

receptor antagonist naltrexone (NTX; 0, 1 or 10 mg/kg), and movement duration (MD; a validated proxy for NWS) was measured using Noldus Ethovision. Concentrations of BUP, NorBUP, and their glucuronide conjugates in the brains of neonatal littermates not undergoing withdrawal testing were determined using LC/MS/MS. Two-way ANOVA and multiple linear regression analyses tested for interactions between BUP and NorBUP on MD and related brain concentrations to MD, respectively. **RESULTS/ANTICIPATED RESULTS:** There was no interaction effect between BUP and NorBUP on MD for either sex or at any dose of naltrexone. In females, but not males, BUP (1 mg/kg/day) significantly increased NorBUP-induced MD by 58% following an injection with 1 mg/kg NTX. A multiple linear regression model that included BUP and NorBUP brain concentrations as predictors of MD was significant and well-fitting [FEMALES:  $F(2, 40) = 23.97$ ,  $P < .0001$ , adj  $R^2 = 0.52$ ; MALES:  $F(2, 40) = 5.84$ ,  $P = .0059$ , adj  $R^2 = 0.19$ ]. There was a differential contribution of NorBUP brain concentrations to MD based on sex. The partial regression coefficient for NorBUP was 51.34 ( $p < .0001$ ) for females and 19.21 ( $p = 0.093$ ) for males. The partial regression coefficient for BUP was similar for females and males (FEMALES:  $\beta_{BUP} = 10.62$ ,  $p = .0017$ ; MALES:  $\beta_{BUP} = 11.38$ ,  $p = .009$ ). **DISCUSSION/SIGNIFICANCE:** We show for the first time a differential contribution of NorBUP to BUP-associated NWS in each sex, suggesting sex differences in NorBUP susceptibility and implicating that treatment strategies reducing prenatal NorBUP exposure may be more effective for females than males.

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### Modeling gastric mucus layer physiology using human organoids

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**OBJECTIVES/GOALS:** Our goal is to explore the extent to which organoids can serve as models for the protective mechanisms of the stomach—the mucus barrier and the pH gradient across it. We aim to first optimize and validate an organoid-based model of the gastric mucus layer, and then define the cellular mechanisms by which the gastric pH gradient is maintained across it. **METHODS/STUDY POPULATION:** We have developed a method for the in vitro engineering of gastric mucus by growing epithelial cells at the air-liquid interface (ALI). We use microrheology with fluorescent microspheres to define and compare the biophysical and viscoelastic properties of our lab-grown mucus to those of native mucus. We will perform CryoFE-SEM to compare the internal heterogeneity of our lab-grown mucus to fresh mucus obtained from patient tissue. For our mechanistic studies, we will use a pH-sensitive dye (methyl red) to assess the ability of our lab-grown mucus to maintain an artificial pH gradient in a microfluidic device. Next, we will use a pH microelectrode to measure proton flux through our mucus in vitro, investigating the potential for a physiological gradient in both 2D and 3D organoid models. **RESULTS/ANTICIPATED RESULTS:** Here we show that gastric organoids and their corresponding epithelial monolayers produce a mucus gel that does indeed mimic in vivo functions. Immunohistochemical staining, electron microscopy, microrheology, and particle tracking analyses revealed that our gastric organoid mucus is viscoelastic and structurally heterogeneous—both properties that are crucial to the stomach's mucosal first line of defense. Mechanically similar mucus was also engineered using two-dimensional air-liquid interface cultures of the same epithelia. Lastly, live

confocal imaging revealed that *H. pylori* motility—an important virulence factor—was drastically hindered by our lab-grown mucus. **DISCUSSION/SIGNIFICANCE:** We describe a novel method for the in vitro engineering of gastric mucus and highlight biophysical properties that contribute to our stomach's defense against pathogens. This work will lead to an improved understanding of gastric physiology and may contribute to the development of novel drug delivery systems to tackle diseases of the gastric mucosa.

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### Molecular Mechanisms of Type II Spiral Ganglion Neuron Development

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**OBJECTIVES/GOALS:** 30,000,000 people in the U.S. have hearing loss, negatively impacting quality of life and work. Understanding auditory axon guidance for spiral ganglia neurons (SGNs) will aid development of new therapies. I study role of Eph/Ephrin signaling in mediating type II SGN turning events, and how planar cell polarity (PCP) signaling modulates this process. **METHODS/STUDY POPULATION:** This quantitative study was conducted on *Efn3* and *Vangl2* null mice possessing *Neurog1CreERT2* and *R26RtdTomato* mutations. Spontaneous Cre activity within the Neurogenin 1 *CreERT2* line causes recombination and expression of fluorescent Rosa26 Reporter (*R26R*) *tdTomato* in a restricted number of SGNs, including type IIs. Together these lines permit SGN sparse labeling. Bulk-labeling was used for *Efn3*;*Vangl2* double knockout (DKO) mutants. Immunostaining and confocal imaging was used to analyze dsRed in *Efn3*; *Vangl2* and NF-200 in DKO to quantify type II SGN turning. In combination, 3D rendering in Imaris software was used to quantify type II SGN turning, branching and other growth and navigation characteristics. 5-6 cochleae per genotype were analyzed and compared by t-test to wild-type controls. **RESULTS/ANTICIPATED RESULTS:** *EPHRIN-A3* is expressed on the membranes of outer pillar and Deiters' cells of the cochlear epithelium. *Efn3* nulls showed a small rise in type II SGNs incorrectly turning toward the apex at an error frequency of 16.9% compared to controls ( $n=6$ ;  $p=0.05$ ). *Efn3* nulls had reduced branch number/fiber compared to controls, 4.14 and 7.22, respectively ( $n=129$ ;  $p$ ). **DISCUSSION/SIGNIFICANCE:** Our results suggest that Eph/Ephrin signaling acts parallel of PCP signaling to mediate type II SGN guidance during development. The clinical implications of these findings are that therapeutics targeting *EPHRIN-A3* and/or *VANGL2* in this pathway could stimulate new outer hair cell innervation by type II SGNs following auditory damage.

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### Nasal-derived Extracellular Vesicles (EVs) carry a cargo of antiviral and immunomodulatory molecules<sup>†</sup>

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**OBJECTIVES/GOALS:** The goals of this project are to: i) investigate the cargo such as immune mediators (cytokines) and small non coding RNAs (sncRNAs) of EVs derived from nasopharyngeal secretions

(NPS) of children with episodes of viral infections and exposed to SHTS; ii) test the biological activity of EVs released from upper airway mucosa on target/recipient lung cells. **METHODS/STUDY POPULATION:** EVs were isolated from ten NPS samples of children with episodes of acute respiratory infections. EVs were characterized by particle sizing (size and concentration), EV markers, and protein arrays for interferons, cytokines, and other immune mediators content. RNA was extracted from ten samples of NPS-derived EVs by column for next generation high throughput sequencing (NGS) to identify sncRNAs in EVs. In studies currently in progress, EVs will be isolated from RSV-infected human nose organoids (HNOs) cells in air liquid interface (ALI) culture, with or without pre-exposure to tobacco smoke. EVs will be then tested for antiviral activity on recipient RSV-infected lower airway cells. Viral titers will be measured in recipient infected lung cells. **RESULTS/ANTICIPATED RESULTS:** We isolated EVs from NPS samples and confirmed by immunoblot EV markers CD63 and Alix. The average size of NPS-derived EVs of virus positive and negative patients was 170 nm and 145 nm, respectively. We determined the particles number of EVs, concentrations of IFN- $\beta$  and IFN- $\gamma$  in NPS and NPS-derived EVs of these children. While IFN- $\beta$  levels were below the limit of detection in both NPS and NPS-derived EVs of all children, IFN- $\gamma$  was detected in NPS and NPS-derived EVs from infected patients, except for the two patients with no viral infections. We extracted RNA from control-, virus infected- and/or SHTS- EVs and found that piR-36511, piR-40926, piR-49645, piR-32679 and piR-53263 were all significantly enriched in EVs derived from NPS of children exposed to TS compared to those not exposed. **DISCUSSION/SIGNIFICANCE:** RSV leads to approximately 22,000 hospitalizations of children due to second-hand smoke. A vaccine is not currently available for RSV infection. EVs represent a novel translational approach to target undruggable. Airway mucosa EVs in viral respiratory infection function as antiviral messengers and tobacco smoke impairs the EV antiviral activity.

<sup>†</sup>This abstract has been updated since the original publication. A corrigendum detailing these changes has been published (doi:10.1017/cts.2024.687).

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### **Nursing Professionals' Experiences during the COVID-19 Pandemic in Puerto Rico: A Phenomenological Study**

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**OBJECTIVES/GOALS:** The COVID-19 pandemic has impacted nursing frontline professionals. The aims of this study were to explore experiences of nursing professionals in Puerto Rico during the pandemic, examine the impact on their health and provide research development opportunities enhance research capacity. **METHODS/STUDY POPULATION:** This interpretative phenomenological study recruited graduate nurses who participated in one in-depth semi-structured virtual interviews. Interviews were audio recorded and transcribed. The data analysis process was guided using the following steps: 1. Reading and re-reading, 2. Initial noting, 3. Developing emergent themes, 4. Searching for connections across emergent themes, 5. Moving to the next case, 6. Looking for patterns across cases, and 7. Writing up. In addition, Van Manen's thematic structure of four foundations was used as a complement to guide reflection and interpretation. Faculty and students participated throughout the process. **RESULTS/ANTICIPATED RESULTS:** Seven nursing professionals' lived experiences caring for Covid-19 patients were gathered. Their ages ranged from 31 to 45 and had

worked between 2 and 14 years providing direct care. Themes that emerged from narrations include compassion fatigue, teamwork, working beyond clinical role, and gratification. Nurses expressed dealing with a very difficult situation, fear of being infected, or infecting my family, and working together to get through it and better help patients. Nurses also expressed feelings of anxiety and lack of institutional support. Additionally, the impact of working with patients, feeling good for being there, good or bad and support from families. **DISCUSSION/SIGNIFICANCE:** Nurses' narrations point to the complexities of their experiences working during the pandemic. They had to transcend usual demands even though they often lacked needed support. We must recognize the value of nursing and reflect upon changes in healthcare that are essential to move nursing forward.

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### **Outcomes of an Integrated Research Ethics Consultation Service**

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**OBJECTIVES/GOALS:** The need for mechanisms of ethical discourse and guidance has increased as translational research collaborations become more complex. The goal of this project is to analyze the stakeholder engagement and ethical issues our research ethics consultation service (RECS) conducted over a two year period. **METHODS/STUDY POPULATION:** We conducted a retrospective review of our RECS database from 2020 to 2022. We examined the nature of the research and ethical issues of concern from consult requestors, including whether or not consults were preventative. In addition, we assessed the educational outreach conducted during that timeframe as a measure of service awareness. **RESULTS/ANTICIPATED RESULTS:** There was a total of 42 consults conducted over the previous year. There were a wide variety of issues related to informed IRB-related processes (31%), consent (24%), QA/QI determination (12%), authorship (10%), confidentiality (7%), diversity/inclusion (7%), grant preparation (7%). Many of the consults (n=28, 67%) included secondary issues. A few consults (n=4, 10%) were preventative, meaning that the consult was requested in anticipation or consideration of a potential ethical issue. Outreach efforts extended to a diverse array of institutional stakeholders and trainees. **DISCUSSION/SIGNIFICANCE:** The RECS serves numerous constituencies throughout our institution on ethical issues spanning nearly all aspects of research design, conduct, and analysis. These data highlight initiatives to increase study efficiency (in collaboration with institutional research oversight) and helps to direct educational efforts and outreach.

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### **Phagocyte heterogeneity and T cell dependence of cellular host defense mechanisms in tuberculosis**

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**OBJECTIVES/GOALS:** Phagocytes, diverse cells that ingest material, are the primary cell type infected by Mycobacterium tuberculosis (Mtb) and the executors of protective mechanisms. T cells play a critical role by helping phagocytes control the infection. Understanding the precise T cell-dependent mechanisms by which