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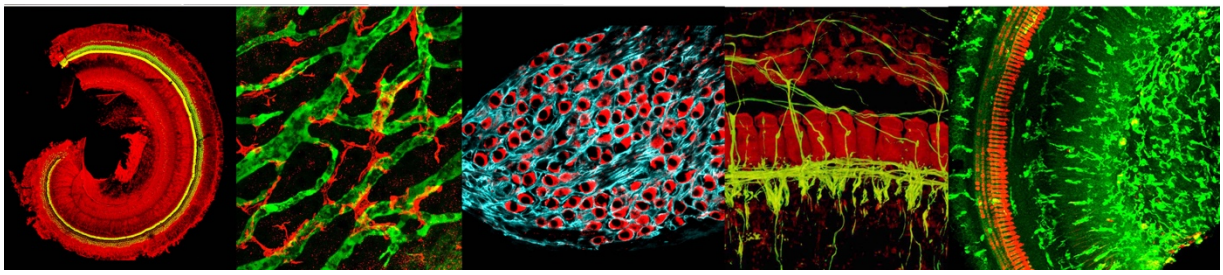
Research Interests:



Cellular and Molecular Mechanisms of Sensorineural Hearing Loss

Genetically modified mouse models and human inner ear tissues have been used for understanding the cellular and molecular mechanisms of auditory nerve survival and degeneration in several pathological conditions, including aging, exposure to noise and ototoxic drugs and genetic defects. We focus on the functional roles of neural crest cell associated transcription factors, RNA binding proteins, complement system, and their related regulatory networks for enhancing remyelination, preventing or protecting the auditory nerve from degeneration and promoting auditory nerve survival and functional recovery after cochlear injury. This translational research program provides an outstanding training opportunity for (1) cutting-edge cellular and molecular procedures in hearing research such as molecular imaging of living cochlear cells, 3D cell culture assay, RT-qPCR, NanoString and RNA-seq analysis of the degenerative auditory nerve; (2) collection and examination of mouse and human temporal bone tissues; (3) contemporary histopathological and high-resolution imaging techniques to assay human and animal inner ear specimens; and 4) evaluation of auditory function using electrophysiological techniques including the measurements of auditory nerve compound action potentials, spontaneous activities of single auditory nerve fiber, and auditory brainstem responses. The study has been supported by grants from NIH/NIDCD for more than 15 years (Lang PI).

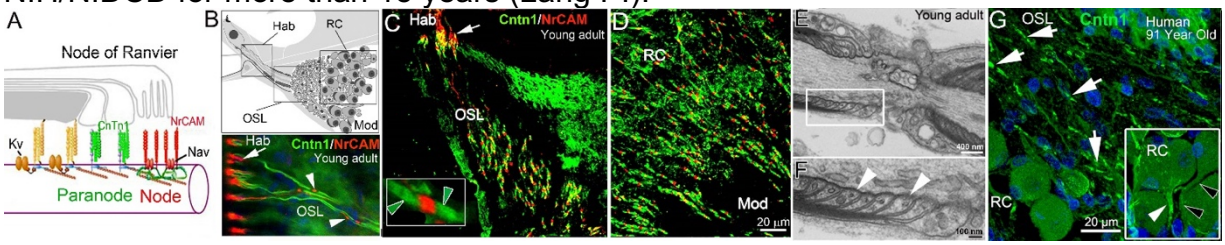


Figure 1. Paranodal structures in the auditory nerve of young adult mice. A: Molecular organization of the node of Ranvier (voltage-gated sodium channel, Nav; voltage-gated potassium channel, Kv). The schematic was modified from Girault and Peles (2002). **B-D:** A schematic (the top panel in **B**) shows the pathway of the spiral ganglion neuron (SGN) neurites at the habenula opening (Hab), osseous spiral lamina (OSL), Rosenthal's canal (RC) and modiolus (Mod). Heminodes (white arrows) and nodes (white arrowheads) were stained with Cntn1 (green arrowheads) and NrCam (red) at the Hab (**B,C**), OSL (**B,C**), RC (**D**) and Mod (**D**) of adult CBA/CaJ mice. The boxed area in **C** shows an enlarged node image. **E-F:** Features of a node of Ranvier in RC of another mouse. **F** is the enlarged image of the boxed area in **E** showing the septate-like axoglial junctions (arrowheads) that connect the paranodal loops with axolemma. **G:** Paranodes (arrows) in the auditory nerve of a 91-year-old donor. Insert shows Cntn1 in the fiber region preceding the SGN soma (white arrowhead) and another similar fiber lacking Cntn1 reactivity (black arrowheads).

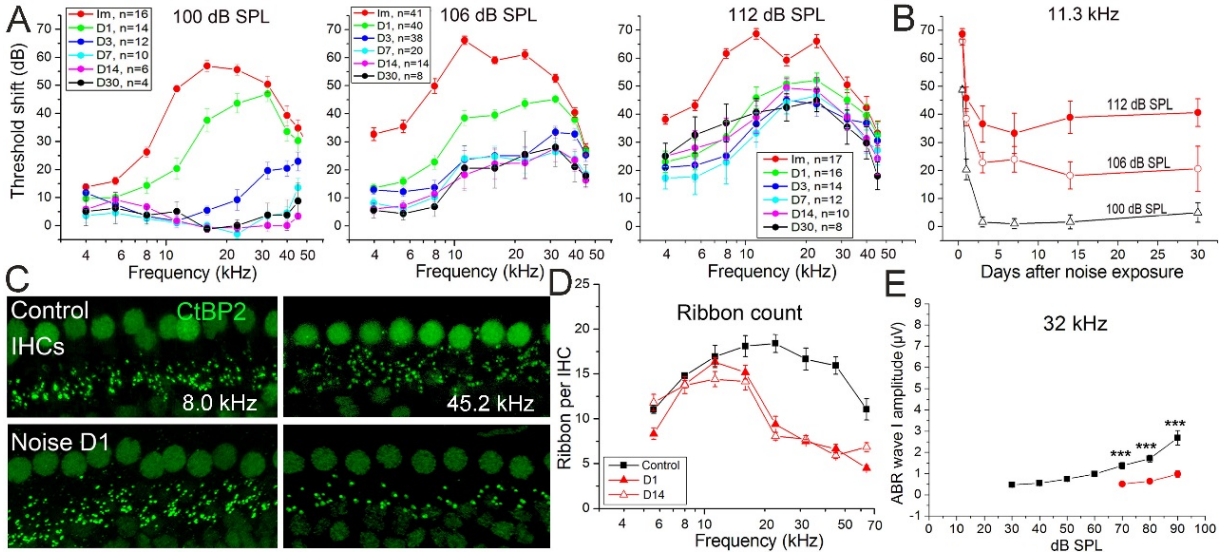


Figure 2. Noise exposure induced pathophysiological alterations in young adult CBA/CaJ mice. A,B: Mean auditory brainstem response (ABR) thresholds revealed a dynamic alteration of auditory function immediately (Im), and at 1, 3, 7, 14 and 30 days (D1-D30) after noise exposure. Adult CBA/CaJ mice were exposed to an octave-band noise (8-16 kHz) at 100, 106 or 112 dB SPL for two hours. **C,D:** There was a marked loss of ribbon synapses (stained with anti-CtBP2 antibody, green) under inner hair cells (IHCs) at frequency regions above 22.3 kHz at both D1 and D14 after 106 dB SPL noise exposure. **E:** ABR Wave I amplitudes were reduced at 32 kHz at D3 after 106 dB noise exposure.

Adult Stem/Progenitor Cell and Auditory Nerve Regeneration/repair

Our recent research on isolation and characterization of adult neural stem/progenitor cells from the adult mouse auditory nerve is aimed at replacing damaged spiral ganglion neurons (SGNs), preventing SGN degeneration and promoting auditory functional recovery. Several lines of studies are ongoing with a focus on remyelination and de-differentiation of adult glial cells after acute auditory nerve injury resulting from noise- or ototoxic drug-exposure. A variety of advanced methods are employed to 1) isolate and expand neural stem/progenitor cells using neurosphere culture assay and auditory nerve micro-dissection; 2) purify and characterize neural stem/progenitor cells using transgenic mouse models and fluorescence-activated cell sorting; 3) identify the molecular characteristics of neural stem/progenitor cells using next generation sequencing, gene expression profiling at the single cell level (e.g., single cell RNA-seq), complementary proteomics assays and super resolution imaging analysis; and 4) directly evaluating functional integration of the transplanted stem cells using

microsurgery and well-established mouse models of auditory nerve degeneration. The research is supported in part by an R01 and an R56 from NIH/NIDCD.

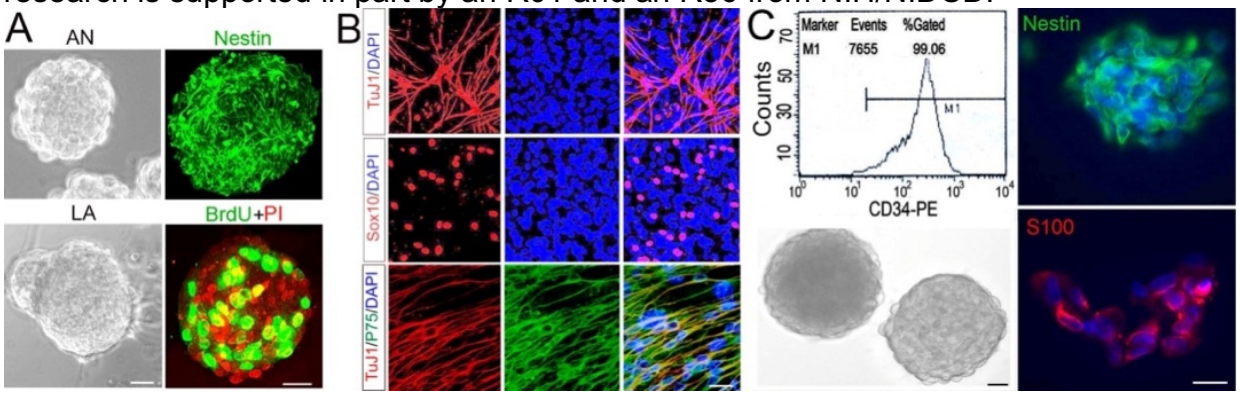


Figure 3. Neural crest-derived stem cells (NCSCs) generated from adult mouse cochlear tissues and human CD 34$^+$ bone marrow cells. **A:** Adult mouse cochlear tissues isolated from auditory nerve (AN) and cochlear lateral wall (LA) give rise to self-renewing spheres. A majority of cochlear sphere-derived cells express the NCSC marker nestin and cell proliferation marker BrdU. **B:** Cochlear NCSCs generate cells expressing the neuronal marker TuJ1 and other neural crest lineage cell markers such as Sox10 and P75 under a neural differentiation condition. **C:** Purified CD34$^+$ cells from adult human bone marrow give rise to self-renewing spheres expressing NCSC markers. Human CD34$^+$ cells were isolated from bone marrow of a 21 year-old donor.

Peripheral auditory system deficits and autism-like behaviors

This is an exciting and newly developed project, which addresses the novel hypothesis that abnormal macrophage related activities, resulting from gene deficiency, lead to hearing loss and that these changes may be associated with communication impairment in Autism Spectrum Disorder and other neurodevelopmental disorders. For example, mutations or deletions in the *MEF2C* gene has recently been linked to Autism Spectrum Disorder. In collaboration with Dr. Christopher Cowan from the Department of Neuroscience and Dr. Bärbel Rohrer from the Department of Ophthalmology, our experiments revealed that Mef2c is highly expressed in cochlear macrophages in postnatal mice and that Mef2c hypofunction results in auditory nerve functional decline and hearing loss. This research is now supported by a research grant from Simmons Foundation (SFARI Pilot Award; MPI; Cowan/Lang/Roehrer).

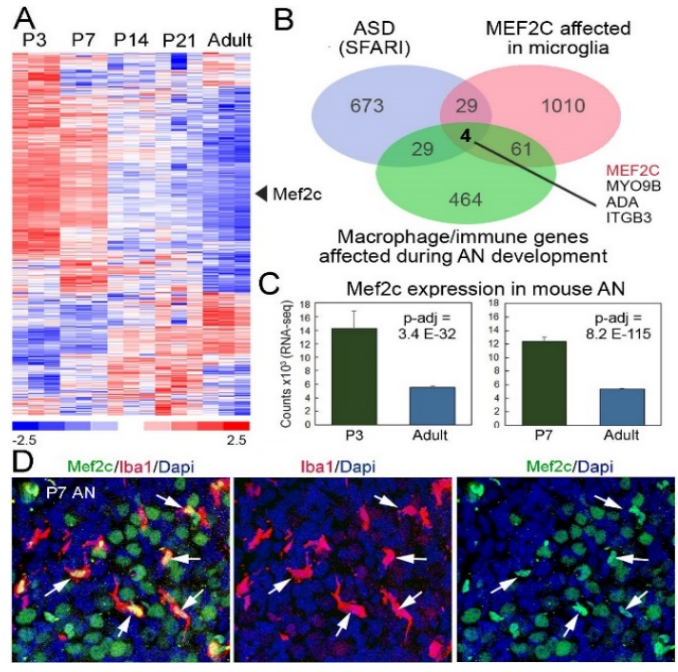


Figure 4. Expression of MEF2C in cochlear macrophages within the auditory nerve (AN) of postnatal mice. (A) Gene expression profiles of 558 macrophage/immune-related genes differentially expressed in AN between P3 and young adult CBA/CaJ mice (log FC >0.3; p-adjusted <0.05). Macrophage/immune-related genes were compiled from the Gene Ontology Database. (B) Venn diagram depicting

overlap among 1) ASD-risk genes (SFARI categories 1-4; https://gene.sfari.org), 2) human orthologs of *Mef2c*-dependent genes identified in mouse microglia (Deczkowska et al., 2017), and 3) human orthologs of macrophage/immune-related genes outlined in (A). *MEF2C* was one of four genes identified in each analysis. (C) Expression of Mef2c mRNA is higher in postnatal AN compared to young adult AN. Graph depicts RNA-seq standardized counts. (D) Expression of Mef2c (green) in macrophages in mouse P7 AN. Macrophages were identified by immunostaining for Iba1 (red).

Selected Recent Publications:

1. Liu T, Li G, Noble KV, Li Yong, Barth JL, Schulte BA, Lang H. Age-Dependent Alterations of Kir4.1 Expression in Neural Crest-Derived Cells of the Mouse and Human Cochlea. Neurobiology of Aging. Neurobiol Aging. 2019;18; 80:210-222. PMCID: PMC6679794

2. Noble KV, Liu T, Matthews L, Schulte BA, Lang H. Age-Related Alterations in Resident Immune Cells of the Human Cochlea. Front Neurol. 2019; doi.org/10.3389/fneur.2019. 00895

3. Noble KV, Reyzer ML, Barth JL, McDonald H, Tuck M, Schey KL, Krug EL, Lang H. Use of Proteomic Imaging Coupled with Transcriptomic Analysis to Identify Biomolecules Responsive to Cochlear Injury. Front Mol Neurosc. 2018 doi.org/10.3389/fnmol. 2018.00243

4. Panganiban CH, Barth JL, Darbelli L, Xing Y, Zhang J, Li H, Noble KV, Liu T, Brown LN, Schulte BA, Richard S, Lang H. Noise-Induced Dysregulation of *Quaking* RNA Binding Proteins Contributes to Auditory Nerve Demyelination and Hearing Loss. The Journal of neuroscience. 2018; 38(10):2551-2568. PMCID: PMC5858596

5. Brown LN, Xing Y, Noble KV, Barth JL, Panganiban CH, Smythe NM, Bridges MC, Zhu J, Lang H. Macrophage-Mediated Glial Cell Elimination in the Postnatal Mouse Cochlea. Frontiers in molecular neuroscience. 2017; 10:407. PMCID: PMC5770652

6. Lang H, Nishimoto E, Xing Y, Brown LN, Noble KV, Barth JL, LaRue AC, Ando K, Schulte BA. Contributions of Mouse and Human Hematopoietic Cells to Remodeling of the Adult Auditory Nerve After Neuron Loss. Molecular therapy. 2016; 24(11):2000-2011. PMCID: PMC5154482

7. Lang H, Xing Y, Brown LN, Samuvel DJ, Panganiban CH, Havens LT, Balasubramanian S, Wegner M, Krug EL, Barth JL. Neural Stem/Progenitor Cell Properties of Glial Cells in the Adult Mouse Auditory Nerve. Scientific reports. 2015; 5:13383. PMCID: PMC4549618

8. Hao X, Xing Y, Moore MW, Zhang J, Han D, Schulte BA, Dubno JR, Lang H. Sox10 Expressing Cells in the Lateral Wall of the Aged Mouse and Human Cochlea. PloS one. 2014; 9(6):e97389. PMCID: PMC4041576

9. Stevens SM, Xing Y, Hensley CT, Zhu J, Dubno JR, Lang H. Heptanol Application to the Mouse Round Window: a Model for Studying Cochlear Lateral Wall Regeneration. Otolaryngology--head and neck surgery 2014; 150(4):659-65. PMCID: PMC4090013

10. Yuan Y, Shi F, Yin Y, Tong M, Lang H, Polley DB, Liberman MC, Edge AS. Ouabain-Induced Cochlear Nerve Degeneration: Synaptic Loss and Plasticity in a Mouse Model of Auditory Neuropathy. Journal of the Association for Research in Otolaryngology. 2014; 15(1):31-43. PMCID: PMC3901858

Complete List of Published Work in MyBibliography:
https://www.ncbi.nlm.nih.gov/myncbi/browse/collection/43924250/?sort=date&direction=descending

The journey is more important than the endpoint. In science or life, little time is spent at 28,000 feet.

-Louis Reichardt