and effective connectivity patterns across major brain networks – Default Mode (DMN), Salience (SN), and Central Executive (CEN) – using graph theoretical metrics, phase-locking values, and multivariate analyses. Identified network patterns will be linked with neuropsychological measures, including the Child Behavior Checklist (CBCL), to inform development of standardized neurofeedback protocol recommendations grounded in developmental neuroscience. RESULTS/ANTICIPATED RESULTS: We anticipate identifying distinct patterns of functional connectivity that predict variation in children's emotional and behavioral functioning. Findings are expected to highlight network patterns – particularly within emotion regulation and executive control circuits – that can guide neurofeedback protocol design. Anticipated products include neurophysiologically informed recommendations for standardized frequency bands, target networks, and protocol parameters to support future clinical trial development. This project represents a key T1 translational step toward establishing a neuroscience-based framework for EEG neurofeedback treatment protocols. DISCUSSION/SIGNIFICANCE OF IMPACT: By integrating neurophysiological and behavioral data to guide protocol development, this work addresses a critical translational gap in the EEG neurofeedback literature. Findings will establish a foundation for standardized, evidence-based neurofeedback interventions that could improve accessibility and efficacy in clinical settings.

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From pixels to pathology: Excitation-scanning hyperspectral imaging for rapid colorectal cancer detection

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OBJECTIVES/GOALS: Colorectal cancer (CRC) disproportionately affects the U.S. Deep South. Socioeconomics, genetics, and healthcare access contribute to increased incidence and mortality. A next-generation endoscope with improved contrast and automatic detection may boost early detection and narrow expertise gaps between top centers and rural clinics. METHODS/STUDY POPULATION: We implemented excitation-scanning hyperspectral imaging (Ex-HSI) to discriminate normal and cancerous mouse colorectal tissues by detecting changes in autofluorescence. Ex-HSI permits detection of all emitted light above a cutoff wavelength. Ex-HSI reduces acquisition time and improves signal-to-noise ratio compared to traditional emission scanning. We used azoxymethane/dextran sodium sulfate (AOM/DSS) to induce colitis and nodule formation in the mouse CRC model. Ex-HSI data was complemented with transmitted light and confocal images. Histology sectioning with H&E staining was obtained for "gold standard" validation. RESULTS/ANTICIPATED RESULTS: Inflammation and bleeding were observed, consistent with AOM/DSS treatment. Nodules were visible using 4x and 20x objectives. Background-subtracted Ex-HSI data was corrected to a flat spectral response. Excitation spectra were extracted from select regions within each field of view. Excitation spectra displayed multiple peak excitation wavelengths, suggesting multiple autofluorescent molecules present. Further investigation will utilize spectral unmixing, principal component analysis, and neural networks to interpret image data. DISCUSSION/SIGNIFICANCE OF IMPACT: A hyperspectral endoscope that detects early-stage cancers and precancers would enhance CRC screening in the Deep South. This research is supported by NIH P01HL066299, NIH UL1TR00141, S10RR027535, S10OD02860, NSF MRI1725937, CCTS T32TR004767, and the Center for Lung Biology, University of South Alabama.

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Macrophage regulation of inflammation in type 2 diabetes

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OBJECTIVES/GOALS: Type 2 diabetes is a metabolic disease characterized by non-resolving inflammation. Our objective is to investigate how macrophages contribute to this dysregulation. We hypothesize that diabetic macrophages have impaired response mechanisms and are more sensitive to metabolic flux, limiting their ability to effectively resolve inflammation. METHODS/STUDY POPULATION: To study this, we isolated and cultured bone marrow-derived macrophages from WT and db/db mice *in vitro* and stimulated them with maturation, inflammatory, and resolving cues. We also isolated human monocytes from PBMCs from patients with type 2 diabetes and age-matched healthy controls and stimulated them *ex vivo* and cultured them *in vitro*. We assessed their timeline of maturation through changes in cell surface markers, their functional inflammatory response through phenotypic marker changes and cytokine release, and their sensitivity to metabolic flux by culturing in the presence or absence of extracellular glutamine. Together, these responses can allow us to understand intrinsic defects in diabetic macrophages' ability to regulate inflammation. RESULTS/ANTICIPATED RESULTS: Notably, diabetic and healthy macrophages display distinct maturation timelines. Overall, diabetic macrophages lag behind healthy macrophages in expression of CD45 and F4/80 but eventually acquire a similar level of mature cell surface markers by day 7-8 in culture. In addition, diabetic cells have a higher proportion of Ly6Chi cells earlier in culture, cells that are generally more poised to be pro-inflammatory. Both healthy and diabetic mature macrophages displayed similar levels of inflammatory response cytokines, inflammatory gene expression changes, and phenotypic marker expression. Interestingly, stimulating these macrophages in the absence of glutamine revealed underlying impairments in their response to inflammatory and resolving cues. DISCUSSION/SIGNIFICANCE OF IMPACT: Patients with diabetes suffer significant complications, such as susceptibility to infections and non-healing wounds, driven by impairments in resolution of inflammation. This research begins to explore the cell-mediated inflammatory aberrations in type 2 diabetes and may reveal targetable mechanisms to improve patient outcomes.

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