Effects of Early Treatment With a Colony Stimulating Factor 1 Receptor Inhibitor are Corrected with Microglial Repopulation

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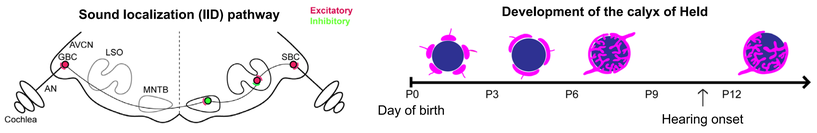
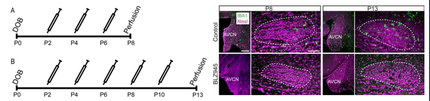
Introduction

Sound localization relies heavily on the speed, precision and reliability of acoustic signal propagation in the auditory brainstem. Communication between neurons and glia is required for the establishment of precise and fully functional neural circuits. In this project we investigated the role of microglia in auditory brainstem circuit assembly. We focused on the maturation of the pathway from the anteroventral cochlear nucleus (AVCN) to the medial nucleus of the trapezoid body (MNTB), which terminates in the calyx of Held. This projection matures during early postnatal development. MNTB neurons are initially polyinnervated, then excess synapses are pruned until a single input remains. We tested the role of microglia on synapse maturation and pruning in MNTB, and auditory function using microglial depletion and subsequent repopulation.

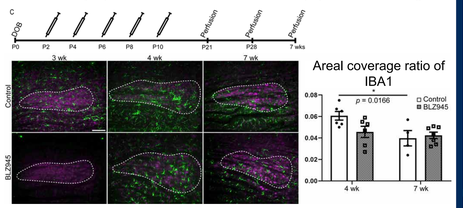


Methods

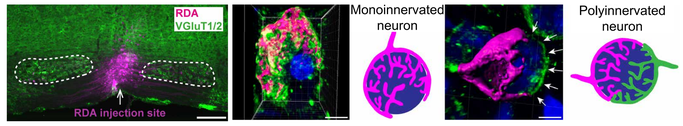


Microglia depletion. Microglia were pharmacologically depleted early in development using repeated injections of BLZ945, an inhibitor of colony stimulating factor 1 receptor (CSF1R), which is essential for microglia survival. BLZ945 dissolved DMSO was administered subcutaneously in C57BL/6 mice which were then perfused at P8 and P13, before and after hearing onset, respectively (A and B). DMSO-only injections were used as controls. Repopulation of microglia was investigated after cessation of treatment at P10.

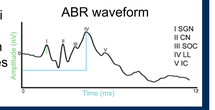
Immunohistochemistry. We stained brainstem sections for the synaptic proteins: vesicular glutamate transporter 1/2 (VGluT1/2), vesicular GABA transporter (VGAT), synaptophysin (Syn) and for the astrocytic markers: aldehyde dehydrogenase 1 family member L1 (ALDH1L1), S100 calcium-binding protein B (S100β) and glial fibrillary acidic protein (GFAP). Microglia were labeled with an antibody targeting a calcium binding protein specific for microglia (IBA1).



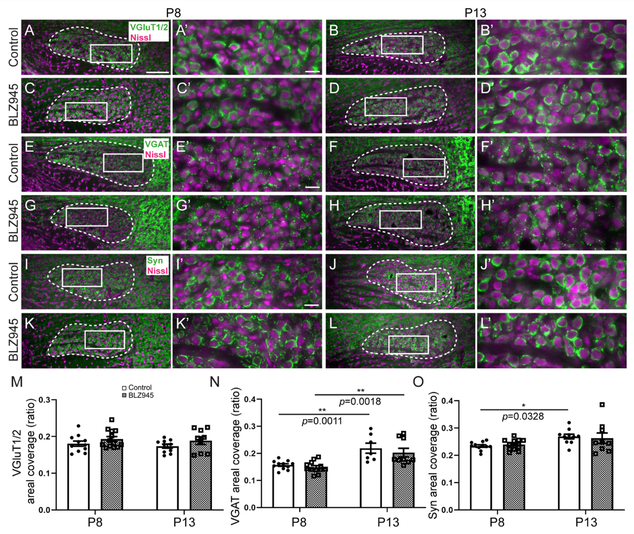
Assessment of polyinnervation. Ventral cochlear nucleus (VCN) axons were sparsely labeled using rhodamine dextran amine (RDA) electroporation in the ventral acoustic stria. Brainstems were sectioned and immunolabeled for the vesicular glutamate transporters 1 and 2 (VGluT1/2). Tissue was analyzed using confocal microscopy and Imaris software. Surfaces of RDA-labeled calyces of Held were reconstructed and their surface area and volume measured. VGluT1/2 staining was used to determine whether the neuron of interest received an additional calyceal input. For each MNTB neuron with an RDA-labeled calyx, we scored the neuron as monoinnervated if we found little or no presynaptic proteins outside the labeled calyx. If the MNTB neuron had presynaptic protein labeling outside the RDA-labeled calyx, we scored the neuron as polyinnervated.



Auditory brainstem recordings (ABRs). ABRs were performed on mice at 4 wk and at 7 wk of age. Stimuli were presented for 3 ms with sound level decreasing in 5 dB SPL steps from 80 to 10 dB SPL. ABRs were first recorded in response to100 µs click stimuli followed by 4, 8, 12, 16, 24, and 32 kHz tone stimuli. At each sound level, sample responses were averaged to attain an averaged response. Auditory thresholds, latencies and amplitudes were determined for peaks I - IV and compared between control and BLZ945 treated mice.



1. Effects of microglia depletion on synaptic protein expression in the MNTB.



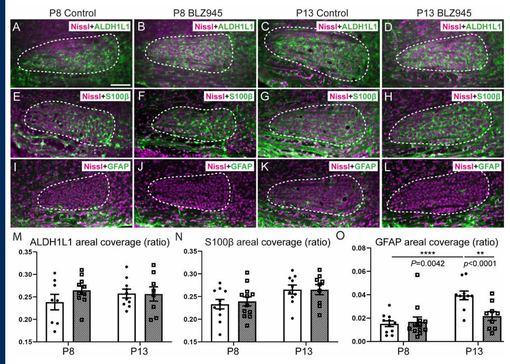
Tissue was stained for Nissl (magenta) and either VGLUT1/2, VGAT or Syn (green).

There was no significant difference between the expression levels of any of the three synaptic proteins tested in the MNTB (dashed line) of control and microglia depleted mice.

Some age related changes in VGAT and Syn expression was observed within a control and/or BLZ945 group.

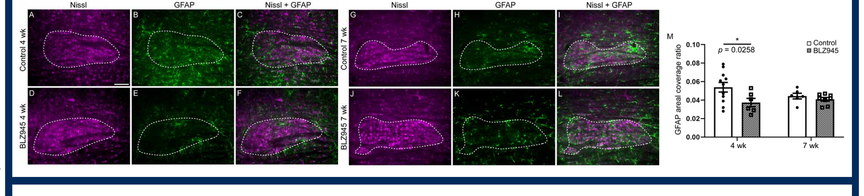
Scale bar in A = 100 µm and applies to panels A-L. Scale bar in A' = 20 µm and applies to panels A'-L1.

2. Effects of microglia depletion and repopulation on expression of astrocytic markers in the MNTB.



Depletion. No significant effect was observed on the areal coverage of either ALDH1L1 or S100β astrocytic markers in the MNTB. GFAP (mature astrocyte marker) expression **(I-L)** was significantly decreased in BLZ945 treated animals after the onset of hearing.

Repopulation. GFAP expression was significantly lower in BLZ945 treated than control mice at 4 wk but matched to that of control levels at 7 wk.

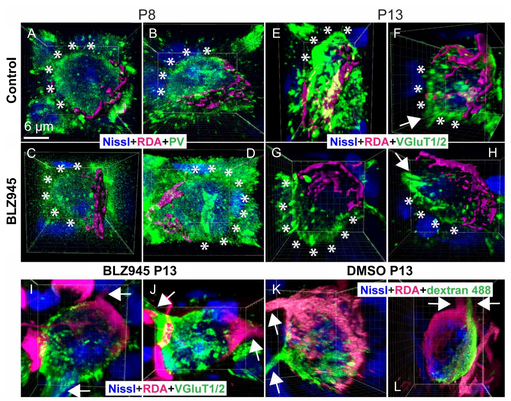


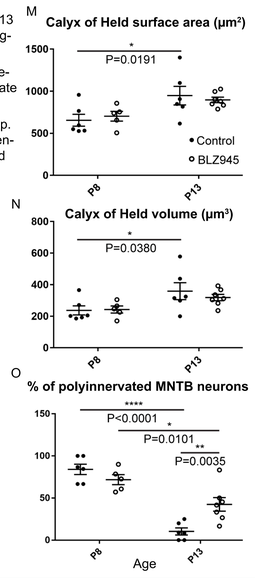
3. Microglia depletion increases the number of polyinnervated neurons in the MNTB.

Polyinnervated principal neurons were found in control and BLZ945-treated mice at both P8 and P13 (indicated with asterisks). Some examples of polyinnervation included visible axons of both converging calyces (arrows in **F, I-L**).

We electroporated three control mice at P13 with both RDA and Alexa 488 dextran at P13, which resulted in some cases of labeling of converging calyces of Held on the same neuron with two separate dyes **(K, L)**.

Surface area **(M)** and volume **(N)** of calyces of Held significantly increased with age in control group. **(O)** The relative number of polyinnervated neurons significantly decreased at P13 in both experimental groups but there were significantly more polyinnervated neurons remaining in microglia depleted mice.

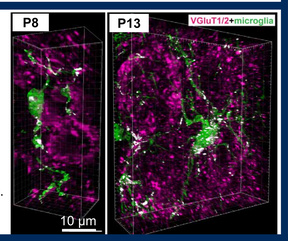




4. VGLUT1/2-stained synaptic proteins colocalize with microglial processes and are also found internalized by microglial cells.

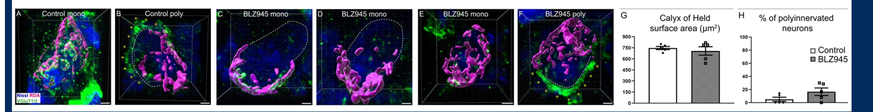
CX3CR1$^{+/GFP}$ microglia express EGFP. White patches indicate locations where EGFP (green) and VGLUT1/2 (magenta) overlap.

Colocalization is seen at microglia branches, which may be in contact with developing presynaptic terminals. Smaller regions of colocalization are also seen inside the microglial cell bodies.

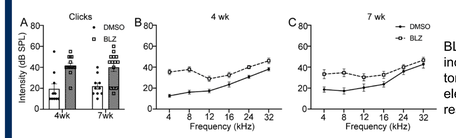


5. Polyinnervation of MNTB neurons after microglia depletion is temporary.

At P28, when microglial IBA1 expression in BLZ945 treated mice is comparable to control levels, majority of principal neurons in the MNTB were monoinnervated although we did encounter some neurons that were contacted by more than one calyx of Held.

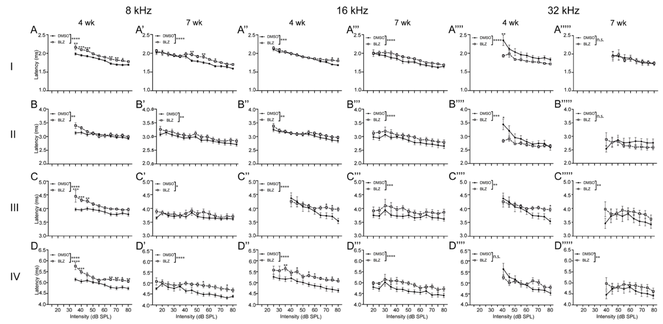


6. Temporary microglia depletion elevates auditory thresholds, increases ABR peak latencies and reduces amplitudes.

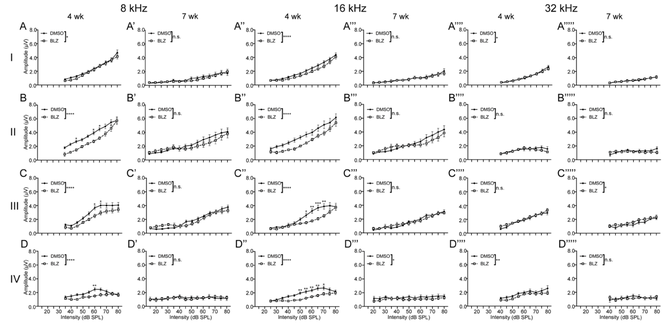


BLZ945 treatment early in development leads to a significant increase in **thresholds** in response to click and low frequency tone stimuli. By 7 weeks of age, microglia depleted mice still show elevated thresholds although significance is maintained only in response to 8 kHz.

Treatment with BLZ945 early in development resulted in elevation of some of peak latencies at various frequencies tested. Most of defects recovered by 7 wk.



Temporary injections of BLZ945 resulted in a significant decrease in amplitudes at most of the peaks and frequencies tested at 4 wk. By 7 wk of age, amplitudes were comparable to control levels.



CONCLUSIONS

- Treatment with BLZ945 during the early postnatal period eliminates microglia from the auditory brainstem.
- Microglia repopulate the auditory brainstem after the cessation of treatment.
- Elimination of microglia results in delayed expression of GFAP astrocytic marker, suggesting a function for microglia in maturation of astrocytes.
- Microglia depletion early in development leads to a temporary increase in polyinnervation in MNTB, suggesting a role for microglia in synaptic pruning.
- Microglia contact and internalize VGluT1/2-positive terminals in the MNTB of CX3CR1 heterozygous mice.
- Elimination of microglia early postnatally leads to temporary defects in auditory function in adult mice.

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