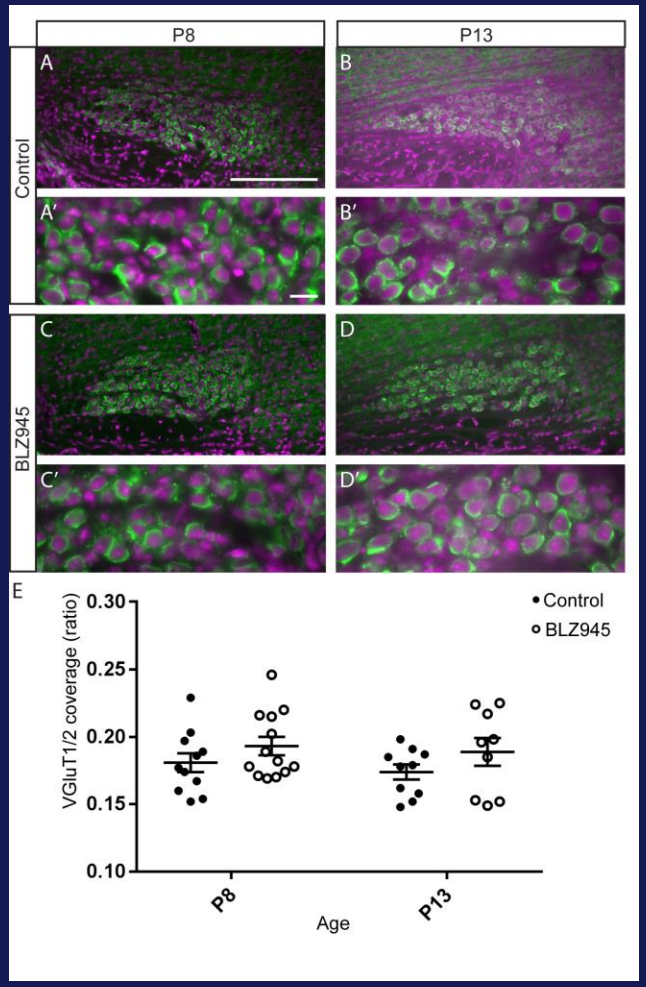
GFAP expression significantly decreases with treatment.

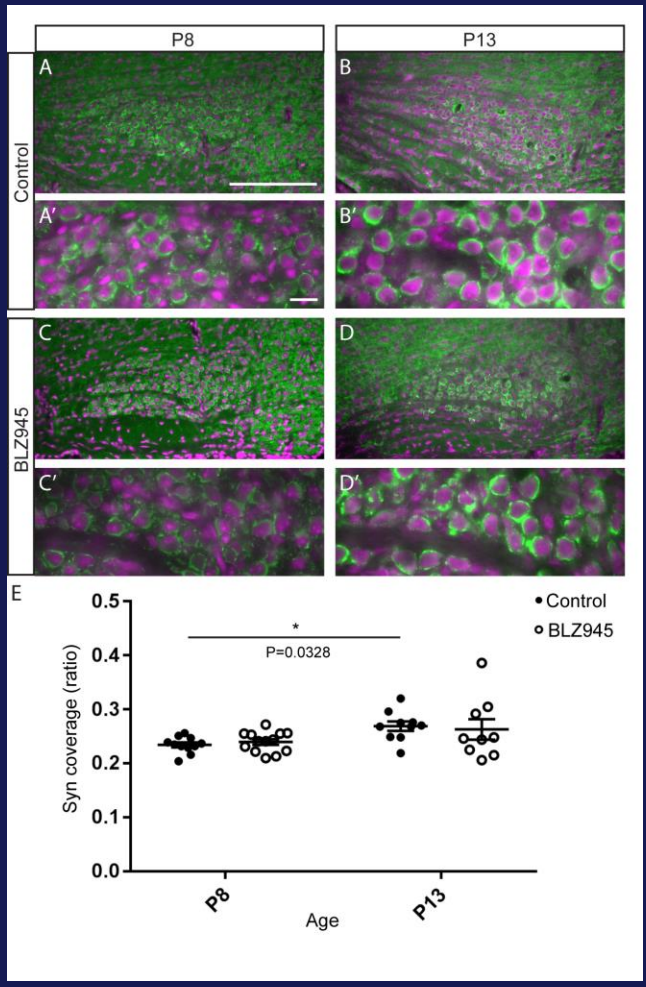
Delayed astrocyte maturation with microglia depletion



- Astrocyte markers show high degree of co-localization
- GFAP is a marker for mature astrocytes that is present at P13
- Suggests that microglia depletion delays astrocyte maturation

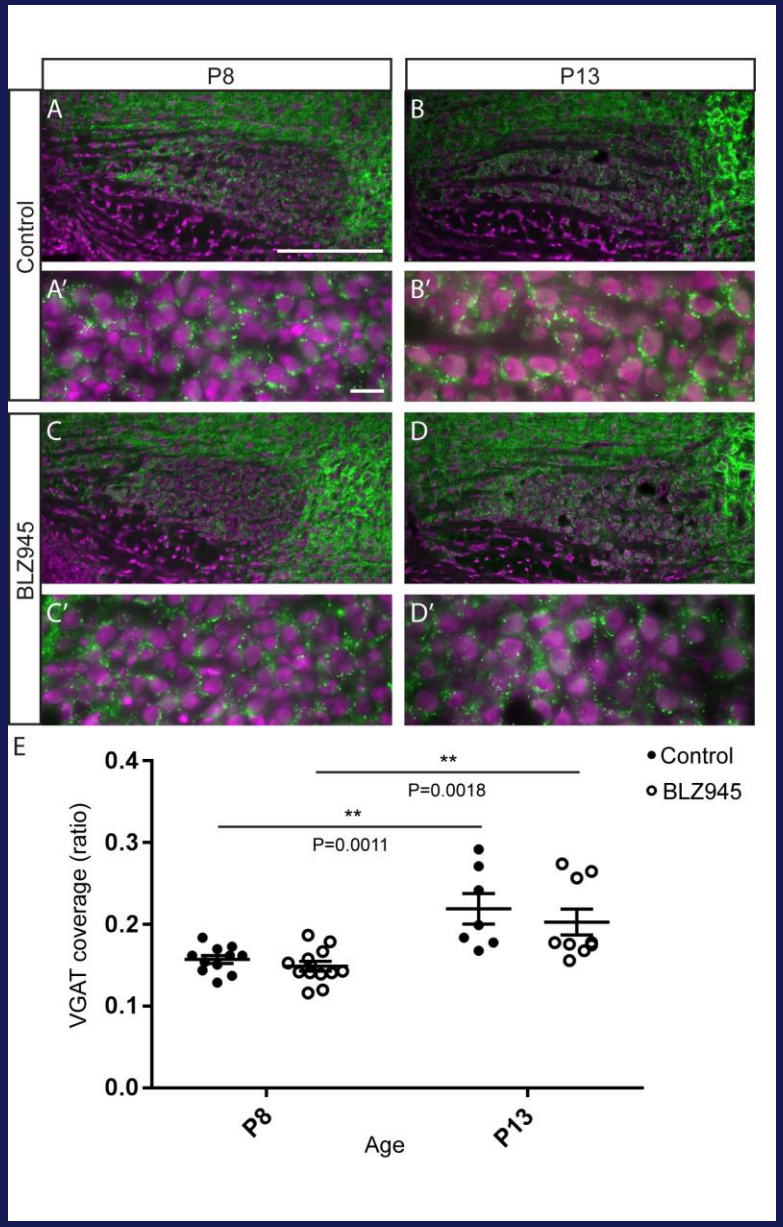
Effects of microglia depletion on excitatory inputs to MNTB





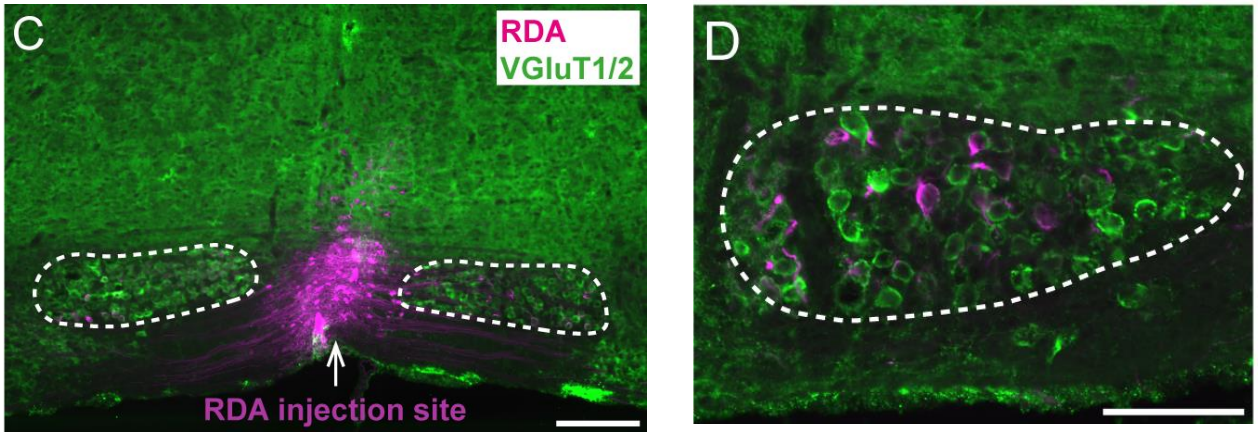
- VGLUT 1/2 and synaptophysin, percent coverage tested at P8, P13
- No significant effect when microglia depleted.

Effects of microglia depletion on inhibitory inputs to MNTB

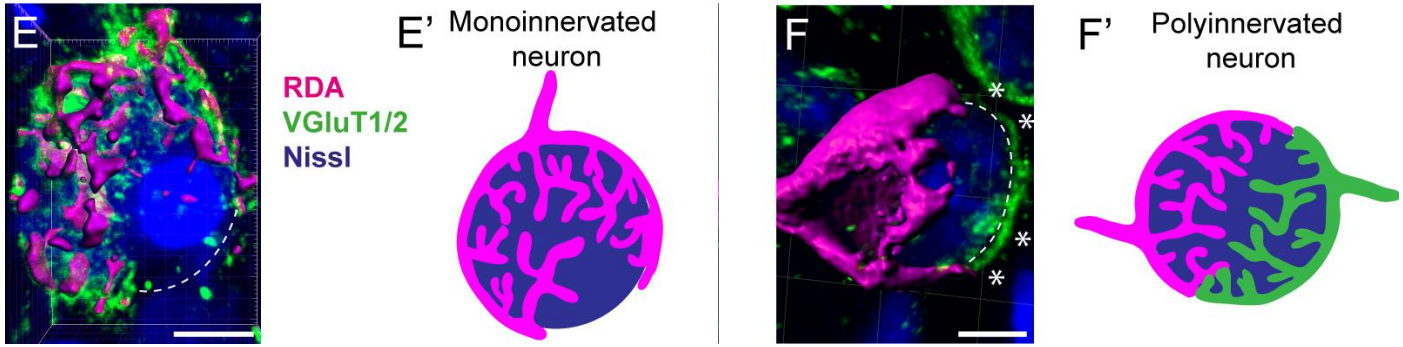


- VGAT is a marker for inhibitory inputs to MNTB.
- Inhibitory puncta increase with age.
- No significant effect when microglia depleted.

Assessment of pruning

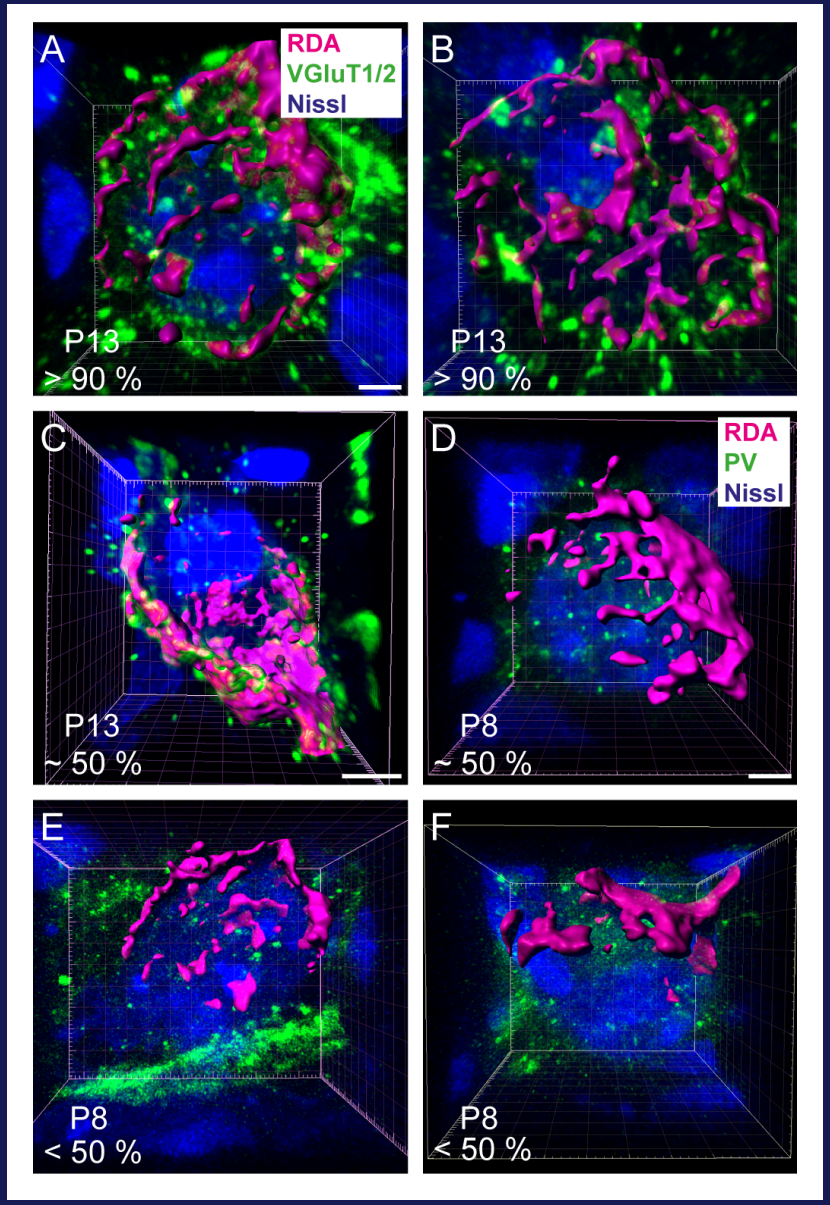


Sparse RDA label in midline + VGluT immunofluorescence

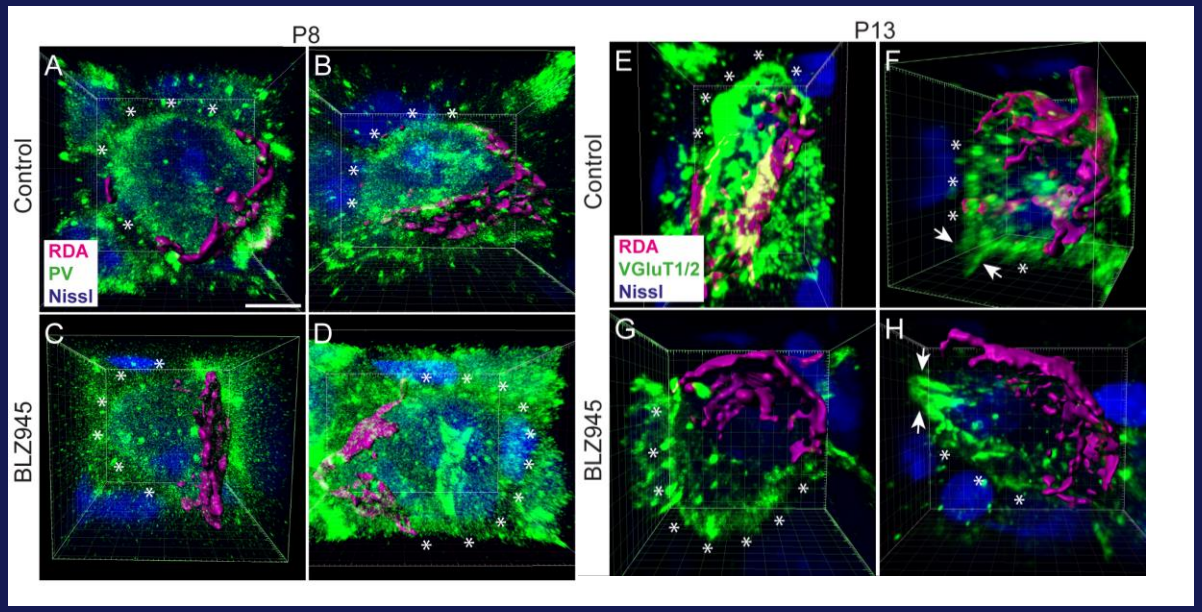


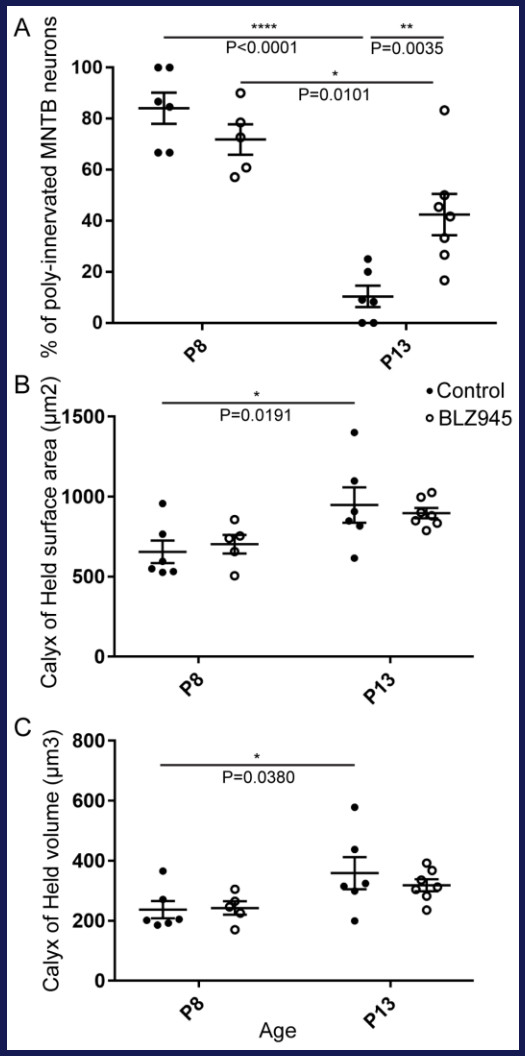
Assessment of pruning

- As expected, we found monoinnervated MNTB neurons at both P8 and P13



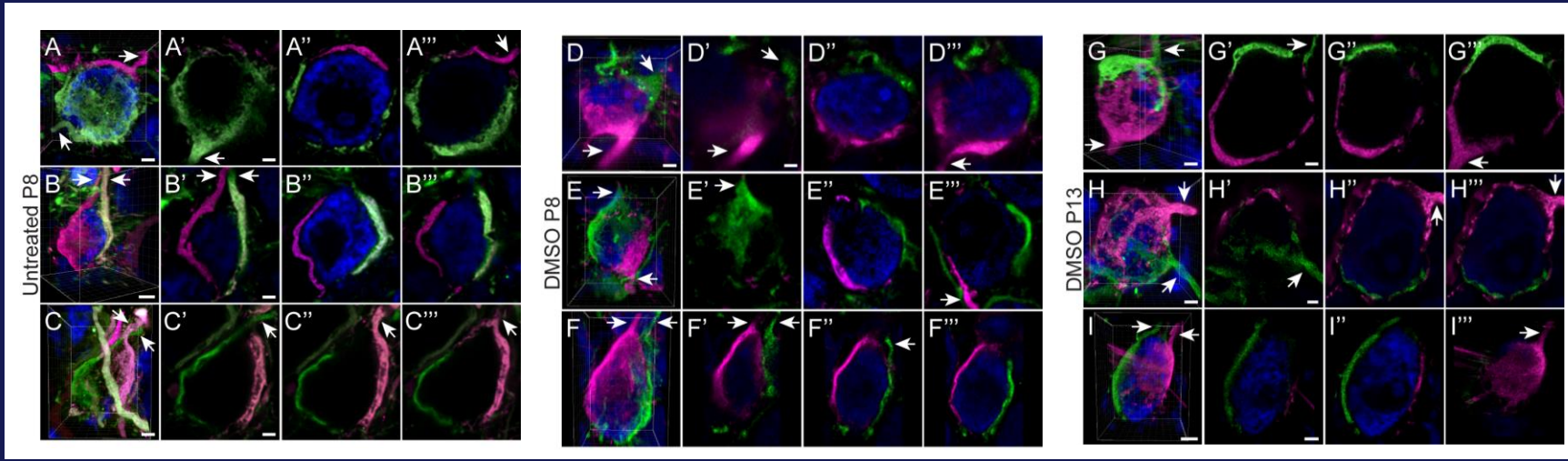
Assessment of pruning





- We found polyinnervated MNTB neurons at both ages.
- **Significantly more polyinnervation was seen in treated animals at P13.** No difference in calyx size.

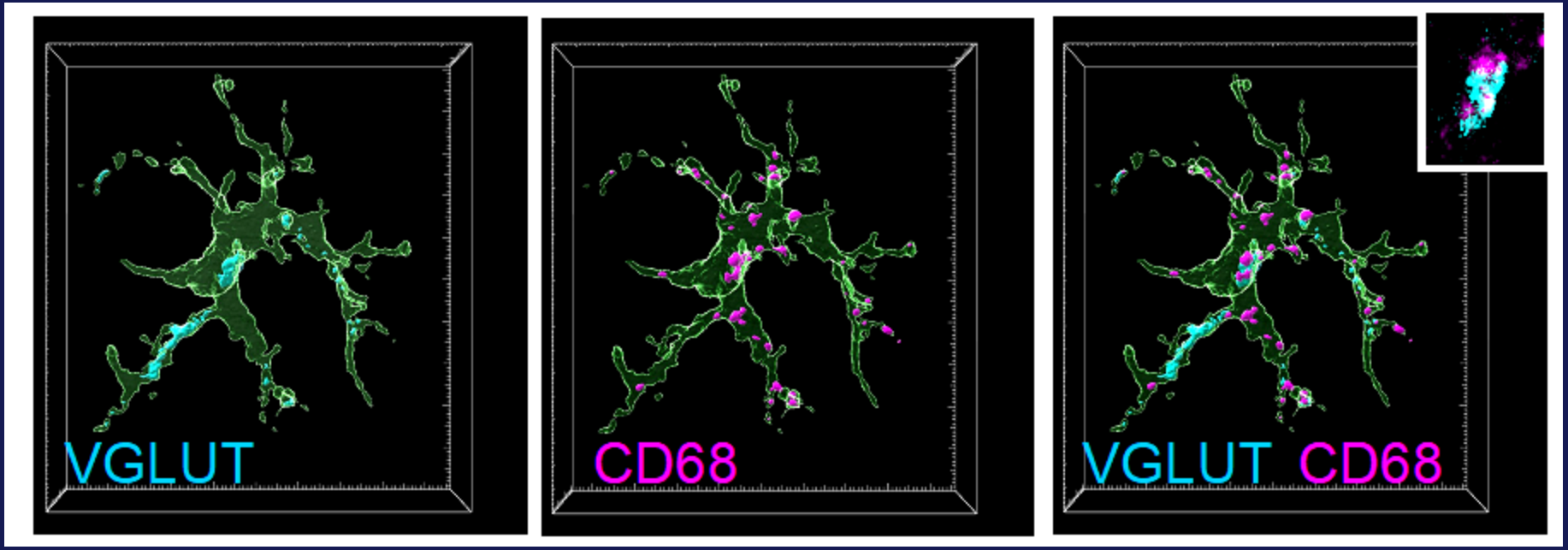
Sequential dye electroporation reveals multiple inputs



- RDA and Alexa488 dextran amine were used sequentially in individual brains to sparsely label calyces.

- Multiple inputs together with preterminal axons seen in all brains.

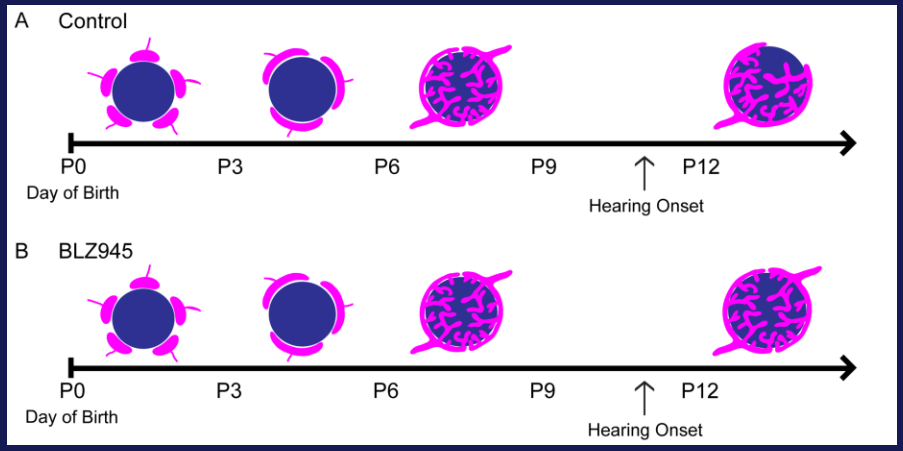
Engulfment of presynaptic protein in microglia



- VGluT1/2 labeled in *Cx3cr1+/-* mice. We used deconvolved confocal images to identify regions of colocalization with GFP.
- VGLUT colocalized with lysosomal marker CD68 in microglial cell bodies and branches.
- **Consistent with a direct role for microglia in pruning calyces**

Summary: Early treatment with CSF1R inhibitor

- Treatment with BLZ945 during the postnatal period eliminates microglia from the auditory brainstem.
- At the later time point, microglia depleted animals show significantly impaired synaptic pruning in MNTB. Presynaptic proteins are engulfed by microglia during development.
- The results suggest that microglia play a role in the late stages of pruning.
- Late pruning effects may also relate to delayed maturation of astrocytes.



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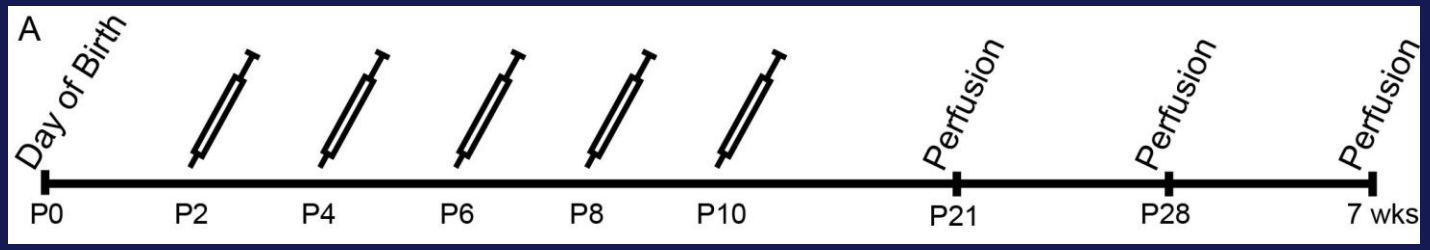
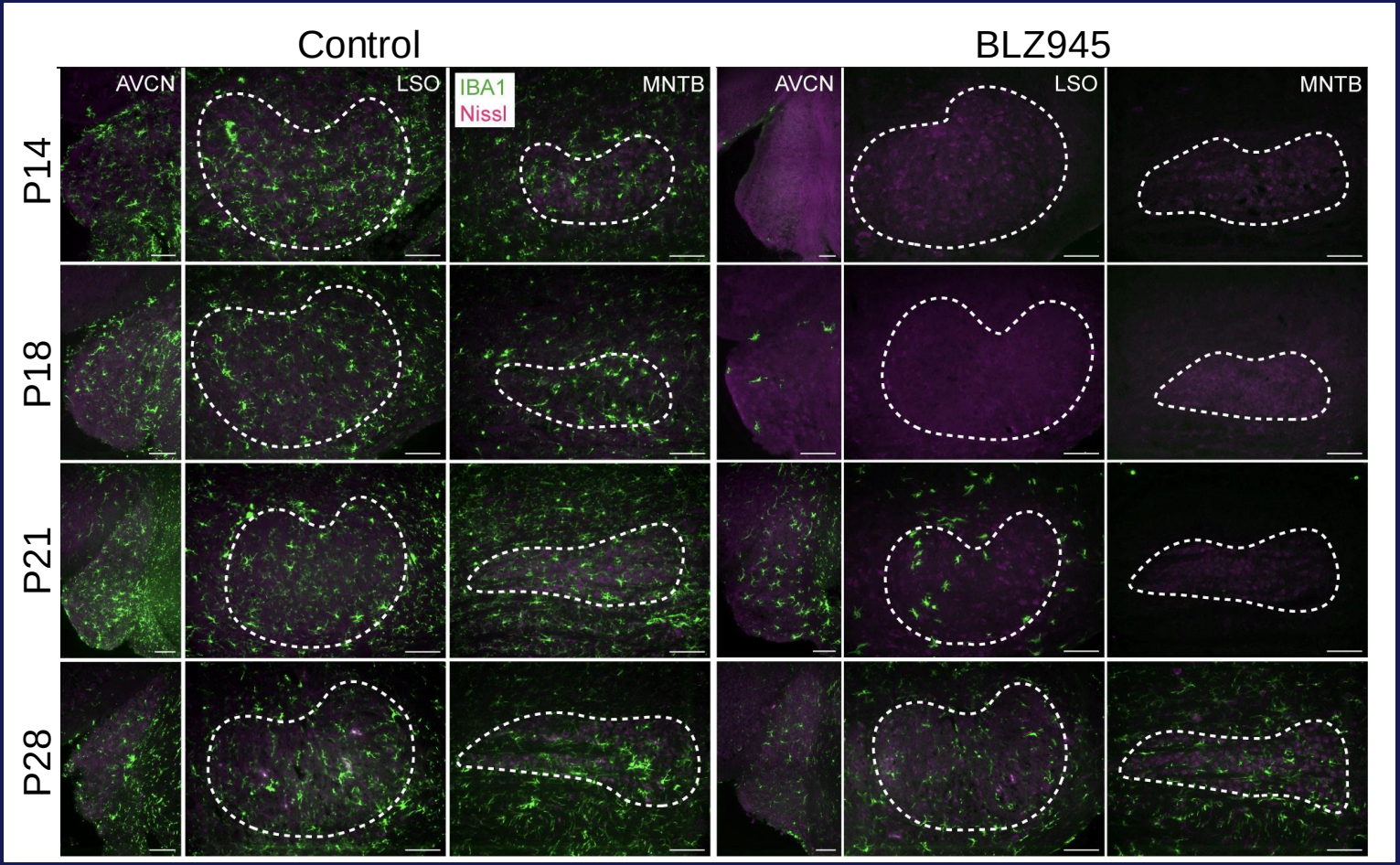
Microglia depletion and repopulation



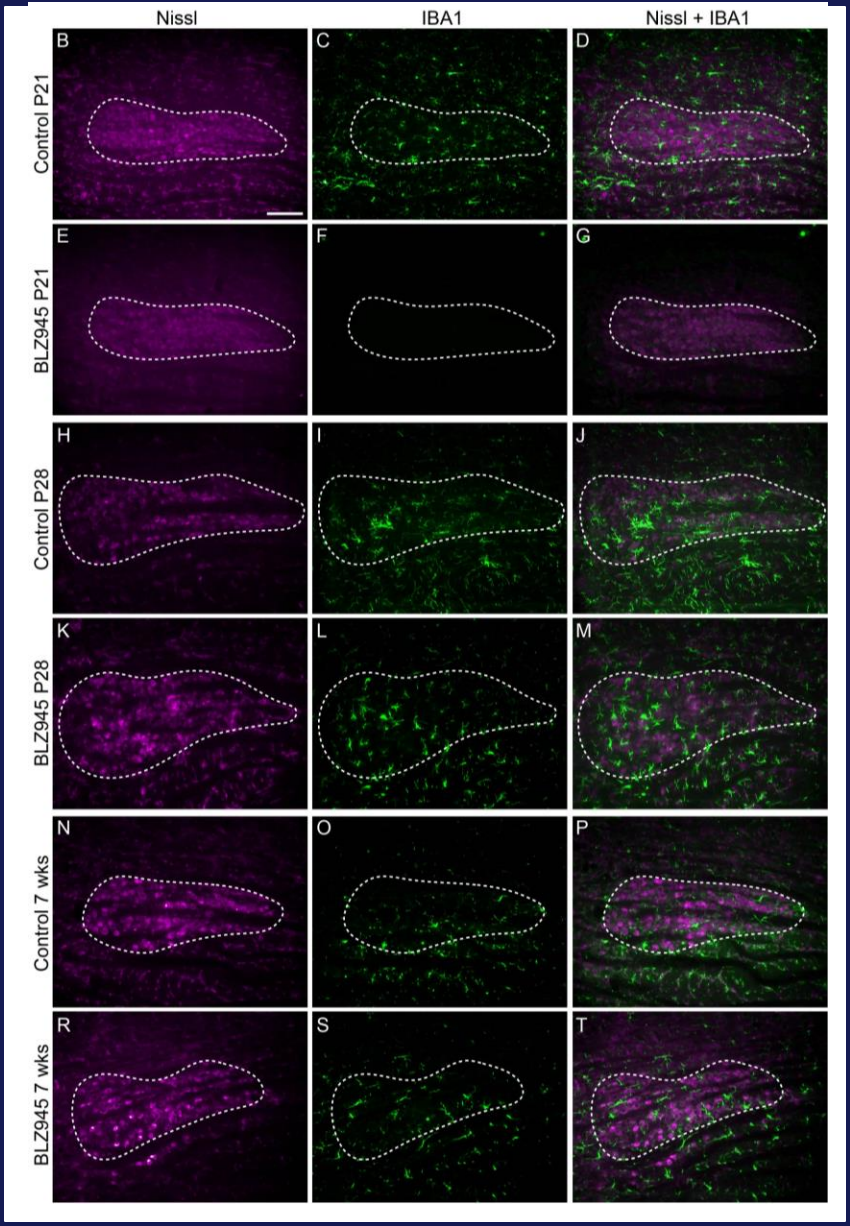
- Schedule of treatment: continue through P10, allow microglia to return.
- Assessment at 3 wks, 4wks, and 7 wks.

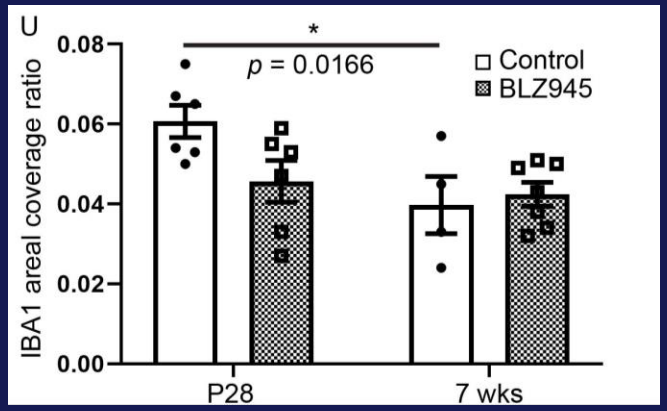
Microglia depletion and repopulation



- Microglia repopulate in VCN then in MNTB; seem to come in from lateral parts of brainstem
- Appear in VCN at 3 weeks, and in MNTB at 4 weeks

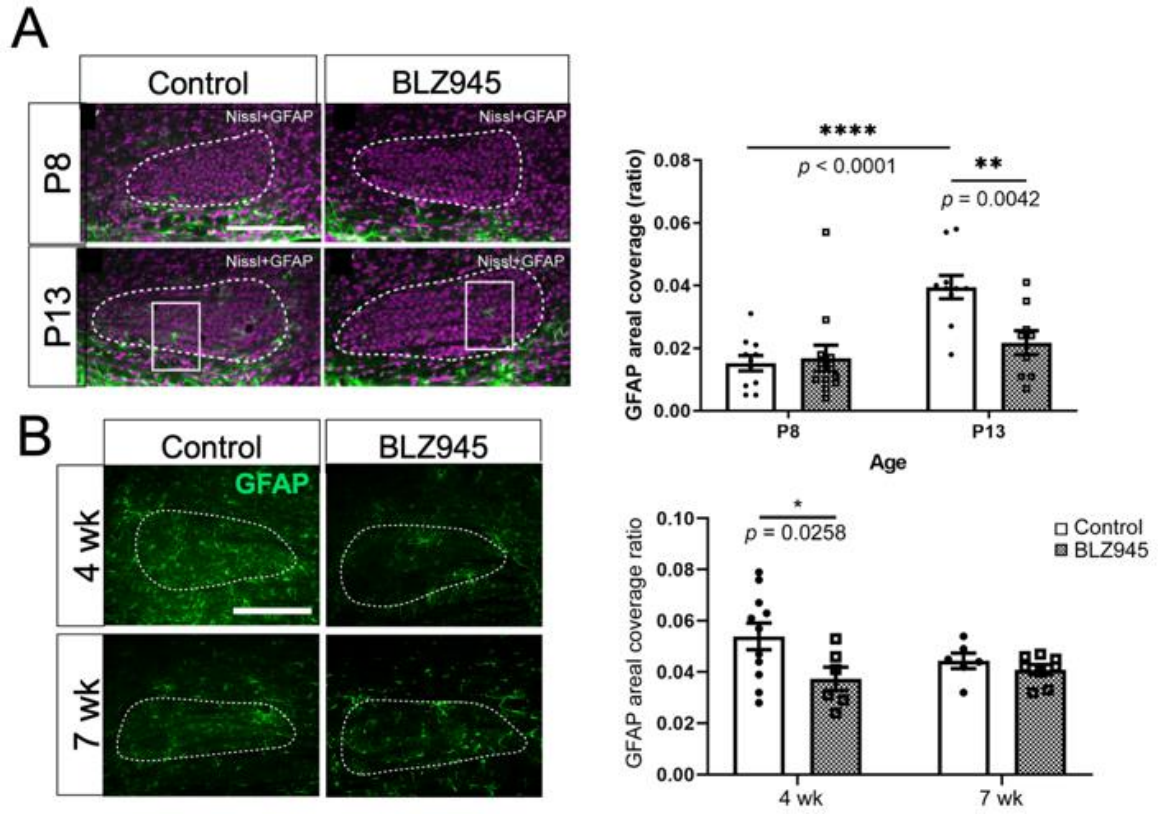
Microglia depletion and repopulation





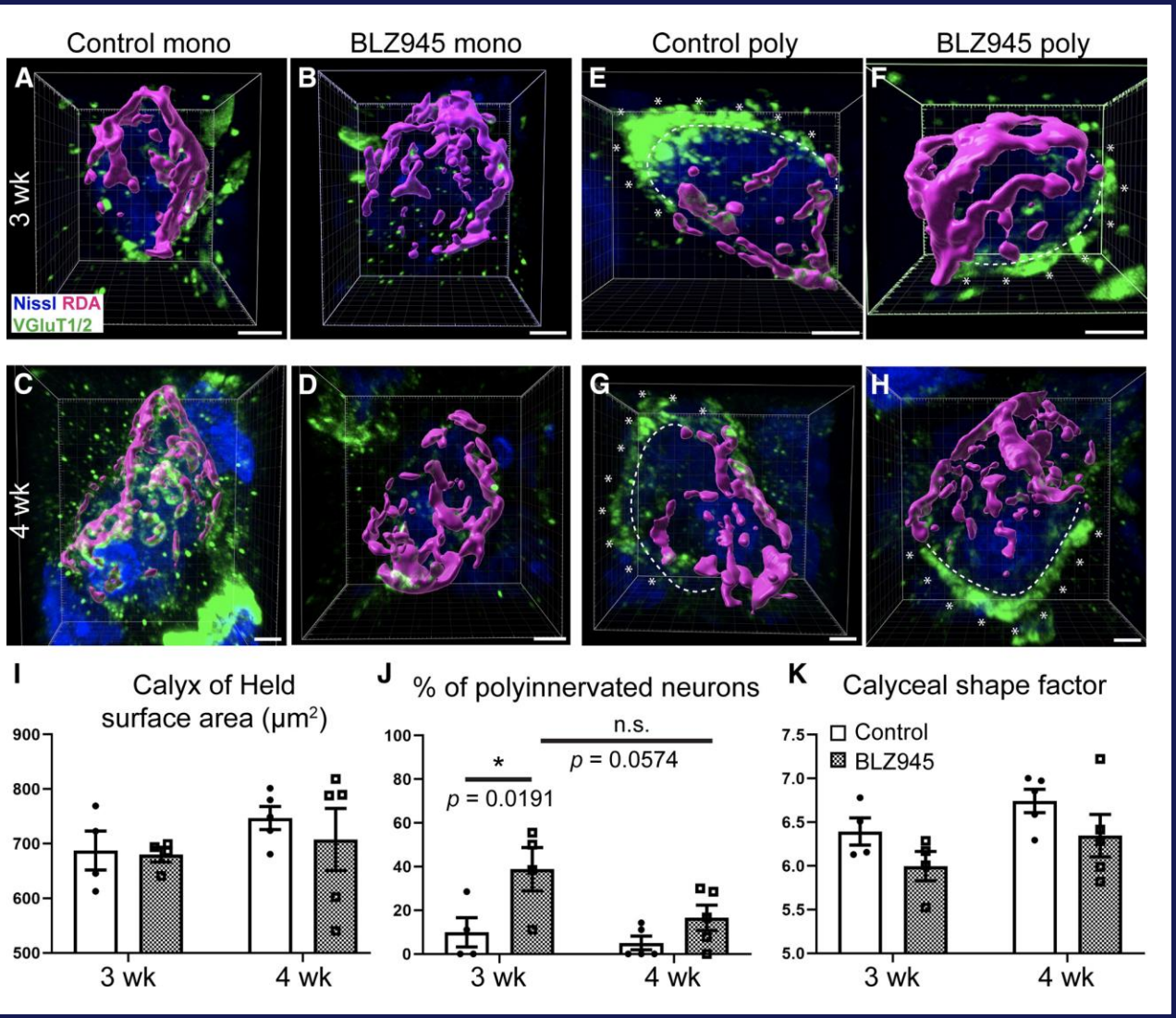
- Microglia populate MNTB at 4 weeks (but not 3 weeks).
- Levels of Iba1 decrease after 3 weeks, and are the same as controls at 7 weeks.

Microglia depletion and repopulation: GFAP



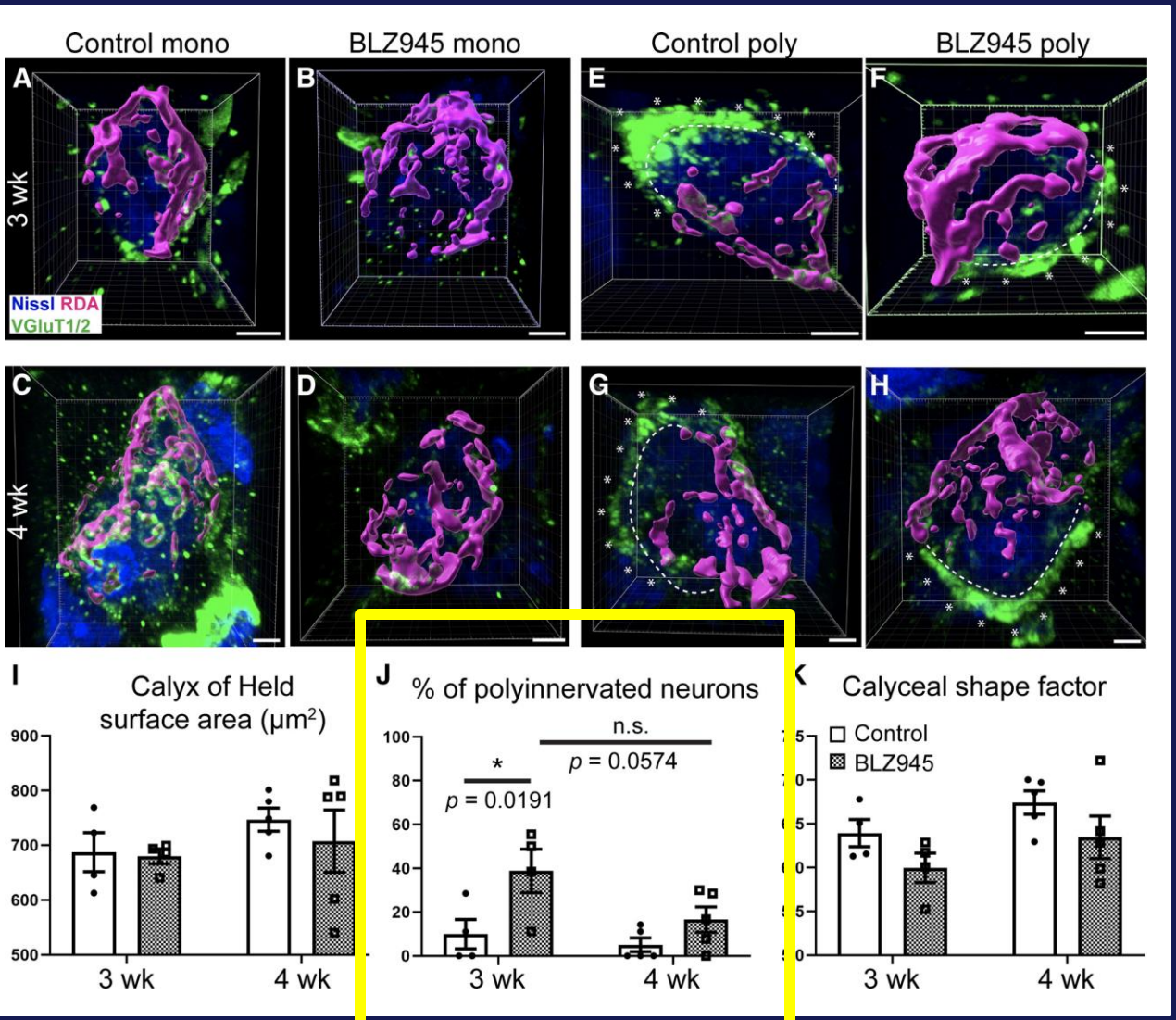
- GFAP remains slightly reduced at P28, but similar to controls at 7 weeks.
- Reflects decrease in GFAP in controls.

Microglia depletion and repopulation: calyx of Held pruning



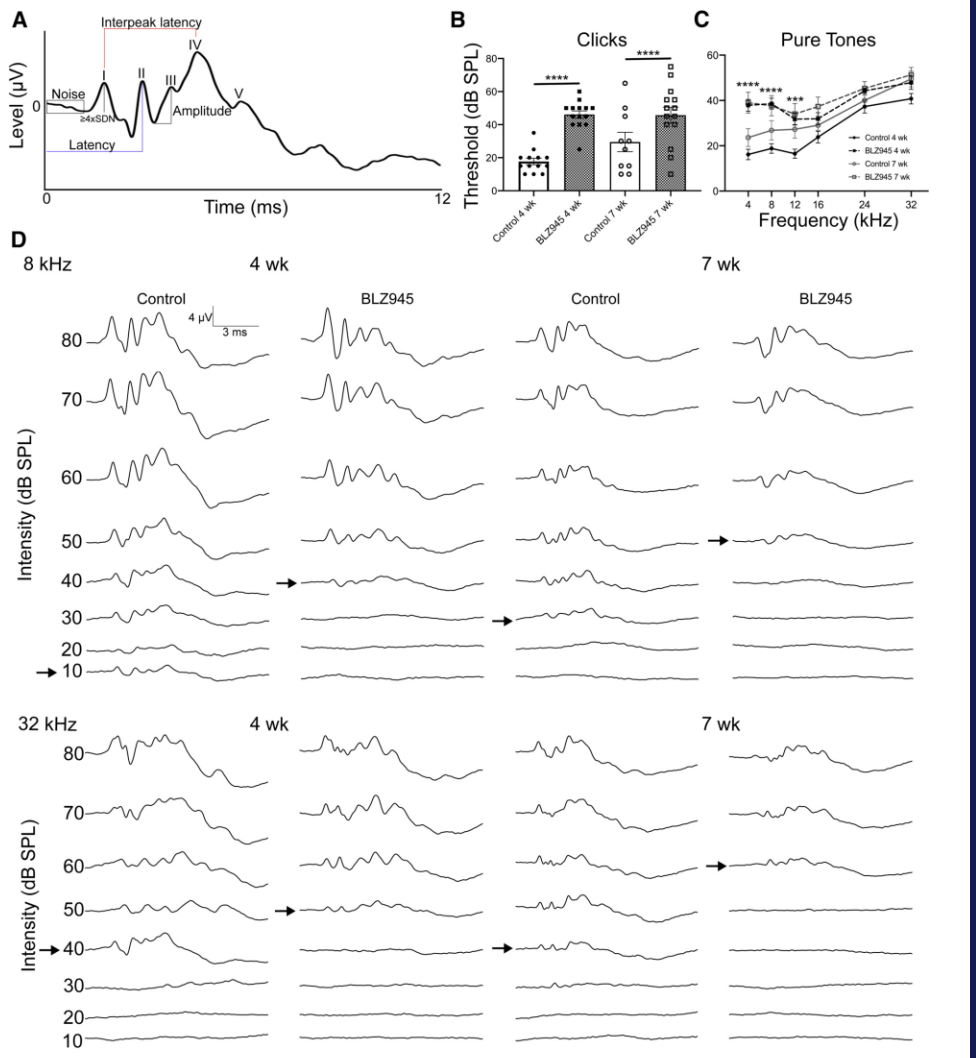
By P28, MNTB neurons were mostly monoinnervated

Microglia depletion and repopulation: calyx of Held pruning



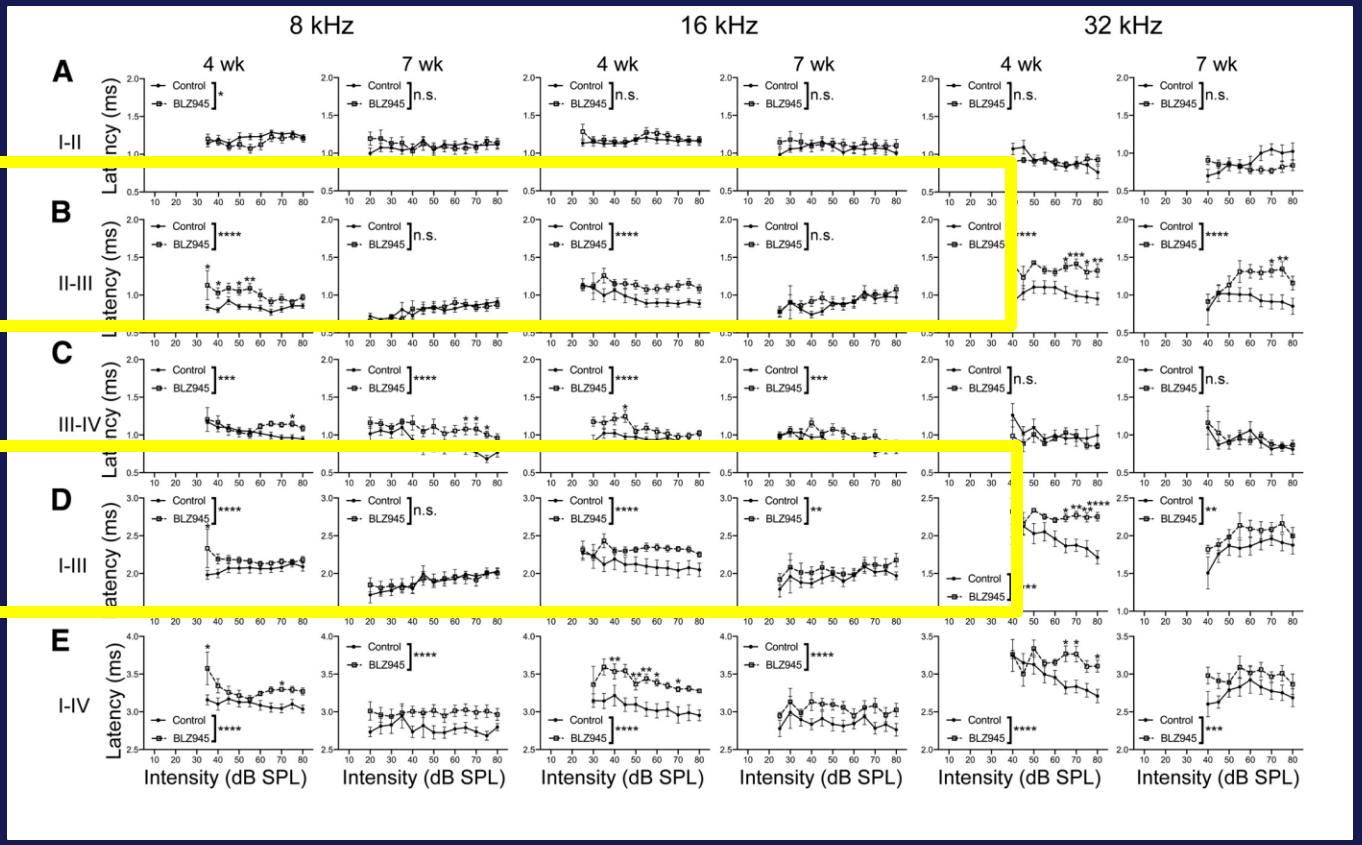
By P28, MNTB neurons were mostly monoinnervated

Microglia depletion and repopulation: Auditory Function



- Early BLZ treatment leads to increase in thresholds in response to click and low frequency tone stimuli.

Microglia depletion and repopulation: Auditory Function



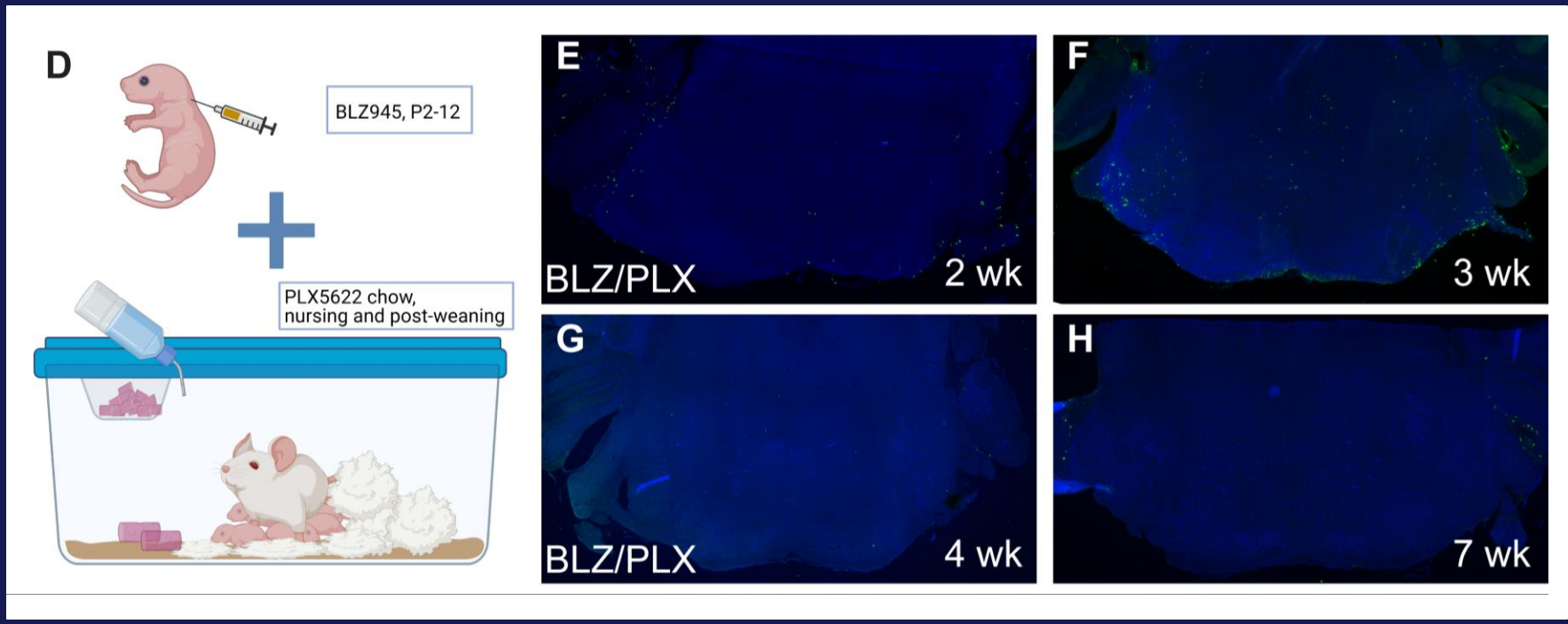
- **Interpeak latencies II-III and I-III** were elevated at 4 weeks, less so at 7 weeks.

Summary – Repopulation by microglia

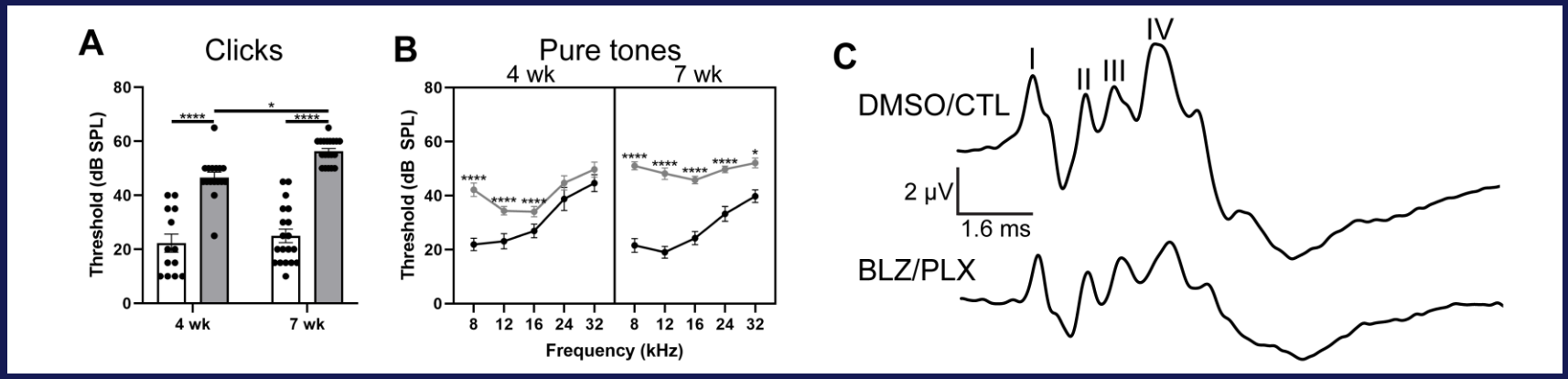
- Recovery after BLZ treatment through P10 leads to repopulation by microglia, recovery of pruning in MNTB, and normal levels of GFAP in the auditory brainstem.
- BLZ treatment through P10 results in elevated ABR thresholds at P28. Thresholds remain elevated at 7 weeks.
- Increased latencies (peak I) and reduced peak II-IV amplitudes are corrected by 7 weeks.

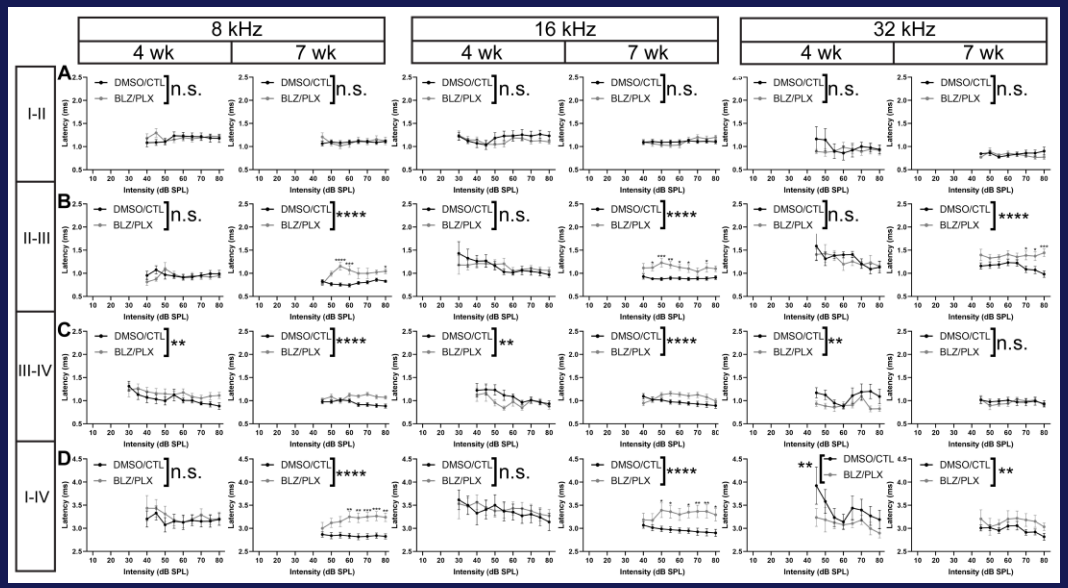
Long-term Microglia Depletion

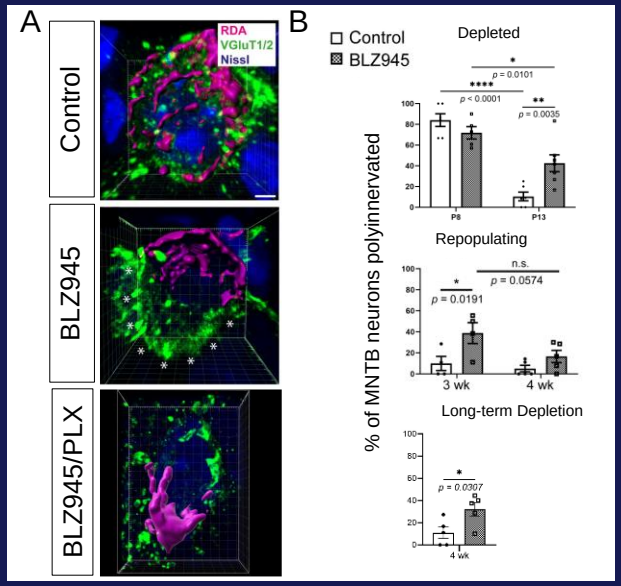
- Subcutaneous BLZ945 did not work for long-term.
- PLX5622 rodent diet not effective through lactation
- BLZ/PLX regimen



Long-term microglia depletion





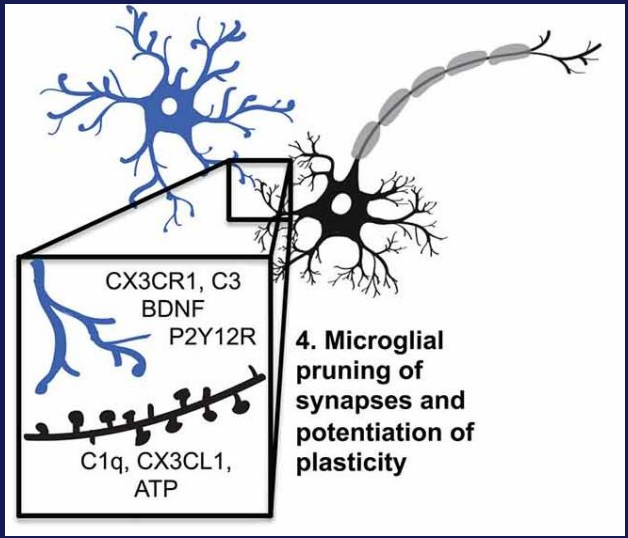


Thresholds increase, amplitudes decrease, and latencies get longer.
Pruning is not restored, with significant polyinnervation in MNTB

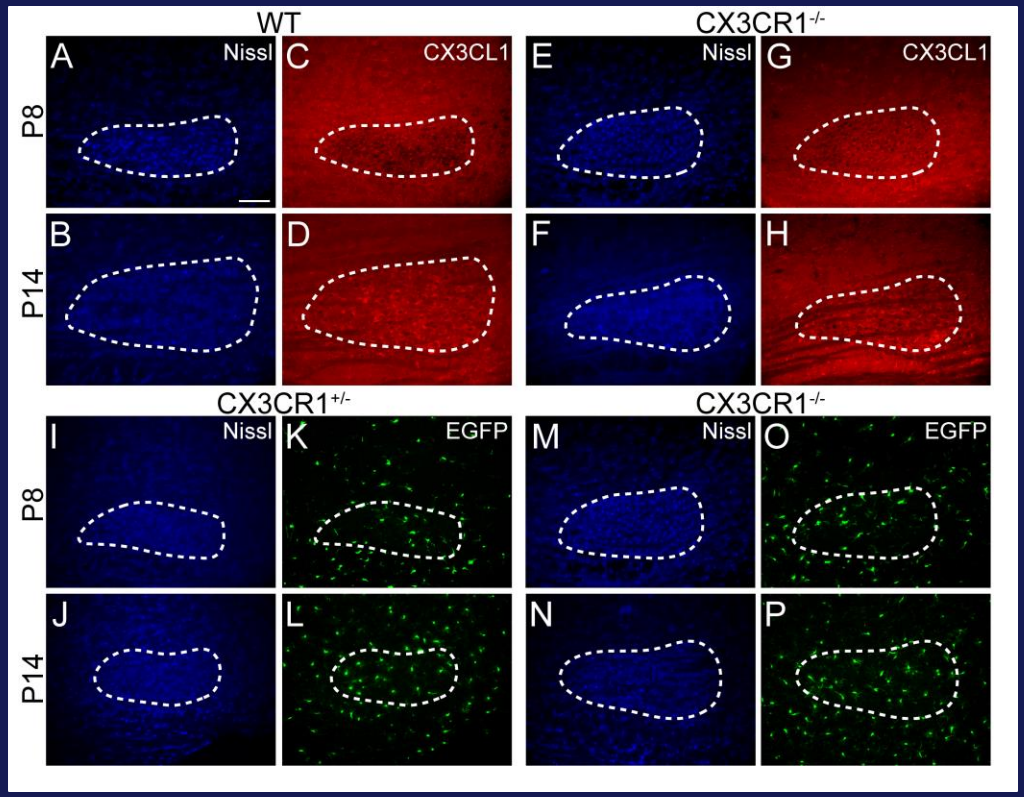
Outline

- Auditory brainstem circuits: how do they work, and what is special about them?
- What are glial cells and how do they contribute to circuits?
- Microglia in the auditory brainstem
  - What happens when you eliminate them during development?
  - What happens when they come back?
  - **How do they work?**
- Conclusions and future directions

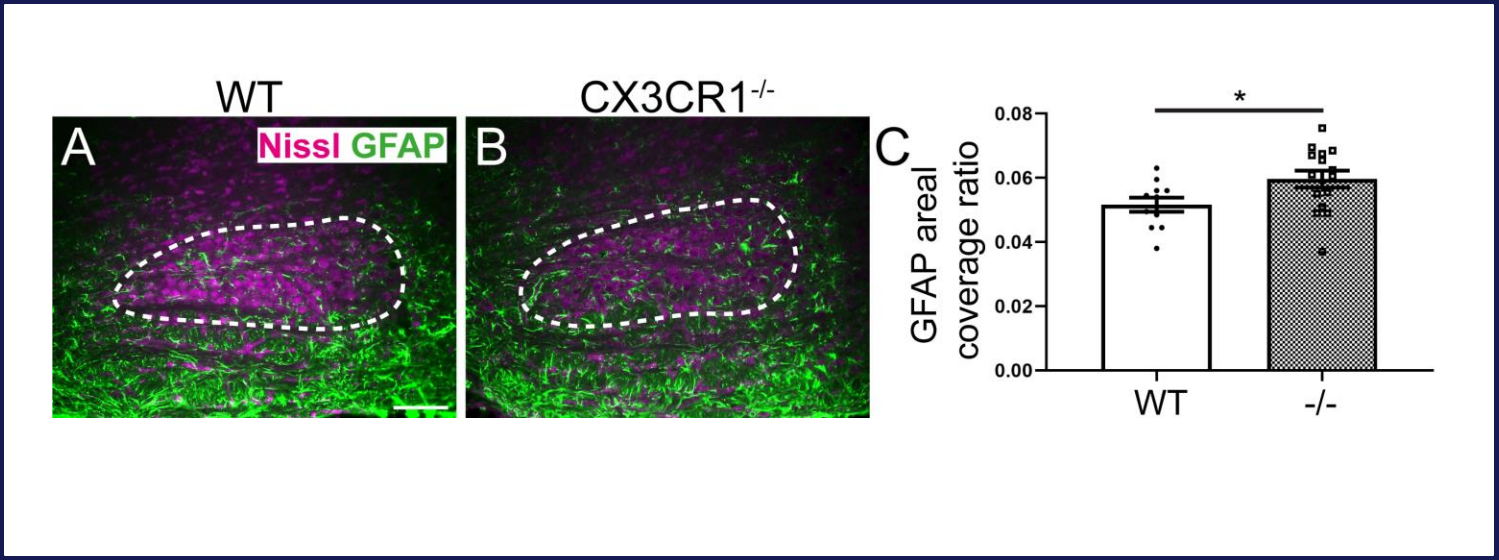
Fractalkine receptor (Cx3cr1)



- Expressed on microglia, responds to fractalkine (CX3CL1), expressed in neurons.
- Expressed in developing auditory brainstem.
- CX3CL1 also expressed.

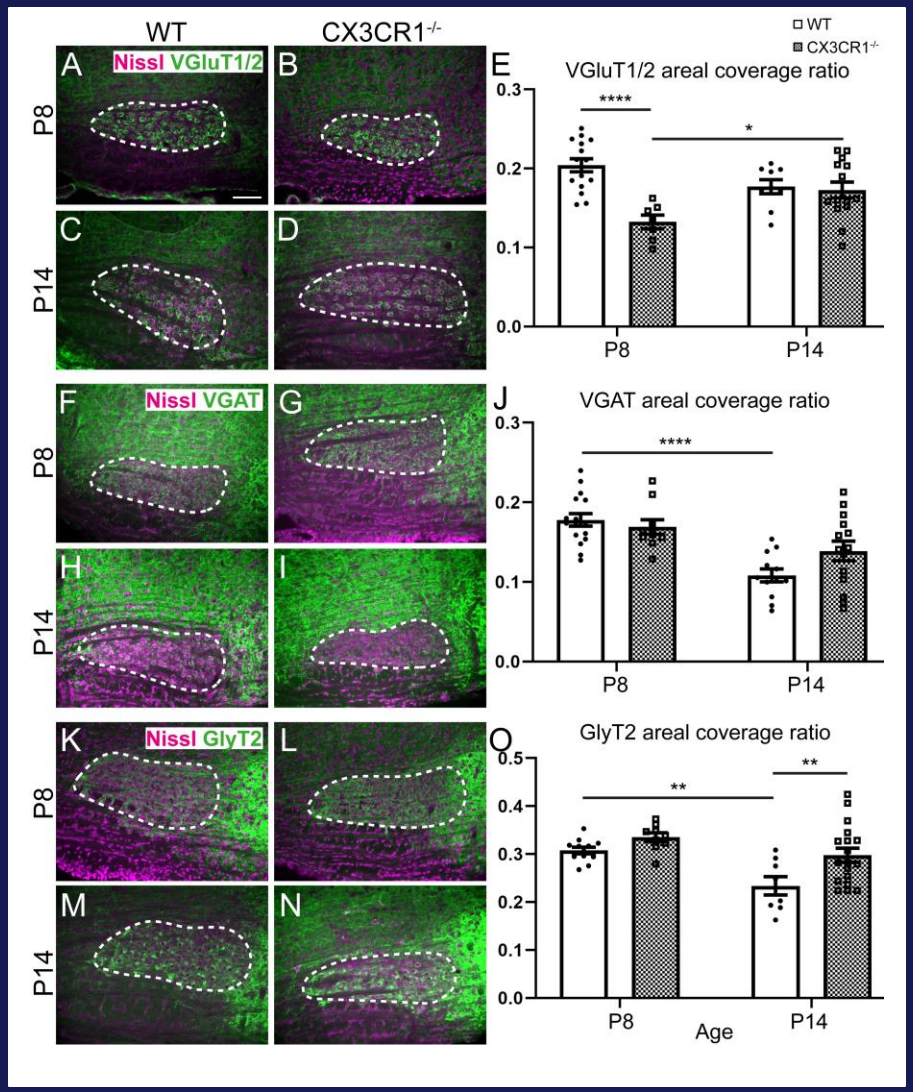


Effects of CX3CR1 mutation on GFAP



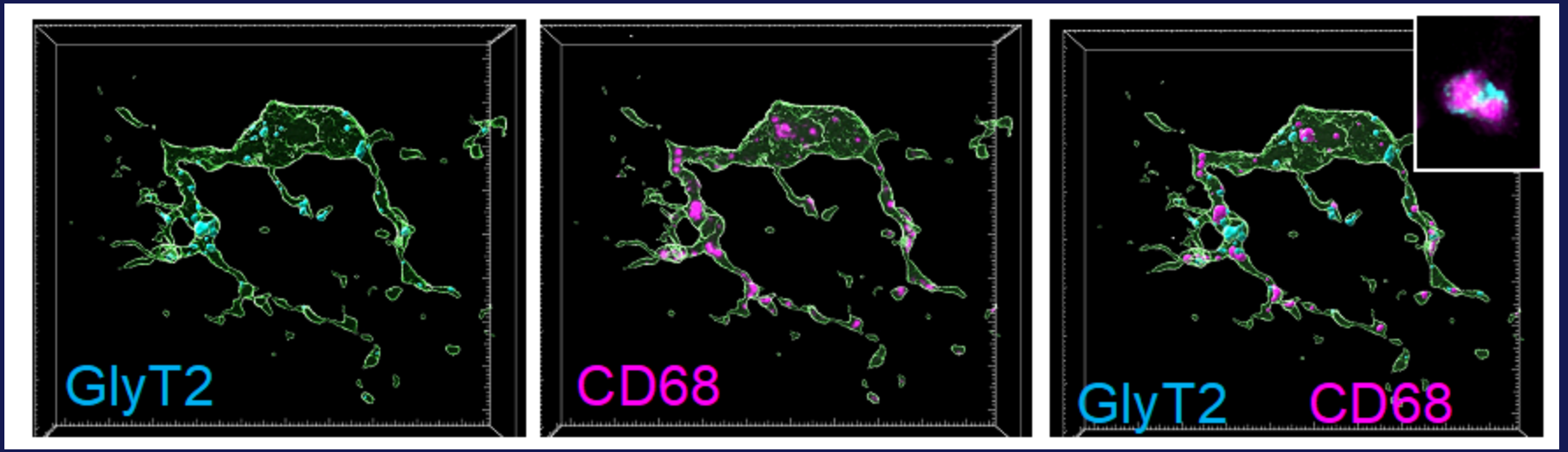
- Microglia elimination resulted in decreased GFAP at P13.
- CX3CR1-/- mice showed *increased* GFAP levels at P14.

Effects of CX3CR1 mutation on synaptic proteins



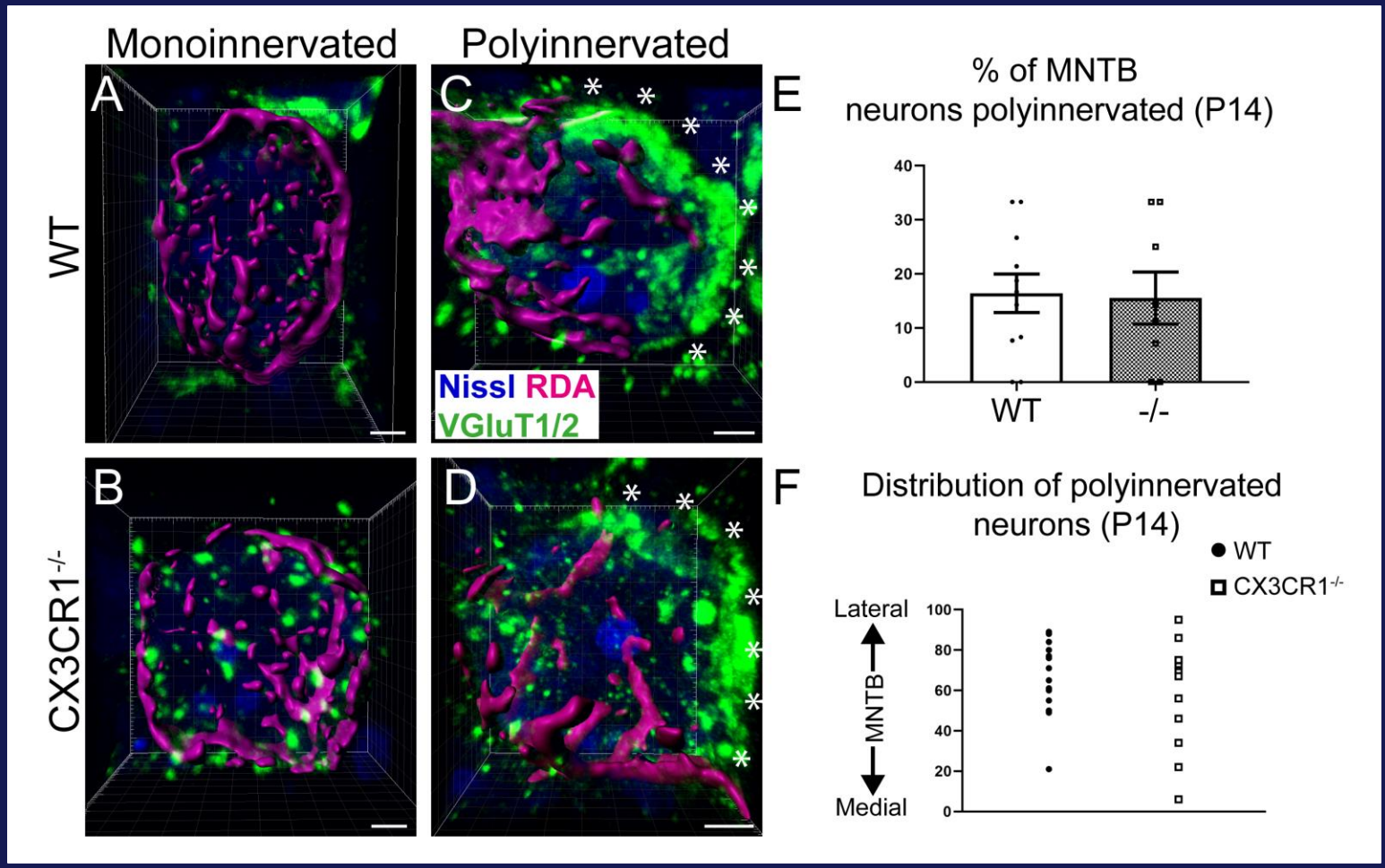
- VGlut1/2 reduced at P8, but not P14.
- VGAT reduced with age, but no effect of genotype.
- GlyT2 is elevated at P14 but not P8. Impaired pruning of inhibitory inputs?

Effects of CX3CR1 mutation: inhibitory synapses



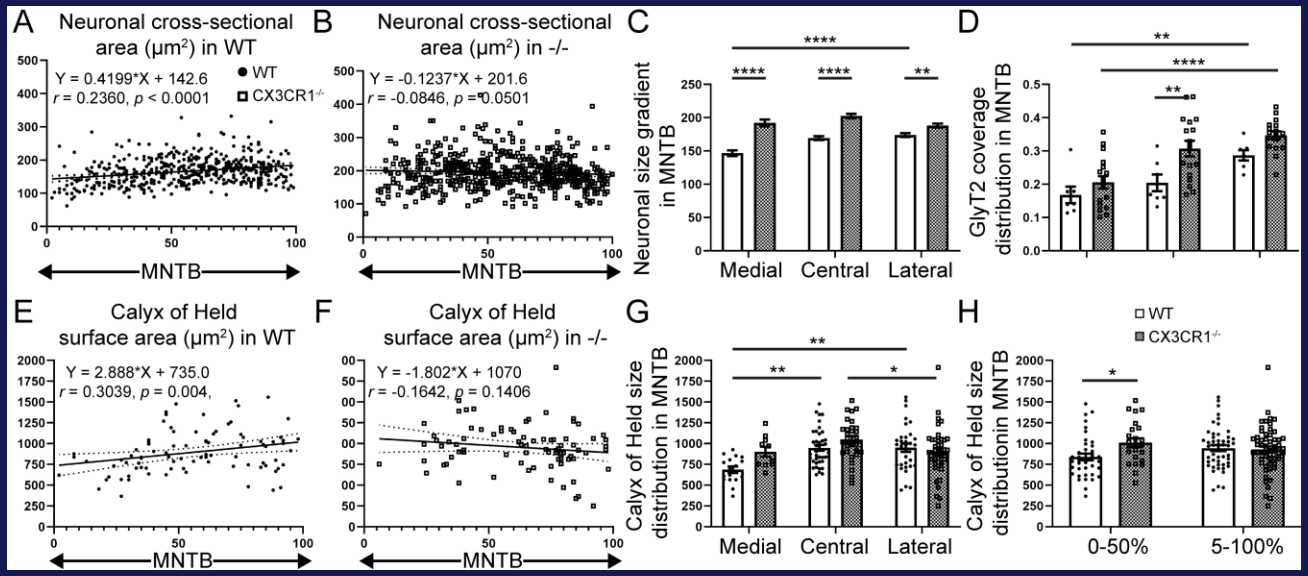
- GlyT2 is engulfed in microglia; lots of overlap with lysosomal marker CD68

Effects of CX3CR1 mutation on pruning



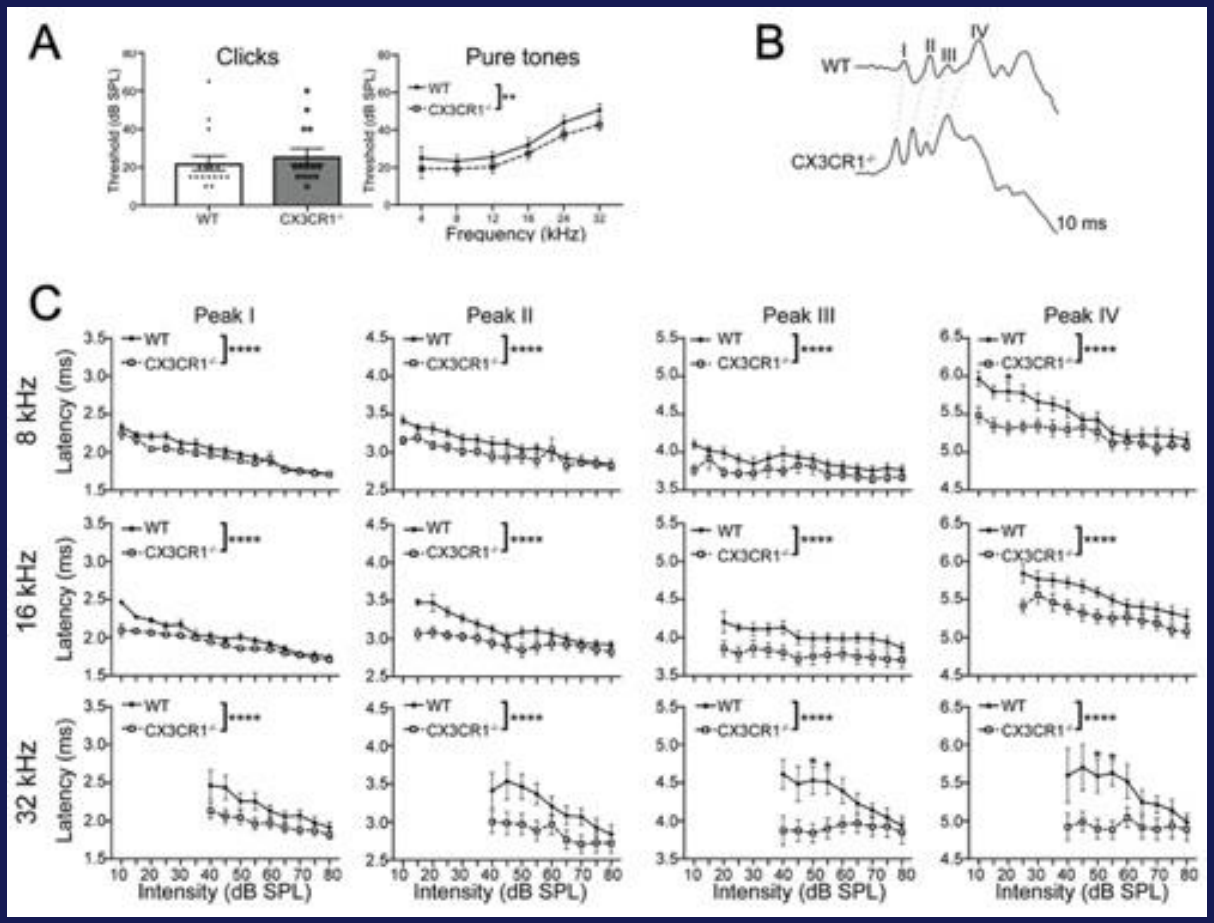
- Calyces reduced to monoinnervation as in controls

Effects of CX3CR1 mutation on MNTB gradients



- Gradient of cell body size is impaired in mutant animals. CX3CR1-/- seem to have larger sizes, particularly in medial region.
- Calyx gradient was also seen in WT animals; not in CX3CR1-/- mice. This was mainly because of larger calyces in medial region.

Effects of CX3CR1 mutation on ABRs



- *CX3CR1*$^{-/-}$ mice have normal click ABR thresholds and slightly **lower** tone thresholds than WT mice.

- ABR latencies are **shorter** in *CX3CR1*$^{-/-}$ mice than in WT mice.

Summary – Role of CX3CR1

- Does not account for effects of microglia depletion.
- Calyx pruning is not impaired, and GFAP is elevated.
- Suggests a role for CX3CR1 that opposes or impedes maturation.

Conclusions and future directions

- Microglia have important roles in maturation of auditory function. Alterations in microglia affect astrocyte maturation, pruning, and auditory function.

- Both peripheral and central effects are seen. Future studies will explore how these are distinct vs. related.

- Multiple signaling molecules are important for neuron-microglial communication. Which signaling pathways are important and how do they promote maturation?

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