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-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.

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 peri-neuronal net integrity and working memory in a mouse model of Tat-induced neuroinflammation. METHODS/STUDY POPULATION: Stereotoxic Injection: C57BL/6J mice were injected bilaterally with HIV-1 IIIIB 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.

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 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 ICS50 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.

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
innervation to the hippocampus is critical for normal learning and memory and is severely degenerated in Alzheimer’s disease. To understand the molecular events underlying this loss, we optimized a primary septal-hippocampal co-culture system that facilitates the study of central cholinergic synapses. METHODS/STUDY POPULATION: We developed an optimized in vitro septal-hippocampal co-culture system modified from previously published protocols. Briefly, hippocampal and septal tissue were harvested from embryonic day 19 (E19) Sprague-Dawley rats, digested with 0.1% trypsin, and an equal number of cells from each region plated onto coverslips coated with poly-D-lysine and laminin at a final density of 300 cells/mm². We use immunostaining with validated primary antibodies and a fluorescent binding assay, together with confocal microscopy, to determine the structure of cholinergic synapses that are 1) native, 2) mammalian, 3) CNS derived, 4) comprised of physiological synaptic partners, and 5) developmentally mature. RESULTS/ANTICIPATED RESULTS: After DIV21, co-cultures maintained a healthy morphology. A subpopulation of neurons strongly expressed the cholinergic markers vesicular ACh transporter (vAChT), choline acetyltransferase (ChAT), and the high-affinity choline transporter (ChT1), whereas most neurons lacked vAChT expression and were presumably glutamatergic or GABAergic. The percentage of cholinergic neurons attained in the co-culture is ~5-7%. The size of these cholinergic neurons is strikingly similar to that reported for BFCNs in the intact brain (mean 30 μm, range 18-43 μm). All sampled cholinergic neurons (28/28 neurons) also expressed molecular machinery necessary for GABA release. Staining for a cholinergic postsynaptic marker shows that 63% of the contacts made with are synaptic. DISCUSSION/SIGNIFICANCE OF FINDINGS: Primary septal-hippocampal co-cultured neurons have not been exploited extensively in the field, perhaps due to the difficulty in maintaining such cultures for extended periods. Here, we optimized an in vitro septal-hippocampal co-culture system, a powerful tool to comprehensively analyze central cholinergic synapse formation and dysfunction.

**Data Science/Biostatistics/Informatics**

**Devices Engineered to Collect Exhaled Breath Condensate (EBC) and their Applications**

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ABSTRACT IMPACT: Human exhaled breath is rich in metabolomic content that represents pulmonary function and gas exchange with blood, which can provide insights into an individual’s state of health. OBJECTIVES/GOALS: Human exhaled breath is rich in metabolomic content that represents pulmonary function and gas exchange with blood. It contains a mixture of compounds that offer insight into an individual’s state of health. Here, we present two novel non-invasive breath sampling devices for use in basic medical practice. METHODS/STUDY POPULATION: The two breath samplers have a disposable mouthpiece, a set of inhale and exhale one-way flap valves to allow condensation of exhaled breath only, and a saliva filter. The housing is constructed out of Teflon®, a chemically inert material to reduce chemical absorbance. The first device condenses exhaled breath into a frozen condensate using dry ice pellets and the other is a miniaturized design that liquifies exhaled breath on a condenser surface with micropatterned features on a cooling plate. Both designs have individual strategic and analytical advantages: frozen exhaled breath condensate (EBC) has high retention of analytes and sample volume; EBC collected in liquid phase offers facilitated sample collection and device portability. RESULTS/ANTICIPATED RESULTS: We investigated if breath aerosol size distribution affects the types or abundances of metabolites. We modified the geometry of the first device to redirect aerosol trajectories based on size. The trapping of larger aerosols increases with filter length, thus altering the aerosol size distribution although no significant changes in the metabolite profiles were found. With the miniaturized device, metabolite abundances were measured in a small cohort of healthy control and mild asthmatic subjects. Differences among subjects were found, as well as main differences between control and asthmatic groups. All analyses of EBC were performed with liquid chromatography - mass spectrometry. Inflammatory suppression found in asthmatic subjects can be explained by prescribed daily use of inhaled corticosteroids. DISCUSSION/SIGNIFICANCE OF FINDINGS: Breath collection devices can be used in intensive care units, outpatient clinics, workplaces, and at home. EBC analysis has been used to monitor asthma and chronic obstructive pulmonary disease. It can be applied to infectious respiratory diseases (e.g. influenza, COVID-19) and for monitoring environmental and occupational chemical exposures.

**30432**

**Novel insights from single-cell RNAseq analysis of the stromavascular fraction of human adipose tissue**

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ABSTRACT IMPACT: Characterizing the cellular composition of human adipose tissue may contribute to the prevention and/or treatment of obesity-associated metabolic diseases. OBJECTIVES/GOALS: Our aims in this study were to use single-cell techniques 1. to characterize cell types within the stromavascular fraction of human adipose tissue, 2. to identify subsets of cells within each type (sub-clustering), 3. to identify gene sets and pathways that may provide information on the function and significance of each cell cluster. METHODS/STUDY POPULATION: Abdominal subcutaneous adipose tissue samples from n=6 healthy volunteers (1M, 5F, age 28-38 y, BMI 24.5-63.0 kg/m2) were collected by aspiration or during surgery. In 3 subjects, all females, paired femoral samples were also collected. After collagenase digestion approximately n=10,000 cells/sample were used for single-cell RNA sequencing using the 10X Genomics platform. After QC and downstream analysis, data were analyzed in Seurat v3.1.5. We identified first different cell types and then subclusters in an unbiased fashion. Gene Set Enrichment Analysis (GSEA) was used for pathway analysis. RESULTS/