and frequency in the past 90 days, and the Alcohol Use Disorders Identification Test (AUDIT), which measures alcohol use and consequences. IV-ASA measures included average and peak breath alcohol concentration (BrAC). The effect of rs16969968 was tested using a dominant model based on the presence of the A allele, and the influence of the rs16969968 polymorphism and smoking on alcohol phenotypes was assessed using t-tests and two-way ANOVA. RESULTS/ANTICIPATED RESULTS: There was a main effect of rs16969968 genotype with A-allele carriers (AA/AG) showing higher AUDIT-Dependence scores compared to the GG group. A main effect of smoking was observed on all the TLFB and AUDIT measures, with smokers showing greater alcohol consumption and problems compared to non-smokers. In the rs16969968 AA/AG group, smokers reported significantly more drinking days ($p<0.0001$), and greater number of drinks ($p<0.0001$), as well as higher AUDIT scores than non-smokers. IV-ASA measures did not show any difference between genotype groups or between smokers and non-smokers. DISCUSSION/SIGNIFICANCE OF FINDINGS: This study identifies both independent and interactive effects of CHRNA5 gene variation and smoking on alcohol drinking measures and provides strong evidence for the effect of smoking on alcohol drinking and its consequences.

**52500**

## Characterization of a Series of 1,4-diaryl-pyrazolo-pyridinones as Anti-Leishmanial Agents*

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ABSTRACT IMPACT: The first-line chemotherapies used to treat leishmaniasis are highly toxic intravenous antimonials yet drug resistance has begun to develop, causing the use of oral treatment options with high price tags; there is a strong need for new, safe, and effective chemotherapeutic agents to treat leishmaniasis. OBJECTIVES/GOALS: This study was conducted in order to identify novel chemical compounds that exhibit anti-leishmanial activity and to further characterize their efficacy and toxicity in in vitro and in vivo systems in the hopes of future chemotherapeutic developments. METHODS/STUDY POPULATION: A total of 28 unique 1,4-diaryl-pyrazolo-pyridinone (1,4-DAPP) compounds were synthesized and anti-leishmanial efficacy and host cell toxicity were determined using L. donovani mCherry-expressing amastigotes and THP-1 macrophages. Additional pharmacokinetic analyses of a potent 1,4-DAPP compound were conducted, revealing a potential metabolite structure. A select group of the novel compounds were screened in a cutaneous leishmaniasis (CL) murine model using L. major mCherry-expressing parasites and female Balb/C mice. The treatment consisted of 10 intralesional injections of compound over a period of 4 weeks, while lesion growth was monitored via fluorescence and manual measurements. RESULTS/ANTICIPATED RESULTS: Four experimental compounds had IC50 values less than 5 micromolar, providing similar anti-leishmanial activity to Miltefosine. Compound 9279817 had a clearance almost twice the rate of normal hepatic blood flow and had a relatively high volumes of distribution, indicating this compound is rapidly cleared and distributes into tissues. In vitro rat liver microsome assays suggest a rapid metabolism of 9279817 and MS/MS results suggest this metabolite is most likely formed via oxidation of the sulfur on the lower aryl ring. This sulfoxide metabolite has similar efficacy as the parent compound and does not exhibit toxicity in vitro. Three of the experimental compounds behaved similarly to the antimony positive control in the murine CL model. DISCUSSION/SIGNIFICANCE OF FINDINGS: This study revealed a novel structural class of compounds that have anti-leishmanial activity. Experiments show compounds with similar efficacy to Miltefosine while having significantly less cytotoxicity, suggesting that the 1,4-DAPP structural class could be further developed as a potential chemotherapeutic.

**60404**

## HIV Tat Induced Neuroinflammation

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ABSTRACT IMPACT: Demonstrate the role of astrocyte released MMPs in response to pathogenic HIV protein Tat. OBJECTIVES/GOALS: In the presence of the pathogenic HIV protein Tat, astrocytes have been demonstrated to adopt an inflammatory phenotype as well as release extracellular matrix degrading enzymes, MMPs. Our work aims to identify whether MMPs alter perineuronal net integrity and working memory in a mouse model of Tat-induced neuroinflammation. METHODS/STUDY POPULATION: Stereotaxic Injection: C57BL6/J mice were injected bilaterally with HIV-1 IIIB Tat 5ug in 5uL or Vehicle (0.2M KCl, 5mM DTT, 50mM Tris, pH 8.0), into the hippocampus (CA1; -1.9mm AP, ±1.6mm ML, -1.5mm DV from pial surface). All outcome measurements were performed 14-days post injection. Behavior: T-maze was used to assess working memory following Tat exposure. qRT-PCR: TaqMan probes were used according to manufacturer on extracted whole hippocampus mRNA. IF: GFAP and CD68 immunofluorescence was used to determine inflammation post injection. Inhibitory interneurons (parvalbumin positive) and peri-neuronal nets (WFA positive) were quantified. WB: Synaptosomes from whole hippocampi (Syn-PER) were isolated and synaptic excitatory markers were quantified (PSD-95, synaptophysin, GluR2a). RESULTS/ANTICIPATED RESULTS: Tat exposure resulted in impairments in working memory as measured by T-maze alternations and an increase in hippocampal mRNA expression of MMP-13 and IL-1β, indicative of neuroinflammation. We also noted an increase in GFAP+ injection site width 14 days post-Tat injection, suggesting robust gliosis. While there were no changes in the excitatory pre and post synaptic markers we found a significant decrease in the percent of PV+ interneurons with peri-neuronal nets (PNNs) following Tat exposure. Taken together, this preliminary data supports a role for inflammation and PNN integrity in Tat-induced alterations in working memory. DISCUSSION/SIGNIFICANCE OF FINDINGS: Our findings suggest that Tat contributes to cognitive impairment and that astrogliosis with elevated MMP-13 facilitates the degradation of peri-neuronal nets (PNNs) within the hippocampus. Since PNN degradation can alter neuronal circuitry future studies will focus on Tat-induced changes in hippocampal signaling.

**66108**

## Central Cholinergic Synapse Formation in Optimized Primary Septal "Hippocampal Co'' cultures

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ABSTRACT IMPACT: Optimization of primary septal-hippocampal co-cultures facilitates studying central cholinergic synapse formation and dysfunction OBJECTIVES/GOALS: Septal cholinergic

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