activation following steroid therapy. Although steroids play a significant role in regulating the amount of inflammatory damage that occurs during IBD treatment, our data suggest that they may be limiting pathways required for effective healing as well.

**2326 Successful hand function recovery after stroke**

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OBJECTIVES/SPECIFIC AIMS: Upper-extremity (UE) impairment affects 88% of stroke survivors due to dysfunctional shoulder-hand coordination. Patients may be able to grasp with the arm at rest, but unable to grasp in a functional context (eg, from a high shelf) because shoulder use elicits involuntary hand muscle activity. Further, much rehabilitation research is directed at unsuccessful stroke recovery (patients with persistent UE impairment) but very little towards patients who show successful clinical recovery (such as those with mild UE impairment) even though these patients have attained the desired rehabilitation outcome. We examined the neurophysiological trajectory of successful recovery compared to unsuccessful post-stroke recovery in the context of functional UE movements to clearly identify what factors are necessary for successful recovery of functional UE movements after stroke.

METHODS/STUDY POPULATION: We studied 3 populations: (1) mildly-impaired patients, early (at <17 d, 30 d, 90 d, and 180 d) after stroke as a model of successful post-stroke recovery, (2) moderately-impaired, chronic patients (>6-months post stroke) with persistent hand function impairment, as a model of incomplete post-stroke recovery (unsuccessful recovery), and (3) Healthy age-range matched controls. We used transcranial magnetic stimulation (TMS) in all 3 groups at the given time points to measure corticomotor excitability (motor evoked potentials, recruitment curve), corticomotor inhibition (short-interval intracortical inhibition, long-interval intracortical inhibition), and intracortical facilitation of hand muscles with the shoulder positioned in different degrees of adduction or abduction (these shoulder positions are known to elicit involuntary, undesired hand muscle activation, which leads to UE dysfunction and impairment in individuals with stroke). RESULTS/ANTICIPATED RESULTS: Data collection is in process and will be presented. Preliminary data from controls shows that corticomotor excitability of selected hand muscles is affected by changes in shoulder position. Preliminary findings in controls are consistent with clinical findings in stroke that certain shoulder positions elicit involuntary and undesired hand muscle activation, leading to UE dysfunction and disability. Findings from the stroke groups will be presented. DISCUSSION/SIGNIFICANCE OF IMPACT: We hypothesize that this centrally-facilitated coupling between shoulder and hand muscles is disrupted after stroke, which may play a central role in the inability of patients to perform functional UE movements. By comparing the TMS metrics in mildly-impaired Versus moderately-impaired chronic patients, we will be able to identify the longitudinal change in corticomotor excitability following stroke. We used transcranial magnetic stimulation (TMS) in all 3 groups at the given time points to measure corticomotor excitability (motor evoked potentials, recruitment curve), corticomotor inhibition (short-interval intracortical inhibition, long-interval intracortical inhibition), and intracortical facilitation of hand muscles with the shoulder positioned in different degrees of adduction or abduction (these shoulder positions are known to elicit involuntary, undesired hand muscle activation, which leads to UE dysfunction and impairment in individuals with stroke). RESULTS/ANTICIPATED RESULTS: Data collection is in process and will be presented. Preliminary data from controls shows that corticomotor excitability of selected hand muscles is affected by changes in shoulder position. Preliminary findings in controls are consistent with clinical findings in stroke that certain shoulder positions elicit involuntary and undesired hand muscle activation, leading to UE dysfunction and disability. Findings from the stroke groups will be presented. DISCUSSION/SIGNIFICANCE OF IMPACT: We hypothesize that this centrally-facilitated coupling between shoulder and hand muscles is disrupted after stroke, which may play a central role in the inability of patients to perform functional UE movements. By comparing the TMS metrics in mildly-impaired Versus moderately-impaired chronic patients, we will be able to identify the longitudinal change in corticomotor excitability following stroke.

**2367 Defining critical features of the immune microenvironment in melanoma using multiplex immunohistochemistry and spatial analysis**

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OBJECTIVES/SPECIFIC AIMS: Precise biomarkers are urgently needed to characterize the tumor immune microenvironment in primary melanoma tumors both for prognostication and to predict the benefit of immunotherapeutic intervention. The goal of this work is to define spatial relationships between D8+ T cells, CD8+ macrophages and Sox10+ melanoma cells in order to define features correlating with prolonged survival

METHODS/STUDY POPULATION: Five micrometer slides from either the primary biopsy or subsequent whole exonic procedure were stained using Opal multiplex HIC for DAPI, CD3 (LN10, Leica), CD8 (4B11, Leica), CD68 (KPI, Biogenex), SOX10 (BC34, Biocare), HLA-DR (LN-3, Abcam), and K67 (MBI, Abcam). Cell phenotypes within representative fields preselected by a trained dermatologist were visualized using the Mantra quantitative pathology workstation (PerkinElmer), and analysis of spatial distribution of CD3+ CD8+ cells analyzed using iForm® image analysis software (PerkinElmer), and Spotfire software (TIBCO). In order to test whether mHIC can better characterize the tumor immune microenvironment, we screened databases at the Herbert Irving Cancer Center (HICC) at Columbia University for stage II/III melanoma patients diagnosed between 2000 and 2012, with available FFPE of primary melanoma tissue and documented clinical follow-up. We identified a preliminary population of 57 patients to begin our analysis. Clinical follow-up was available on 35 patients of whom 21 patients were alive with no evidence of recurrence or died with no evidence of recurrence and 14 had died of melanoma. Twenty-four patients had more than 24 months of survival information available but no detailed clinical information to determine cause of death. RESULTS/ANTICIPATED RESULTS: First, we evaluated whether density of immune cells in tumor and stroma predicted prognosis in 35 patients with disease specific survival information. We find that high number of CD8+ + cells in tumor correlates with Disease Specific Survival (p = 0.0232) and CD3 + CD8+ + cells in stroma may also correlate with DSS (p = 0.0671). This is consistent with what is known in the literature regarding tumor infiltrating lymphocytes (TILs). We also found that CD68+ + cells in stroma predict poor prognosis (0.0259). This is consistent with the proposed
deleterious role for macrophages in tumor progression. Next, using nearest neighbor analysis we examined the effect of HLA-DR and Ki67 expression on spatial distribution of CD3+ and CD68+ T cells. We find that CD68+ T cells are closer to myeloid (CD68+) cells expressing HLA-DR. This is consistent with the potential of HLA-DR expressing cells to present antigens to T cells, and suggests that T cells may preferentially interact with HLA-DR expressing myeloid cells. Conversely, we find that Ki67 expression on tumor (SOX10+) cells correlates with increased distance from CD3+ CD68+ T cells relative to SOX10+ Ki67+ tumor cells. This finding is consistent with the observation that more advanced tumors with higher mitotic rates have decreased T cell infiltrates, and suggests that dividing melanoma cells are less likely to interact with T cells. In addition, we performed analysis to determine whether spatial relationships defined above impact prognosis. Clinical oncology follow-up was available on 35 of the 57 patients evaluated above. We compared proximity of CD3+ CD8+ cells to both myeloid (CD68+) and tumor (SOX10+) cells in patients who recurred and those with no evidence of recurrence. We found that CD3+ CD8+ cells in patients who had recurrence were closer to CD68+ HLA-DR− cells than in patients who had no recurrence (t-test, p = 0.0371), this correlated with DSS (p = 0.003). Conversely, distance from CD3+ CD8+ to CD68+ HLA-DR+ in relationship to recurrence was not significant with a trend towards CD3+ CD8+ T cells being closer in nonrecurrent patients (t-test, p = 0.1362). DISCUSSION/SIGNIFICANCE OF IMPACT: Consistent with the literature, we find that densities of CD8+ T cells correlates with favorable outcomes in early stage melanoma. We also find that density of CD68+ macrophages in stroma correlates with poor outcome. If proximity is a determinant for interactions of these data indicate that Ki67+ cells interact less with CD8+ T cells than do Ki67− melanoma cells. Further, HLA-DR expression on CD68+ infiltrating cells likely enhances their interaction with T cells. Interestingly, on further analysis, CD3+ CD8+ cells were significantly closer to CD68+ HLA-DR− cells in patients who recurred, implying that interactions between these cell types may not be favorable. This analysis demonstrates that spatial analysis may be useful in predicting prognosis in early stage melanoma, and this is the first report of this type of analysis predicting outcomes in primary tumor specimens to our knowledge. Further staining and analysis of the complete patient cohort (n = 120) is ongoing.

Understanding epicardial fat biology by imaging
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OBJECTIVES/SPECIFIC AIMS: The goal is to understand the underlying mechanism of epicardial fat biology and its response to cardiometabolic disease. We are using quantitative multi-echo balanced echo-planar (mDixon) of water and lipid sequence, T2* blood-oxygen-level-dependent (BOLD) sequence of iron metabolism, and magnetic resonance (MR) characteristics. We will then investigate whether epicardial fat tissue is increased in subjects with previously measured serum and clinical data to identify pathways of interest. The present study seeks to determine whether PAC promotes or inhibits neural signals that underlie attention. METHODS/STUDY POPULATION: Six adult epilepsy patients with implanted electrodes participated in a cued attention task. Subjects participated in a cued attention task where they oriented attention to one side of the screen at a time and discriminated between stimuli as fast as possible with mouse clicks. Perception-related electrodes discriminated the location and/or shape of the target. These were determined with a cluster-based permutation test. Behavior-related electrodes predicted reaction time (RT) with neural activity prior to target appearance. These were determined with correlations between PAC and RT. PAC was calculated using the modulation index (MI). RESULTS/ANTICIPATED RESULTS: We found 47 perception-related electrodes that discriminated location and/or shape of target (p < 0.05, FDR corrected). We found 27 behavior-related electrodes where PAC prior to the target predicted RT (p < 0.05 FDR corrected). There was little overlap between the perception-related and behavior-related electrodes (3%). PAC also did not discriminate left-sided and right-sided cues. In addition, behavior-related electrodes had less local neural activity and higher PAC during the period of cued attention than perception-related electrodes. DISCUSSION/SIGNIFICANCE OF IMPACT: PAC minimally facilitates perception-related attention and facial response speed. We suggest that PAC might improve response speed by “quieting” task irrelevant neural activity. For the same reason, PAC is absent in electrodes that are actively processing meaningful streams of visual data. These findings highlight separable aspects of the human attention system and how PAC contributes to both. Future directions include determining differences in PAC for attentional disorders like ADHD and neurological neglect.

Metabolite and biomarker predictors of WTC-lung injury: An integrated multiplex platform pilot analysis
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OBJECTIVES/SPECIFIC AIMS: In this pilot case-control study, the metabolome was quantified in subjects with previously measured serum and clinical biomarkers. The serum metabolome was then integrated with existing serum and clinical biomarkers of WTC-exposed firefighters to identify pathways significant to loss of lung function following acute PM-exposure. This robust subset of metabolite and serum biomarkers may be clinically relevant to predicting progression to lung disease in a larger cohort. METHODS/STUDY POPULATION: Serum drawn within 6 months of 9/11 was analyzed in this pilot. Clinical measures were obtained from electronic medical records. Never-smoking, male, WTC-exposed firefighters with normal pre-9/11 lung function were segregated based on FEV1 percent predicted (FEV1 %Pred) at symptomatic presentation. Cases of WTC-LI (FEV1 %Pred < LLN, n = 15) and controls (n = 15) were identified from previous cohorts. Ultra-high performance liquid chromatography tandem mass spectrometry quantified the metabolomic fingerprints of a group with previously assessed (by multiplex panels; ELISA and Luminex) serum chemokines and cytokines. High-dimensional data analysis and dimension reduction techniques integrated metabolites, cytokines, chemokines, and clinical data to identify pathways of epicardial fat volume detected by anatomic imaging. This study will impact the management of patients at risk for cardiovascular disease because it will demonstrate that quantification of epicardial fat status by MR identifies fat tissue changes validated by histology at lower cardiovascular disease risk quartile than CT.

Perception- and behavior-related attention systems distinguished by phase amplitude coupling and high-gamma power
Ravi Varkki Chacko, Kenny Kim, Kate Jung, Gordon Shulman, Maurizio Corbetta and Eric Leuthardt

OBJECTIVES/SPECIFIC AIMS: Attention is a cognitive function that binds perception and behavior. Recent evidence suggests that attention involves phase-amplitude coupling (PAC) of neural signals. PAC occurs when the amplitude of one frequency (frequency for amplitude) is maximal at particular phases of another frequency (frequency for phase). However, some studies suggest PAC improves attention, while others maintain that PAC inhibits attention. The present study seeks to determine whether PAC promotes or inhibits neural signals that underlie attention. METHODS/STUDY POPULATION: Six adult epilepsy patients with implanted electrodes participated in a cued attention task. Subjects participated in a cued attention task where they oriented attention to one side of the screen at a time and discriminated between stimuli as fast as possible with mouse clicks. Perception-related electrodes discriminated the location and/or shape of the target. These were determined with a cluster-based permutation test. Behavior-related electrodes predicted reaction time (RT) with neural activity prior to target appearance. These were determined with correlations between PAC and RT. PAC was calculated using the modulation index (MI). RESULTS/ANTICIPATED RESULTS: We found 47 perception-related electrodes that discriminated location and/or shape of target (p < 0.05, FDR corrected). We found 27 behavior-related electrodes where PAC prior to the target predicted RT (p < 0.05 FDR corrected). There was little overlap between the perception-related and behavior-related electrodes (3%). PAC also did not discriminate left-sided and right-sided cues. In addition, behavior-related electrodes had less local neural activity and higher PAC during the period of cued attention than perception-related electrodes. DISCUSSION/SIGNIFICANCE OF IMPACT: PAC minimally facilitates perception-related attention and facial response speed. We suggest that PAC might improve response speed by “quieting” task irrelevant neural activity. For the same reason, PAC is absent in electrodes that are actively processing meaningful streams of visual data. These findings highlight separable aspects of the human attention system and how PAC contributes to both. Future directions include determining differences in PAC for attentional disorders like ADHD and neurological neglect.