

comfortable for use in human subjects. **DISCUSSION/SIGNIFICANCE OF IMPACT:** This novel technique allows for an inexpensive, noninvasive, and reproducible ocular blood flow imaging platform. By optimizing this technique, we can proceed with our future plans for a pilot study to compare our imaging technique with the current standard, paving the way for future clinical studies.

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### Optogenetic stimulation of corticotropin-releasing hormone expressing neurons in Barrington's nucleus recapitulates the social stress voiding phenotype in mice

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**OBJECTIVES/SPECIFIC AIMS:** Voiding postponement is common cause of LUT dysfunction in which children void infrequently with large volumes. This condition is modeled in mice that are subjected to social stress who show decreased voiding frequency and increased voided volumes along with increases in corticotropin-releasing hormone (CRH) expression in Barrington's nucleus (BN) (i.e., the pontine micturition center). Optogenetics is a technique to selectively stimulate cells or neurons of interest via light activated channel receptors [i.e., channel-2 rhodopsin (ChR2)]. Here we examined the effects of optogenetic manipulation of CRH BN neurons on the in vivo voiding phenotype and urodynamics in awake mice. We hypothesized that stimulating these neurons at higher frequencies (10–50 Hz) would lead to CRH-dependent alterations in voiding phenotype (i.e., larger voided volumes and longer intermicturition intervals). **METHODS/STUDY POPULATION:** Double transgenic mice expressing ChR2 in CRH cells were generated using the Cre-lox recombinase system and had fiberoptic probes implanted into BN at 8 weeks of age. The mice also underwent simultaneous catheter placement into the bladder for in vivo cystometry. In vivo cystometry before and during optogenetic stimulation at various frequencies was performed 5–7 days postoperatively. Saline was perfused at 10  $\mu$ L/minute and baseline stable voiding cycles were established. Bladder capacity, threshold pressure, voiding pressure, and voided volume were recorded at baseline and at each optogenetic setting. In some mice, the protocol was repeated in the presence of CRH antagonist (NBI 30775). **RESULTS/ANTICIPATED RESULTS:** Fiberoptic stimulation (470 nm at 25 and 50 Hz) produced a significant rise in the intermicturition interval, bladder capacities and increased void volumes. This effect was especially pronounced in females in whom bladder capacity and intermicturition interval more than doubled at 50 Hz stimulation. Fluoroscopic images confirmed complete bladder emptying with each void. The increased bladder capacity at higher frequencies (25 and 50 Hz) was CRH-dependent as injection of a CRH antagonist (NBI 30775) blocked the optogenetic effect. Control non-double mice showed no effects from optogenetic stimulation. **DISCUSSION/SIGNIFICANCE OF IMPACT:** Our results suggest that optogenetic stimulation of CRH-expressing neurons in BN at high frequency (25 and 50 Hz) inhibits micturition and recapitulates the voiding phenotype seen in socially stressed mice (large, infrequent voids). Lower frequencies of optogenetic stimulation (2 and 10 Hz) had no effects on cystometry parameters or voiding phenotype. In addition, females had a greater response to optogenetic stimulation compared with males with larger bladder capacities and longer intermicturition intervals. The changes in voiding phenotype seen were CRH dependent as blockage of the CRH receptor prevented changes in cystometry parameters with optogenetic stimulation. Further elucidation of these and other neural subpopulations in BN are warranted to understand micturition and how it may be manipulated in disease states such as voiding postponement and acute urinary retention.

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### Personalized models of distal airway epithelial-stromal unit in COPD

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**OBJECTIVES/SPECIFIC AIMS:** The objective of this study is to develop patient-derived "personalized" organotypic models of human distal airways, in which basal

stem cells (BCs) isolated from the pre-/terminal conducting airway region are co-cultured with autologous stromal cells from the same region to reproduce patient-specific distal airway epithelial-stromal units and their remodeling in COPD. **METHODS/STUDY POPULATION:** We established a protocol to isolate and propagate epithelial BCs, fibroblasts, and endothelial cells from the distal airways of normal and COPD lung donors. Heterogeneous cellular and molecular phenotypes in the human distal airways were characterized using immunofluorescence and single-cell RNA sequencing. Patient-specific distal airway epithelial-stromal units were reconstructed by co-culturing BCs and autologous stromal cells using an air-liquid interface-based airway wall model and a bronchosphere-based 3D distal airway organoid assay. **RESULTS/ANTICIPATED RESULTS:** Histologic analysis of derived epithelial-stromal units revealed heterogeneous patient-specific phenotypes characterized by hypo-/hyper-/metaplastic lesions (hypo-regenerative phenotype, mucous cell hyperplasia, squamous metaplasia, distal-to-proximal repatterning) in the epithelial compartment, accompanied, in some samples, by stromal remodeling. Candidate epithelial-stromal cross-talk mechanisms were identified using quantitative real-time RT-PCR analysis of autologous epithelial and stromal compartments of established patient-specific distal airway unit models. **DISCUSSION/SIGNIFICANCE OF IMPACT:** Epithelial and stromal cells isolated from distal airways of subjects with and without COPD can be assembled into functional, organ-level tissue which mimics the architecture of human distal airways and, in patients with COPD, reproduces several distal airway remodeling phenotypes. Patient-specific models of distal airway epithelial-stromal cross-talk established in this study can be used to identify candidate pathways that mediate disease-relevant airway remodeling and potentially utilized as pre-clinical platforms for developing personalized therapeutic approaches to suppress the progression of distal airway remodeling in chronic lung diseases, including COPD.

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### Pharmacokinetics of phosphatidylethanol 16:0/20:4 homolog in human blood after consumption of 0.4 and 0.8 g/kg alcohol in a laboratory clinical study

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**OBJECTIVES/SPECIFIC AIMS:** The purpose of this study was to characterize the pharmacokinetics of phosphatidylethanol (PEth) 16:0/20:4 homolog in uncoagulated, human blood samples taken from 18 participants in a clinical laboratory setting after consumption of 2 doses of ethanol. **METHODS/STUDY POPULATION:** Male and female participants received either 0.4 or 0.8 g/kg oral doses of ethanol during a 15-minute period. Blood samples were collected before and throughout 6 hours immediately after alcohol administration, then after 2, 4, 7, 11, and 14 days of administration day. PEth 16:0/20:4 levels were quantified by liquid mass spectrometry. Breath ethanol concentrations were measured concurrently with each blood collection during the administration day, as well as transdermal ethanol concentrations monitored constantly before, during and after ethanol administration day. **RESULTS/ANTICIPATED RESULTS:** (1) Single doses of 0.4 and 0.8 g ethanol/kg produced proportional increases in BrAC and PEth 16:0/20:4 levels; (2) the increase of PEth 16:0/20:4 from base line to C<sub>max</sub> was less than either PEth 16:0/18:1 or PEth 16:0/18:2 during the 6-hour period after ethanol administration; (3) the mean rate of formation of PEth 16:0/20:4 was lower than those of the other 2 homologs; (4) the mean half-life of PEth 16:0/20:4 was 2.18 days, which was shorter than that of either PEth 16:0/18:1 and PEth 16:0/18:2, which were 6.80 and 6.62, respectively. **DISCUSSION/SIGNIFICANCE OF IMPACT:** The results of this study further confirm that PEth homologs are a sensitive biomarker for ethanol consumption. The measurement of three PEth homologs appears to provide additional information about the level and time frame of drinking.

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### Predictive cytological topography (PiCT): A radiopathomics approach to mapping prostate cancer

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**OBJECTIVES/SPECIFIC AIMS:** The objective of this study is to use machine Learning techniques to generate maps of epithelium and lumen density in MRI