

supplementation. Ten participants are enrolled (target 30 participants). Renal blood velocity (RBV) in the renal and segmental arteries will be measured in the decubitus position using Doppler ultrasound during a 3-minute baseline and 3-minute cold pressor test. We will measure brachial BP with an automated oscillometric BP monitor. RVR will be calculated as mean BP divided by RBV. Statistical analyses will include ANOVA and correlations with  $\alpha$  set at  $\leq 0.05$ . RESULTS/ANTICIPATED RESULTS: We anticipate attenuated RBV and increased BP during the cold pressor test, particularly following high salt loading, leading to greater RVR. We hypothesize ketone supplementation will attenuate the high salt induced increase in RVR during the cold pressor test. In addition to RVR we will examine renal vascular conductance which is the ease with which blood flows through arteries, calculated as RBV divided by mean BP. Additional hemodynamics such as heart rate and systolic and diastolic BP will be reported and correlated with primary outcomes. DISCUSSION/SIGNIFICANCE: Dietary salt plays a role in hypertension, cardiovascular disease, and chronic kidney disease, which are leading causes of death. Ketone supplementation may be a promising approach to counteract the detrimental effects of high dietary salt and improve cardiovascular health in adults.

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### Microglial Behavior and Iba-1 Expression: Evaluating the Cognitive Impact of Vascular Dementia and Long COVID

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OBJECTIVES/GOALS: The study aims to explore the role of microglial behavior in cognitive impairment associated with vascular dementia (VaD) and long COVID. Using immunohistochemistry (IHC) and quantitative PCR (qPCR), we will assess the expression of Iba-1, a microglial activation marker, in subjects with VaD and SARS-CoV-2 infection. METHODS/STUDY POPULATION: Out of 48 female C57BL/6 mice, 24 had surgical intervention in the form of bilateral carotid artery stenosis (BCAS) for experimental induction of vascular dementia. After 2 weeks, 12 BCAS and 12 non-BCAS were infected with 1E4 PFU of mouse-adapted 10 (MA10) strain of SARS-CoV-2. 2 weeks post-infection, 4 weeks post-operatively, all animals were euthanized and tissues were processed for cDNA and histology. Immunofluorescence and RT-qPCR used to quantify microglia via Iba-1, BBB integrity via claudin-5 as well as occludin, GFAP, and integrin  $\alpha 5$ . RESULTS/ANTICIPATED RESULTS: We anticipate observing distinct patterns of microglial behavior in subjects with vascular dementia (VaD) and those with long COVID. Through immunohistochemistry (IHC), we expect to see increased Iba-1 expression, indicative of microglial activation. Quantitative PCR (qPCR) will likely corroborate these findings, showing elevated levels of Iba-1 mRNA. Lastly, we anticipate that the data will reveal interactions between microglia and the blood-brain barrier (BBB). These interactions could provide insights into how microglial behavior influences BBB integrity and, consequently, cognitive function in VaD and long COVID. DISCUSSION/SIGNIFICANCE: This study aims to clarify the role of microglia in cognitive decline linked to vascular dementia and long COVID. By categorizing patients based on microglial activation, we can better tailor treatments. The findings could lead to targeted therapies that address cognitive impairment in these conditions.

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### Eph/Ephrin Signaling Influences Innervation of Outer Hair Cells in Cochlea

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OBJECTIVES/GOALS: 48,000,000 people in the U.S. have hearing loss, negatively impacting quality of life and work. Unveiling axon guidance for auditory type II spiral ganglia neurons (SGNs) will aid development of new therapies. I study the role of Eph/Ephrin and planar cell polarity (PCP) signaling during type II SGN turning and outer hair cell (OHC) innervation. METHODS/STUDY POPULATION: This quantitative study was conducted on *Efna3* and *Vangl2* null mice possessing *Neurog1CreERT2* and *R26RtdTomato* mutations. Spontaneous Cre activity within the *Neurogenin1CreERT2* 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. Immunostaining and confocal imaging were used to analyze dsRed in *Efna3* and *Vangl2* mice and quantify type II SGN turning. In combination, Imaris 3D renderings were used to quantify type II SGN turning, branching, navigation features and temporal effects of EPHRIN-A3-Fc on type IIs via cochlear cultures (a gain-of-function manipulation). For both sexes, 5-6 cochleae per genotype were analyzed and compared by t-test to wildtype (WT) controls. RESULTS/ANTICIPATED RESULTS: *Efna3* nulls showed a small rise in type II SGNs incorrectly turning toward the apex at an error rate of 16.0% compared to WT (n=6; p=0.05). *P0 Efna3* nulls had reduced branch number compared to WT, 4.1 and 7.2, respectively (n=129; p<0.0001), suggesting EPHRIN-A3 acts as a positive growth cue. In cochlear cultures, EPHRIN-A3-Fc led to type II SGN collapse at E15.5, indicating a repulsive function. However, at *P0*, EPHRIN-A3-Fc treatment led to type II SGNs with elevated branch numbers compared to Control-Fc treatment: 18.1 and 11.4, respectively (n=116; p<0.0001). This indicates a positive growth function. At E16.5, EPHRIN-A3 protein immunoreactivity on Deiters' and pillar cells appears reduced in *Vangl2* nulls compared to WT cochleae, suggesting that EPHRIN-A3 acts downstream of PCP signaling. DISCUSSION/SIGNIFICANCE: Results suggest that Eph/Ephrin signaling acts downstream of PCP signaling to mediate type II SGN guidance and EPHRIN-A3 switches its mode of activation. The clinical implications of these findings are that therapeutics targeting EPHRIN-A3 and/or VANGL2 in their given pathways could stimulate new OHC innervation following auditory damage.

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### Brain Structural Alterations in Metabolically Healthy and Unhealthy Obesity: A Quantitative Comparison Using Coordinate-Based Meta-Analysis

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OBJECTIVES/GOALS: The primary research goal was to identify brain alterations reliably associated with obesity using coordinate-based