Issue No. 1 | October 2021

Creighton University School of Medicine

Translational Hearing Center
Creighton University
Boys Town National Research Hospital
University of Nebraska Medical Center

Message From Our Program Director

Welcome to the Translational Hearing Center, an NIH/NIGMS-funded Center of Biomedical Research Excellence (CoBRE) committed to developing a cadre of translational auditory/vestibular research scientists developing biomedical and ototherapeutic solutions that preserve or restore hearing and vestibular function. We currently support 3 Research Projects Leaders (each at $200k per year for up to three years) and 3 Pilot Project Awardees (each at $50k for 1 year). More details of these projects are within this newsletter. The Center has 2 specialized Research Cores, the Auditory & Vestibular Technology Core, and the Drug Discovery & Delivery Core, to assist researchers in meeting their research goals. Updates can be found online here: Translational Hearing Center | School of Medicine | Creighton University *

Hearing loss in infants and children results in delayed acquisition of listening and spoken language skills critical for academic achievement and maximal career trajectories. In the aging population, hearing loss and vestibular deficits without appropriate rehabilitation accelerates aging and cognitive decline.

The Center is located at Creighton University, with nearby Boys Town National Research Hospital (BTNRH) and the University of Nebraska Medical Center (UNMC) as institutional partners. Our Overall goal is to build a critical mass of academi. The Translational Hearing Center is open to all researchers at Creighton, BTNRH and UNMC developing strategies to preserve or restore hearing and vestibular function. The Center is an inter-departmental, inter-school and inter-institutional collaborative research environment.

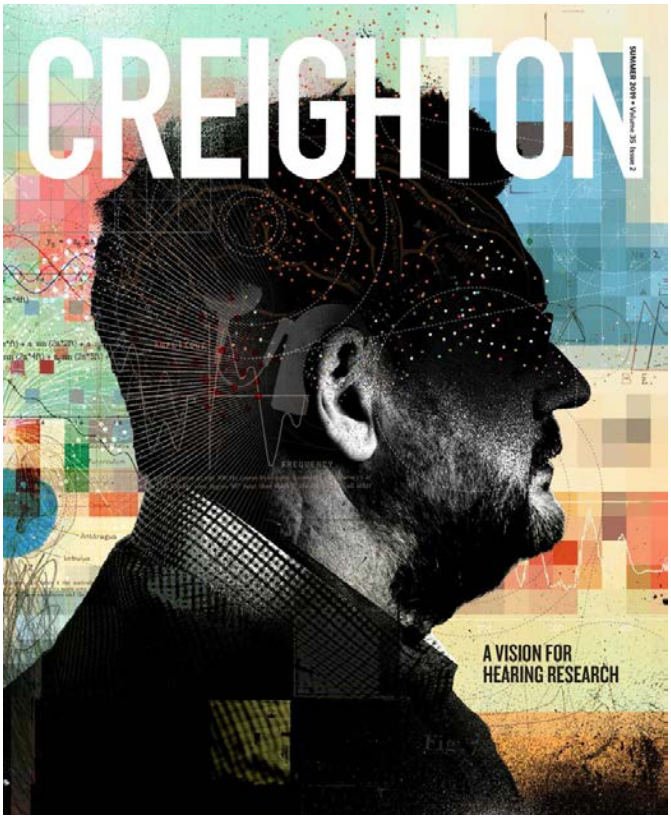
In this issue, we will highlight the aims of the center, individual project summaries of Research Projects Leaders and the Pilot Project Awardees, as well as a look back at the 2021 Bellucci Symposium. We are thrilled to share this progress with you.

*Website still under construction

Peter S. Steyger, PhD Director, Translational Hearing Center

Table of Contents

Featured articles



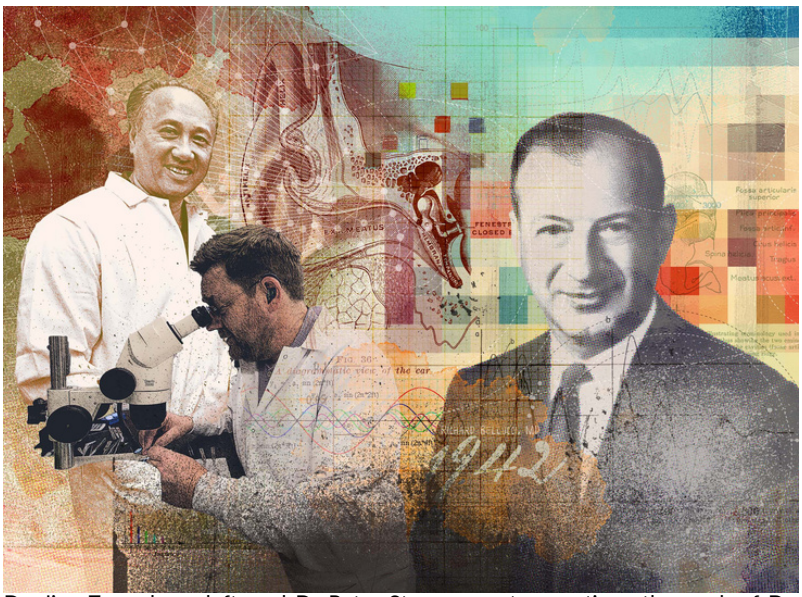
The Center was featured in the summer issue of Creighton Magazine 2019

Aim 1: Develop the infrastructure and expertise base for translational auditory and vestibular research

COBRE funding will enable an Administrative Core to provide a unique, transformational research environment for junior investigators to translate their basic science discoveries into therapeutic strategies that preserve or restore hearing and vestibular function. The Administrative Core will coordinate interactions between project leaders with their Mentors, and the External Advisory Committee. The Administrative Core will develop a Drug Discovery & Delivery Core to coordinate the necessary drug screens, production of optimized lead compound derivatives and their delivery to the inner ear and associated central auditory neural pathways, as well as an Auditory & Vestibular Technology Core to validate the efficacy of lead candidate ototherapeutic hits.

Aim 2: Build a critical mass of funded investigators leading translational auditory and vestibular research.

Our researchers examine both peripheral and central mechanisms of hearing loss and/or vestibular dysfunction, and identify pharmacotherapeutic strategies that preserve or restore these sensory functions, with multiple levels of research funding for investigators. We have an outstanding Mentoring Plan for junior investigators, complementing their expertise with senior investigators as Internal Mentors and biostatistical support, as well as outside investigators with translational and clinical expertise as External Mentors. Additional mentoring is provided by Research Core personnel, grantsmanship classes and Mock Study Sections of proposals prior to submission for federal review. Evaluations of research progress and other Center activities are also key to optimize the Center's success. The Center will benefit from the burgeoning translational research environment in Omaha, Nebraska. Future plans call for expanding the Center to submit Investigational New Drug applications, safety and efficacy studies, as well as human studies and clinical trials in partnership with patient populations served by Creighton University's academic medical center, Catholic Health Initiatives (CHI) Health system, BTNRH and UNMC



Dr. Jian Zuo, above left, and Dr Peter Steyger, center, continue the work of Dr. Bellucci at Creighton through hearing research

Research Cores

Auditory & Vestibular Technology (AVT) Core

The Auditory & Vestibular Technology (AVT) Core will provide instrument and technical support to Research Project Leaders and principal investigators associated with the Translational Hearing Center to conduct auditory and vestibular research across the full range of experimental model systems, from single molecule analysis to whole organism models. Between project leaders with their Mentors, and an External Advisory Committee. The AVT Core facilities and its personnel can also enhance the scope of technical options, foster collaboration for multidisciplinary research, play an important role to prepare talented new investigators to submit new research proposals, and in the technical training of graduate students and post-doctoral fellows. The AVT Core will continue to incorporate new methodologies as Core options to provide leading-edge technologies to center investigators.

Meet the AVT Core Directors

David Z. He, MD, PhD
Professor of Neuroscience

Michael Nichols, PhD
Professor of Physics

D. David. Smith, PhD
Professor of Biochemistry

The AVT core offers a broad range of services:

Electrophysiology (directed by David He) Our electrophysiology facilities offer both non-invasive and invasive electrophysiological procedures (cochlear potentials). Current instruments include: two complete sets of TDT RZ6 for measuring auditory and vestibular function (evoked potentials and otoacoustic emissions) from rodents. In addition, two Axopatch 200B integrating patch clamp amplifiers and 1440A Digidata boards a record cochlear potentials (CM, CAP, EP) from small mammals and microphonic responses from zebrafish.

Molecular Biology (directed by David He) The Molecular Biology facility is equipped for techniques commonly used for molecular biological investigations. In addition to equipment for genotyping, qPCR and *in situ* hybridization, the facility also has a 10x Genomics for single-cell RNA-sequencing. Services offered by this core include PCR, q-PCR, *in situ* hybridization, RNAScope *in situ* hybridization, RNA extraction, quality examination, and preparation for RNA-seq and DNA-sequencing.

Advanced Imaging (directed by Michael Nichols) The current instruments that the CoBRE Confocal Core currently has are the Zeiss 700 confocal microscope as well as a Zeiss 710 confocal microscope including the nessecary software for imaging.

Mass Spectrometry (directed by David Smith) Current instruments includes a Q Exactive™ hybrid Quadrupole-Orbitrap™ mass spectrometer from Thermo Scientific™. This is a remarkable instrument with a resolving power of up to 140,000 FWHM and a mass accuracy of less than 1 ppm. For LC-MS experiments, the Q Exactive™ is coupled to a Vanquish™ Flex Binary UHPLC System comprised of a biocompatible, binary, high pressure gradient mixing pump, autosampler and heated column compartment. The Q Exactive™ is capable of LC-MS-MS experiments employing collision-induced dissociation (CID) or higher-energy collisional dissociation (HCD) of mass ions to obtain amino acid sequences and quantitative data from isobaric tagged proteolytic fragments. Alternatively, an EASY-nLC™ 1200 instrument, capable of flow rates as low as 100 nL/min, is available for nLC-MS experiments. Data analysis will be aided by the Proteome Discoverer 2.3, Tracefinder 4.1 and Compound Discoverer 3.0 software packages.

Drug Discovery & Delivery (DDD Core)

The Drug Discovery & Delivery Core that will coordinate the necessary drug screens, production of optimized lead compound derivatives and their delivery to the inner ear and associated central auditory neural pathways, as well as coordinating with the Auditory & Vestibular Technology Core to validate the efficacy of lead candidate ototherapeutic hits. Our experienced DDD core staff possess all the professional skills and knowledge base required to undertake essential drug discovery and delivery programs to drive them from target validation, through hit identification, hit-to-lead determination and lead optimization to develop a high-quality and well-characterized drug candidate (Fig. 1).

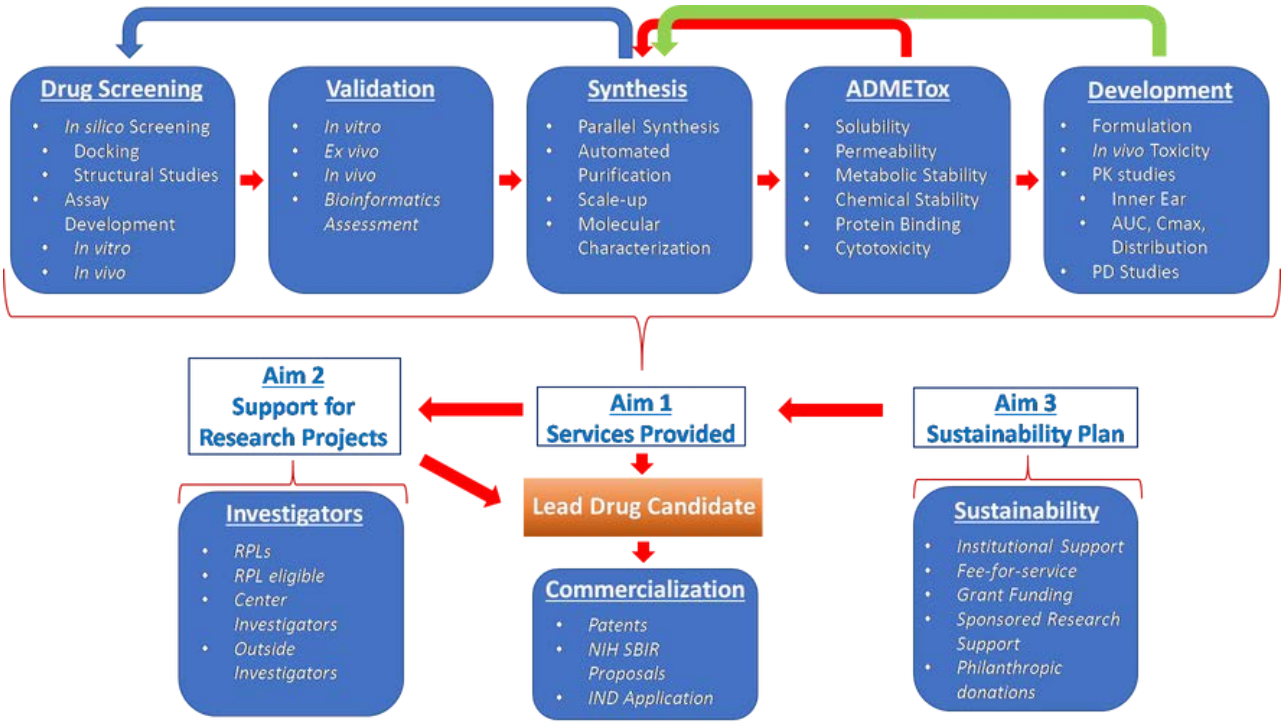


Fig. 1. DDD Core workflow and services (Aim 1) utilized to discover and develop validated and well-characterized drug candidates to support research projects and a sustainable core.

We offer screening approaches to identify hit compounds, including fragment-based screens, peptide-based drug screening, high-throughput screening and complementary in silico approaches. We also provide hit-to-lead services where high-throughput chemistry enables the rapid design and synthesis of libraries of novel compounds. Successive use of *in silico* techniques and medicinal chemistry, enables our team to produce high quality leads for further optimization. We will develop in house solutions to deliver robust data for complex projects to fit your needs. Our solutions include a broad range of biophysical, biochemical, cellular and live cell imaging platforms as well as translational assays. We can integrate early ADMETox profiling, PK/PD correlations, to predict optimal human dose.

Meet the DDD Core Directors

Director:
Dr. Jian Zuo
Chair BMS

Co-Director:
Dr. Sandor Lovas
Professor BMS

Co-Director:
Dr. Alekha Dash
Chair Pharmacy Sciences

Core Manager:
Dr. Gopal Jadhav
Assistant Professor

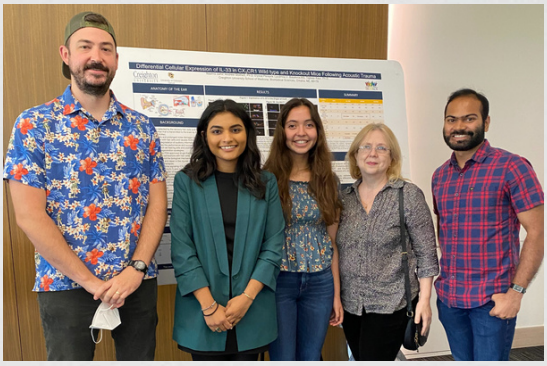
Research Project Leaders

Jeffery North

Aminoglycosides (AG) have broad antibiotic spectra against aerobic gram-positive and gram-negative bacteria as well as mycobacterial pathogens. AG toxicities include kidney tubular necrosis, vertigo, and, most notably, hearing loss. AG are used to treat multidrug-resistant tuberculosis (MDR-TB) and *Mycobacterium abscessus* complex (MABSC) infected patients (e.g. cystic fibrosis, bronchiectasis or chronic obstructive pulmonary disease). Studies have shown that 55-58% of patients infected with MDR-TB who received amikacin as part of their therapy, experienced hearing loss due to its ototoxic effects. Likewise, up to 27% of cystic fibrosis patients infected with *M. abscessus* who received AG therapy experienced hearing loss. To date, there is no FDA-approved therapy available to prevent or treat hearing loss. A reduced reliance on AG therapy in mycobacterial infections will minimize hearing loss for patients infected with drug-resistant *M. tb* strains and nontuberculous mycobacteria. We have discovered a novel series of small molecules (indole-2-carboxamides and acetamides) that have potent activity against a panel of mycobacteria. Two of our lead candidates had poor oral absorption yet achieved efficacy in a mouse model of *M. abscessus* infection. We propose to discover and develop anti-mycobacterial inhibitors with potent activity with improved pharmacokinetic profiles and no ototoxicity. Using ligand-based drug design and computer aided drug design. *In vitro* bioavailability and toxicity profiles will also be determined. Finally, potent anti-NTM agents with optimized bioavailability and toxicity profiles will be subjected to macromolecular mechanism of action studies, ensuring future compounds remain on target as MmpL inhibitors. Our lab has developed novel MmpL3 (Mycobacterium membrane protein Large 3) inhibitors showing excellent promise for the treatment of mycobacterial infections, including *Mycobacterium tuberculosis*, the causative pathogen for tuberculosis. The design and synthesis of a novel series of MmpL3 inhibitors led us to identify a number of analogs with 0.06-8 µg/mL potency against various slow- and fast-growing mycobacterial pathogens of clinical interest. Consistent with earlier findings in *M. tuberculosis*, our preliminary evidence indicates that ICs (Indole-2-carboxamide) kill *Mycobacterium abscessus* isolates through the inhibition of the essential mycolic acid transporter, MmpL3. Mycolic acids, which are long α-alkylated β-hydroxylated fatty acids, are primary constituents of the mycobacterial outer membrane (also referred to as mycomembrane) and inhibition of translocation across the plasma membrane through the inhibition of MmpL3 has a rapid bactericidal effect on the cells. We believe MmpL3 inhibitors are an important discovery of a new chemotype that can be used for the treatment of mycobacterial infections.

Tejbeer Kaur

Hidden hearing loss" is damage to the cochlear nerve fibers connecting the sensory cell receptors of the inner ear to the brain, that occur before changes in hearing thresholds, and is likely a major contributor to difficulties understanding speech in a noisy environment as well as tinnitus and hyperacusis. Tinnitus is the ringing noise that some people experience in their ears that others cannot hear which is caused by underlining conditions such as age-related hearing loss. Hyperacusis is when people experience normal noises as being too loud and loud noises cause extreme pain and discomfort. We will to examine the effect of fractalkine exposure and macrophage depletion in preserving or restoring nerve fiber connections after noise trauma. Such knowledge will advance our understanding on the role of immune system in damaged ears and is essential to developing novel therapeutic strategies to reconnect nerve fibers to sensory cells and restore hearing function.



Pictured is Tejbeer Kaur's lab team

Padmashri Ragunathan

Exposure to alcohol during pregnancy produces fetal alcohol spectrum disorders (FASD) that are associated with sensory and cognitive deficits. Individuals with FASD have impaired auditory processing and also frequently exhibit atypical auditory behaviors. It is therefore important to determine the molecular mechanisms that govern auditory processing in normal and developmentally abnormal brain. We will examine auditory processing in mice prenatally exposed to alcohol, perform in vivo imaging in the primary auditory cortex to track AMPARs α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid.) and dendritic spines over days, and perform electrophysiological recordings to study AMPAR-mediated synaptic transmission. The goal of this study is to provide a mechanistic basis for the altered auditory processing observed in FASD and examine the therapeutic potential of the BDNF-TrkB (Brain-Derived Neurotrophic Factor-tyrosine kinase receptor B) signaling to preserve or restore central auditory processing following prenatal alcohol exposure.

Pilot Project Awardees

Annemarie Shibata

Series bacterial infections are often treated with aminoglycoside antibiotics that are associated with hearing loss in up to 20-50% of recipients. Hearing loss in young children can delay language, social, and cognitive development. The socioeconomic cost of hearing loss makes the development of effective treatments critically important. To develop novel therapeutics that prevent or ameliorate ototoxicity, the molecular mechanisms regulating the earliest stages ototoxicity in the cochlea must be identified and effectively targeted. Inflammatory immune responses can contribute to the hearing loss caused by aminoglycoside antibiotic treatments. Long non-coding RNA (lncRNA) are RNA transcripts longer than 200 nucleotides that do not code for protein. LncRNAs are emerging as important tissue-specific regulators of gene transcription and translation. We have identified lncRNAs that regulate the transcription of inflammatory genes following activation of immune cells with the gram-negative bacteria immunogen, lipopolysaccharide (LPS). Macrophages and microglia are important immune cells that respond quickly to bacterial infection and LPS. We have recently shown that Nostrill (iNOS Transcriptional Regulatory Intergenic LncRNA Locus) is upregulated in LPS-stimulated microglia in culture and in the cortical tissue of LPS injected mice. Silencing of Nostrill decreased inducible nitric oxide synthase (iNOS) expression, nitric oxide and MCP-1/CCL2 production, and microglial neurotoxicity. Resident macrophages, microglial-like cells (MLCs) and lymphocytes are found throughout the cochlea and vestibular organs of rodents and humans. Interestingly, systemic exposure to LPS induces cochlear immune responses that upregulate nitric oxide and MCP-1/CCL2 production. This likely generates proinflammatory processes that exacerbate the ototoxicity of aminoglycoside antibiotics. These processes also influence endocytotic and transcytotic trafficking of aminoglycosides. Our preliminary data demonstrate that Nostrill and other lncRNAs are upregulated in the cochlea of LPS-injected mice. We hypothesize that lncRNAs, such as Nostrill, act as primary regulators of gene transcription controlling cochlear immune responses and are potential therapeutic targets for early inflammatory events in the cochlea that exacerbate hair-cell loss upon aminoglycoside treatment. To address this hypothesis *in vivo*, LPS- treated mice will be used to identify differentially expressed lncRNAs in immune-responsive cells of the cochlea (Aim 1) and the biological function of differentially expressed lncRNAs in the mechanism of proinflammatory responses will be elucidated using in *vitro* and *ex vivo* methods (Aim 2). The results of these studies will be directly applicable to understanding the mechanisms of systemic inflammation that contribute to aminoglycoside-induced ototoxicity and to the identification of novel lncRNA therapeutic targets for ameliorating hair cell and hearing loss.

Gwen King

Age-related changes increase the risk of hearing loss as well as cognitive decline in humans. Klotho is an age-regulated protein expressed by two similar tissue types, the brain's choroid plexus and the cochlea's stria vascularis. Both the brain and the cochlea show decreased function under klotho-deficient conditions. Our pilot data suggest that Klotho affects cells throughout the brain because it is critical to choroid plexus epithelial cell activities. Klotho-deficient choroid plexus epithelial cells have dramatically decreased expression of transporters critical to cellular function. Choroid plexus epithelial cells provide barrier protection, nutrients, and signal transduction support to the entire brain. The same can be said for the stria vascularis of the cochlea that secretes and maintains the ionic concentration into the endolymphatic cochlear duct and the sensory hair cells required for mechanoelectrical transduction essential for auditory perception. The long-term goal of this pilot proposal is to understand the hearing-related functional consequences of klotho-deficiency in the cochlea. We will use mouse models to interrogate the central hypothesis that klotho-deficiency causes profound dysfunction of the stria vascularis to induce sensorineural hearing loss. In Aim 1, we will use mouse models to determine the role of klotho-deficiency on cochlear structure and function. Aim 2 will focus on the role of oxidative stress in klotho-deficient cochlear phenotypes. The significance of the proposal lies in contributions to understanding the basic biology of klotho as a protein critical for normal cochlear function. The proposal is innovative in approach to study hearing loss induced by loss of stria vascularis function driven by an age-downregulated protein, klotho.

Brian North

Age-related hearing loss (ARHL) is the third most prevalent chronic disease associated with older adults and contributes to social isolation, depression, and is linked to worsening of dementia. Advanced age is the predominant risk factor for hearing loss. However, the precise mechanisms behind the age-related degeneration of cochlear structures and function remains unclear, in part due to the complexity of each causal factor and interaction of the various mechanistic pathways leading to ARHL. This lack of understanding ARHL at the molecular level directly impacts the development of therapeutic strategies to treat or prevent this condition.

Our lab is interested in the role of the protein BubR1, as well as its upstream regulators, have in controlling ARHL. BubR1 protein levels naturally decline in most tissues with age, and mice engineered to express low levels of BubR1 from birth have premature aging and die within a year. BubR1 loss leads to increased senescence, which is an irreversible cell cycle arrest and a major cellular pathway controlling aging as well as ARHL. We have previously identified that the anti-aging pathways mediated by the NAD+-dependent SIRT2 deacetylase regulate BubR1 protein abundance in both young and aged animals. Based on these observations, we hypothesize that the dysregulation of the NAD+/SIRT2/b-TRCP/BubR1 pathway with age contributes to senescence formation and inner ear hair cell dysfunction and age-related hearing loss. To test this hypothesis, we will assess at the cellular level whether the NAD+/SIRT2/β-TRCP1/BubR1 pathway modulates responses to oxidative stress in cochlear hair cells and to identify cellular aging mechanisms mediated by the NAD+/SIRT2/β-TRCP1/BubR1 pathway in controlling ARHL. Through these studies we will establish targeting upstream regulators of the aging process, including the NAD+/SIRT2/b-TRCP/BubR1 pathway, as a therapeutic mechanism to prevent (or treat) ARHL.

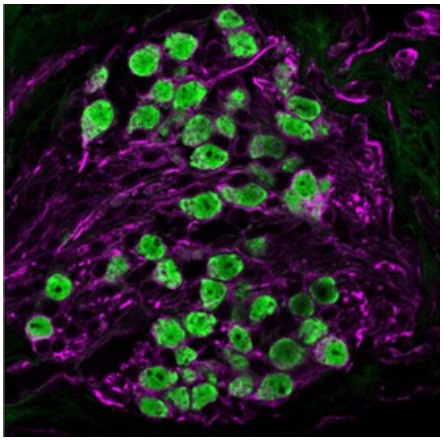
- Brian's lab's website can be found here www.northlaboratory.com

---

3rd Annunal Bellucci Symposium on Hearing Research

In light of the COVID-19 crisis, we combined the 2nd and 3rd annual events into a two-day, virtual symposia on Hearing Research. The Symposia focused on drug therapeutics for hearing loss. We had 370+ registrants from 23 countries and territories join us virtually for two days of amazing presentations on research and development being undertaken. In addition to twelve guest speakers addressing different aspects on drug therapeutics for hearing loss, this year's virtual Symposia included academic poster sessions as well as representatives of several prominent national and local companies who presented on their recent progress on drug development and hearing loss.

More details to be found at: https://belluccisymposium.weebly.com



Confocal image of spiral ganglion neuron's cell body and axons. Courtesy of Dr. Tejbeer Kaur's lab

Bellucci Prize and Trainee Award Winners

The first keynote address was given by 2020 Bellucci Prize winner Penelope Brock, MD, PhD, FRCPCH from University College *London From: symptoms to solutions: an example of international collaboration*. The second keynote address was given by the 2021 Bellucci Prize Winner Lisa Cunningham, PhD: *Atorvastatin reduces cisplatin-induced hearing loss*.

2020 Bellucci Prize Winner

Peneloope Brock, MD, PhD, FRCPCH

2020 Bellucci Trainee Award Winner

Stephanie Rouse, BA

2021 Bellucci Prize Winner

Lisa Cunningham, PhD

2021 Bellucci Trainee Award Winner

Nopporn Jongkamonwiwat, PT, PhD

About Dr. Richard J. Bellucci

Dr. Bellucci's mission in starting the Bellucci DePaoli Foundation was to ensure the important work of hearing restoration continues. The Foundation offers stipends to impressive PhD candidates, making important contributions in auditory research, plus support for acquiring necessary research equipment. Dr. Bellucci is best known for a surgical operation he pioneered, the stapedectomy, and as the inventor of the surgical tool used during this operation: the Bellucci Micro Ear Scissors. The operation was developed in the late 1950s and was one of the first uses of a microscope during surgery. During the procedure, the stapes (a tiny bone in the ear) is removed and replaced by a prosthetic device, gifting patients with certain types of hearing loss to regain their hearing. Dr. Bellucci was Chair of Otolaryngology at the Manhattan Eye, Ear & Throat Hospital (1963-79) and Chairman of Otolaryngology at New York Medical College (1966-80), completing his residency at the former. He trained many ear, nose, and throat specialists who practice today throughout the United States, Canada, and beyond. Dr. Bellucci was also the Director of several impressive residency programs. In addition to running his own private practice and serving as a longtime president of the American Otological Society, he volunteered time and services in his later years at the Hopital de Sacre Coeur in Milot, Haiti, exemplifying the Jesuit spirit of service.

Visit our website: Translational Hearing Center | School of Medicine | Creighton University

The Translational Hearing Center at Creighton University, Boys Town National Research Hospital and University of Nebraska Medical Center is funded by a CoBRE Award GM139762 from the National Institute of General Medical Science, a component of the National Institutes of Health. This newsletter is solely the responsibility of the authors and does not necessarily represent the official views of any supporting institution.