

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:  $F(2, 40) = 23.97, P < .0001, \text{adj } R^2 = 0.52$ ; MALES:  $F(2, 40) = 5.84, P = .0059, \text{adj } R^2 = 0.19$ ]. 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: $\beta_{\text{BUP}} = 10.62, p = .0017$ ; MALES: $\beta_{\text{BUP}} = 11.38, p = .009$ ). 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 *EfnA3* 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 *EfnA3;Vangl2* double knockout (DKO) mutants. Immunostaining and confocal imaging was used to analyze dsRed in *EfnA3; 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. *EfnA3* nulls showed a small rise in type II SGNs incorrectly turning toward the apex at an error frequency of 16.9% compared to controls ( $n=6; p=0.05$ ). *EfnA3* 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