meta-analysis. A secondary goal was to compare brain alterations in metabolically healthy (MHO) and unhealthy (MUO) obesity. METHODS/STUDY POPULATION: Source data were peer-reviewed studies reporting locations of gray-matter alterations in group-average, case-control contrasts (obese vs. non-obese) cohorts, performed in a whole-brain, voxel-wise manner. Both voxel-based morphometry and voxel-based physiology studies were included. Three coordinate-based meta-analyses were performed: Pooled (MUO + MHO), MHO, and MUO. RESULTS/ANTICIPATED RESULTS: Thirty-two studies reporting a total of 50 case-control contrasts (MHO, 23; MUO, 27) met inclusion criteria, representing 3,368 participants (obese, 1,781; non-obese, 1587). The pooled analysis yielded 8 cerebral foci (3 nuclear, 5 cortical) in regions implicated in reward-seeking, cognitive, and interoceptive behaviors. MHO yielded 7 cerebral foci (4 nuclear, 3 cortical), partially overlapping Pooled results, with similar behavioral loadings. The MUO pattern was distinct, with 3 cerebellar and 1 occipital foci. DISCUSSION/SIGNIFICANCE: Brain alterations occurred reliably in obesity. The dominant pattern (Pooled & MHO) involved cerebral reward-system circuits, evident even in metabolically healthy obesity. Cerebellar alterations occurred exclusively in metabolically unhealthy obesity, a pattern previously reported in metabolic syndrome.

355
Connecting computational models of reading to the brain in post-stroke alexia

Ryan Staples[1], Andrew DeMarco[2] and Peter Turkeltaub[3]
[1]Georgetown-Howard; [2]Georgetown University Medical Center and [3]Georgetown University Medical Center, MedStar National Rehabilitation Hospital

OBJECTIVES/GOALS: Many left hemisphere stroke survivors have a reading disorder (alexia), which is experienced as decreasing well-being. Therapies produce inconsistent results, demonstrating a need for treatment response predictors. We are investigating neural correlates of reading computational models to identify biomarkers to improve therapeutic outcomes. METHODS/STUDY POPULATION: Artificial neural network models of reading, mapping between orthography (visual word form), phonology (auditory word form), and semantics (word meaning), are trained to read single words at a healthy, adult capacity. The models are independently damaged to reflect the individual orthography-to-semantics, semantics-to-phonology, and orthography-to-phonology deficits of a sample of left hemisphere stroke survivors (n = 85). These deficits are measured with cognitive tests assessing the intactness of mappings between representations. Model damage is enacted by removing percentages of the connections between representations. For each type of deficit, the percentages of links removed are entered into a voxel-based lesion symptom mapping analysis to identify areas of cortex associated with that mapping. RESULTS/ANTICIPATED RESULTS: We anticipate that the neural correlates of model layers will be localized to a mostly left-lateralized network. Increased damage to the links between semantics and phonology in the model will likely be related to lesions involving the left posterior superior temporal sulcus and inferior frontal gyrus (IFG). Damaged orthography-to-semantic links will be related to the left fusiform gyrus (FG) and IFG. Finally, damage to the orthography-to-phonology links will be related to the left FG and superior temporal gyrus. DISCUSSION/SIGNIFICANCE: Mapping components of language models onto the brain will improve our understanding of the neural networks supporting language processing. Identifying these neural correlates may also produce biomarkers that can be used in predicting reading impairment at the acute stage or optimizing therapy in the chronic stage of stroke.

356
The Effect of Regional Anesthesia on Breast Cancer Recurrence

Reema Martini[1], Anna Woodbury[1], Kevin Kalinsky[1], Jeffrey Switchenko[2], Sunil Badve[1] and Vinita Singh[1]
[1]Emory University School of Medicine and [2]Emory University Rollins School of Public Health

OBJECTIVES/GOALS: To determine the impact of regional anesthesia (RA) on rates of breast cancer recurrence, breast cancer specific mortality, post mastectomy pain syndrome, and chronic opioid use among patients who received regional anesthesia during primary breast cancer surgery. METHODS/STUDY POPULATION: Study population: Patients who underwent primary breast cancer surgery at Emory University and among a national patient cohort. Methods: This is a retrospective analysis of patients who underwent primary breast cancer surgery. Invasive breast cancer specific survival & breast cancer-specific survival will be estimated using Kaplan-Meier method and compared among patient groups. Cox proportional hazards will be utilized to estimate the unadjusted & adjusted risk of breast cancer recurrence and breast cancer-specific mortality between the two groups. RESULTS/ANTICIPATED RESULTS: We will present on our work comparing rates of breast cancer recurrence among patients who received regional anesthesia compared to patients who did not. DISCUSSION/SIGNIFICANCE: This study aims to establish the effect of regional anesthesia on breast cancer recurrence, breast cancer specific mortality, post mastectomy pain syndrome, and chronic opioid use following primary breast cancer surgery.

357
Effects of Dietary Nitrate Supplementation on Vascular Function in Individuals with Prediabetes

Blaine E. Arney, Timothy J. Fulton, Christopher W. Sundberg and Sandra K. Hunter
Marquette University

OBJECTIVES/GOALS: Impaired vascular function, a subclinical marker of cardiovascular disease, has been identified in prediabetes. Dietary nitrate supplementation has been shown to improve vascular function. However, this has not been studied in prediabetes. The purpose was to determine the effects of dietary nitrate on vascular function in prediabetes. METHODS/STUDY POPULATION: Five individuals with prediabetes (4 men, 1 woman; 55 ± 17 yr; HbA1c $= 5.8 \pm 0.2$) participated in a double-blind, placebo-controlled, repeated measures study. Participants were randomly assigned to a 3-day nitrate supplementation (nitrate-rich beetroot juice, 12.9 mmol, 140 mL), or a placebo supplementation

(nitrate-depleted beetroot juice, 0.05 mmol, 140 mL). Following supplementation, participants reported to the lab for measures of vascular function in the lower limb. Doppler ultrasonography was used to measure flow-mediated dilation (FMD) and post-occlusive reactive hyperemia (RH) of the superficial femoral artery in response to a 5-min bout of leg ischemia. RESULTS/ANTICIPATED RESULTS: FMD did not differ between the nitrate-rich ($2.87 \pm 2.01\%$) and placebo ($2.24 \pm 1.69\%$) conditions (p = 0.48; d = 0.35). Furthermore, peak RH did not differ between the nitrate-rich (1503 ± 443 ml/min) and placebo (1762 ± 414 ml/min) conditions (p = 0.36; d = 0.46). DISCUSSION/SIGNIFICANCE: These preliminary results suggest that dietary nitrate supplementation in the form of beetroot juice does not improve vascular function in individuals with prediabetes.

358
In Vitro Uptake of Harmful Algal Bloom Toxin Microcystin-LR in Human Placental Cells

Michael Campbell, Mokshitkumar Patel, Shuo Xiao, Xia Wen and Lauren M. Aleksunes
Rutgers University

OBJECTIVES/GOALS: Harmful algal blooms (HABs) are increasing in both frequency and intensity due to climate change. HABs release the toxin microcystin-LR (MC-LR) which enters cells via organic anion-transporting polypeptides (OATPs). In this study, we sought to assess the ability of MC-LR to accumulate in trophoblasts, potentially disrupting placental functions. METHODS/STUDY POPULATION: Intracellular accumulation of MC-LR at exposure concentrations of 0.1, 1, and 10 μM over 6 hrs was evaluated in immortalized JAR placental cytotrophoblasts. Western blotting was used to evaluate protein-bound MC-LR accumulation in JAR cells. The function of OATP transporters in JAR cells was determined by pre-incubating cells with 10μM cyclosporin A, a general OATP inhibitor for 1 hr, and then incubated with 1μM OATP substrate fluorescein for up to 40 min. Fluorescence of fluorescein was measured at Ex/Em: 494nm/515nm by spectrophotometry. RESULTS/ANTICIPATED RESULTS: A concentration-dependent increase of MC-LR bound proteins in JAR cells was observed at 6 hrs with the greatest intracellular accumulation of MC-LR at 10μM. In the transporter experiments, a significant decrease of fluorescein uptake by up to 45% into JAR cells was observed following cyclosporin A inhibition of OATPs. These findings are consistent with the functional expression of OATP transporters in JAR placenta cells. Ongoing studies are evaluating whether the cyclosporin A-mediated inhibition of OATPs also inhibits the uptake of MC-LR. DISCUSSION/SIGNIFICANCE: Although MC-LR is well-known for its hepatotoxic and neurotoxic effects, there is growing interest in examining its potential adverse impacts on female reproductive health, particularly during pregnancy. Active uptake of MC-LR into the placenta could interfere with placental and fetal development.

362
Examining Temporal Links Between Distinct Negative Emotions and Tobacco Lapse During A Cessation Attempt

Dusti Jones, Lindsey N. Potter, Cho Y. Lam and David W. Wetter
University of Utah

OBJECTIVES/GOALS: Negative emotions (NE) play a pivotal role in addiction-related processes, including tobacco lapse during a quit attempt. Some NEs (e.g., shame, guilt) are posited to lead to a spiraling effect, whereby lapse predicts increased NEs leading to further lapse. This study goal is to examine associations between NEs and lapse. METHODS/STUDY POPULATION: This study examined associations between tobacco lapse and 13 distinct NEs among people who use tobacco and are trying to quit in two tobacco cessation studies. In Study 1, 220 adult (ages 18-74) cigarette users who identified as Black (50% female) participated in a 14-day study where ecological momentary assessment (with assessments approximately every 4 hours) was used to assess emotions and lapse in real-time and real-world settings. In Study 2, 288 adult (ages 18-71) cigarette users who were low socioeconomic status (51% White, 14% Black, 10% Hispanic, 49% female) participated in a 14-day study with the same study protocol as Study 1. Between and lagged within-person associations testing links between distinct NEs and lapse were examined with multilevel modeling with logistic links for binary outcomes. RESULTS/ANTICIPATED RESULTS: Results from Study 1 suggested that at the between-person level, disgust (OR =1.22, CI: 1.05, 1.42), nervousness (OR=1.23, CI:1.05,1.43), guilt (OR=1.40, CI: 1.16,1.69), and sadness (OR=1.18, CI:1.02,1.36) were predictive of higher odds of lapse, and at the within-person level, shame (OR=1.23, CI:1.04,1.45) was associated with higher odds of lapse. Results from Study 2 were similar and suggested that at the between-person level, disgust (OR=1.35, CI: 1.16, 1.56) and guilt (OR=1.88, CI:1.07,3.30), and at the within-person level, shame (OR =1.31, CI:1.10,1.55), were associated with higher odds of lapse. DISCUSSION/SIGNIFICANCE: The present study uses real-time, real-world data to demonstrate the role of distinct NEs on momentary tobacco lapse and helps elucidate specific NE that hinder the ability to abstain from tobacco use during a quit attempt. Results suggest that disgust, guilt, and shame play consistent roles in predicting lapse among diverse samples of tobacco users.

363
A CRISPR/dCas9 Epigenetic Therapuetic Approach for CASK-Related MICPCH*

Casiana Gonzalez, Julian Halmai and Kyle Fink
University of California, Davis

OBJECTIVES/GOALS: CASK-related microcephaly with pontine and cerebellar hypoplasia (MICPCH) is a rare X-linked neurodevelopmental disorder caused by mutations in calcium/calmodulin-dependent serine protein kinase (CASK). We aim to rescue CASK expression via an CRISPR/dCas9 epigenetic therapeutic and create iPSC-based CASK relevant in vitro model systems. METHODS/STUDY POPULATION: As females have two X-chromosomes, disease causing mutations present with a 50/50 expression of mutant and wildtype, due to the mosaicism caused by random X-chromosome inactivation (XCI). This project will adapt an established CRISPR/dCas9 epigenetic approach to rescue expression from the silenced, wild-type CASK allele. We aim to accomplish this through testing different dCas9 orthologues and a guide RNA screen targeting the CASK promoter. Constructs will be tested for optimal targeting efficacy in vitro and assessed via RT-qPCR. Additionally, epigenetic modifications from our approach will be analyzed through bisulfite sequencing. We also aim to apply this epigenetic rescue technology in disease relevant cell lines and eventually in engineered patient mutation iPSC-derived neurons. RESULTS/ANTICIPATED RESULTS: Our results show the ability to target CASK and assess gene expression changes with CRISPR/dCas9 paired with an epigenetic modifier and transcriptional activator. Additionally, our fibroblast model with nonpathogenic single