

of trauma. RESULTS/ANTICIPATED RESULTS: We demonstrated that neighborhood disadvantage is associated with decreased volume and alterations of resting state functional connectivity of structures implicated in affect processing, including the hippocampus, amygdala, and ventromedial prefrontal cortex. These results held even after controlling for relevant individual variables, including acute post-traumatic stress symptoms and years of education. Moreover, individuals from disadvantaged neighborhoods exhibited heightened activation of these same structures in response to aversive stimuli. Thus, brain regions critical for recognizing and processing negative stimuli are susceptible to the effects of area-level socioeconomic factors. DISCUSSION/SIGNIFICANCE OF FINDINGS: The results offer additional evidence that neurobiological mechanisms clarify how stress 'gets under the skin'. Changes to key brain regions may explain why those living in disadvantaged neighborhoods are at a heightened risk of PTSD. Broadly, these findings should inform future policies and community-driven interventions aimed at reducing poverty.

77822

PSD95-nNOS interaction alters the basolateral amygdala transcriptome following fear conditioning: implications for molecular mechanisms underlying PTSD

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ABSTRACT IMPACT: This research takes a transcriptomic approach to parse genes and molecular pathways that underlie the fear memory circuitry and, in doing so, identifies therapeutic targets that can further be developed into treatments for fear disorders, such as post-traumatic stress disorder. OBJECTIVES/GOALS: Normal fear learning produces avoidance behavior that promotes survival, but excessive and persistent fear after trauma can lead to development of phobias and post-traumatic stress disorder (PTSD). Our goal is to understand the mechanism and identify novel genetic targets underlying fear responses. METHODS/STUDY POPULATION: Involvement of the basolateral amygdala (BLA) in fear acquisition is well established and requires activation of N-methyl-D-aspartic acid receptors (NMDARs). At a cellular level, NMDAR activation leads to production of nitric oxide (NO) by a process mediated by interaction between postsynaptic density protein 95 (PSD95) and neuronal nitric oxide synthase (nNOS). To elucidate mechanisms underlying the role of the PSD95-nNOS-NO pathway in conditioned fear, here we use rodent behavioral paradigms, pharmacological treatment with a small molecule PSD95-nNOS inhibitor, RNA-sequencing, and an AAV-mediated knockdown of the nNOS gene in the BLA. RESULTS/ANTICIPATED RESULTS: We show that treatment with ZL006 attenuates rodent cued-fear consolidation. Additionally, with RNA-sequencing, expression of 516 genes was altered in the BLA following fear expression; of these genes, 83 were restored with systemic ZL006 treatment. Network data and gene ontology enrichment analyses further revealed that cGMP effects, insulin-like growth factor binding, and cognition-related pathways were significantly altered. Finally, we show that a BLA-specific knockdown of nNOS attenuates cued fear consolidation, without adverse effects on other memory and motor behaviors. DISCUSSION/SIGNIFICANCE OF FINDINGS: Via a model of NMDAR-mediated fear consolidation, our results reveal novel pathways and genetic targets that underlie plasticity of fear memory circuitry. Importantly, these results will inform future therapeutic strategies for targeting fear related disorders like PTSD.

96101

Temporal Evolution of Neural Activity in Human Brain Organoids

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ABSTRACT IMPACT: This study will provide the essential characterization of intrinsic neural activity in human brain organoids, both at the single cell and network levels, to harness for translational purposes. OBJECTIVES/GOALS: Brain organoids are 3D, stem cell-derived neural tissues that recapitulate neurodevelopment. However, to levy their full translational potential, a deeper understanding of their intrinsic neural activity is essential. Here, we present our preliminary analysis of maturing neural activity in human forebrain organoids. METHODS/STUDY POPULATION: Forebrain organoids were generated from human iPSC lines derived from healthy volunteers. Linear microelectrode probes were employed to record spontaneous electrical activity from day 77, 100, and 130 organoids. Single unit recordings were collected during hour-long recordings, involving baseline recordings followed by glutamatergic blockade. Subsequently, tetrodotoxin, was used to abolish action potential firing. Single units were identified via spike sorting, and the spatiotemporal evolution of baseline neural properties and network dynamics was characterized. RESULTS/ANTICIPATED RESULTS: Nine organoids were recorded successfully ($n=3$ per timepoint). A significant difference in number of units was seen across age groups ($F(2,6) = 6.4178, p = 0.0323$). Post hoc comparisons by the Tukey HSD test showed significantly more units in day 130 (51.67 ± 14.15) than day 77 (16.33 ± 14.98) organoids. Mean firing rates were significantly different in organoids based on age, with drug condition also trending toward significance ($F(6,12) = 9.97, p = 0.0028$ and $p = 0.08$ respectively). Post hoc comparisons showed a higher baseline firing rate in day 130 ($0.99\text{Hz} \pm 0.30$) organoids than their day 77 counterparts at baseline ($0.31\text{Hz} \pm 0.066$) and glutamate blockade ($0.31\text{Hz} \pm 0.045$). Preliminary network analysis showed no modularity or small-world features; however, these features are expected to emerge as organoids mature. DISCUSSION/SIGNIFICANCE OF FINDINGS: Initial analysis of brain organoid activity demonstrates changes in single unit properties as they mature. Additional work in this area, as well as further network analyses, will confer better sense of how to rationally utilize brain organoids for translational purposes.

Clinical Epidemiology

Basic Science

60941

Vaginal pH predicts cervical intraepithelial neoplasia-2 regression in women living with human immunodeficiency virus

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ABSTRACT IMPACT: The potential to use vaginal pH as a low cost, non-invasive diagnostic test at the point of CIN2 diagnosis to predict

worsening of cervical disease. OBJECTIVES/GOALS: We previously reported that persistence/progression of cervical intraepithelial neoplasia-2 (CIN2) was uncommon in women living with HIV (WLH) from the Women's Interagency HIV Study (WIHS, now MWCCS). Here we examined additional factors that may influence CIN2 natural history. METHODS/STUDY POPULATION: A total of 337 samples from 94 WLH with a confirmed CIN2 diagnosis were obtained from the MWCCS. 42 cervicovaginal HPV types and 34 cervicovaginal cytokines/chemokines were measured at CIN2 diagnosis (94 samples) and 6-12 months prior to CIN2 diagnosis (79 samples). Covariates, including CD4 count and vaginal pH, were abstracted from core MWCCS visits. Logistic regression models were used to explore CIN2 regression (CIN1, normal) vs. persistence/progression (CIN2, CIN3). Log rank tests, Kaplan Meier method, and Cox regression modeling were used to determine CIN2 regression rates. RESULTS/ANTICIPATED RESULTS: The most prevalent HPV types were HPV54 (21.6%) and 53 (21.3%). 33 women (35.1%) had a subsequent CIN2/CIN3 diagnosis (median 12.5 years follow-up). Each additional hr-HPV type detected at the pre-CIN2 visit associated with increased odds of CIN2 persistence/progression (OR 2.27, 95% CI 1.15, 4.50). Higher vaginal pH (aOR 2.27, 95% CI 1.15, 4.50) and bacterial vaginosis (aOR 5.08, 95% CI 1.30, 19.94) at the CIN2 diagnosis visit associated with higher odds of CIN2 persistence/progression. Vaginal pH >4.5 at CIN2 diagnosis also associated with unadjusted time to CIN2 persistence/progression (log rank p=0.002) and a higher rate of CIN2 persistence/progression (adjusted hazard ratio [aHR] 3.37, 95% CI 1.26, 8.99). Cervicovaginal cytokine/chemokine levels were not associated with CIN2 persistence/progression. DISCUSSION/SIGNIFICANCE OF FINDINGS: We found relatively low prevalence of HPV16/18 in this cohort. Elevated vaginal pH at the time of CIN2 diagnosis may be a useful indicator of CIN2 persistence/progression and the rate of persistence/progression.

92180

Prevalence of *Clostridioides difficile* strains found in Texas soil

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ABSTRACT IMPACT: This work investigates *C. difficile* strains in soil as a potential exposure for gut colonization and community-acquired infection of *C. difficile*. OBJECTIVES/GOALS: Identifying environmental sources of *C. difficile* can inform how non-hospital reservoirs can potentially contribute to *C. difficile* exposure and subsequent gastrointestinal colonization. The objective of the study was to identify *C. difficile* and toxin genes across various soil sources. METHODS/STUDY POPULATION: This was a cross-sectional study utilizing soil samples obtained throughout Texas, USA. All samples were collected between August and November of 2019 and 2020. Samples were taken from human and animal high contact areas, such as recreational parks. Samples were stored at -80°C until processing. DNA extractions were performed using the DNeasy Powersoil Pro Kit (Qiagen) per manufacturer's instructions. Real-time PCR was also performed on extracted DNA using the Microbial DNA qPCR Multi-Assay Kit for *Clostridium difficile* Pathogenicity (Qiagen) for the identification of *C. difficile*, toxin A (TcdA), and toxin B (TcdB) genes. RESULTS/ANTICIPATED RESULTS: A total of 137 soil samples including dry dirt, sand, and wet soil near water sources were collected and processed for the presence of *C. difficile*. These included samples from parks and

trails (42.3%), water sources (36.5%), and other public spaces (21.2%). *C. difficile* was identified in 59 (43.1%) soil samples: 6 (4.4%) with Toxin A and 2 (1.5%) with toxin B production. *C. difficile* was most prevalent among samples taken from parks and trails (50.0%), followed by other public spaces (48.3%), and water sources (32.0%). The median (IQR) Cq value for the *C. difficile* gene was 39.24 (33.45-40.47) among samples that tested positive. DISCUSSION/SIGNIFICANCE OF FINDINGS: We identified a high prevalence of *Clostridioides difficile* in soil samples, though toxin gene detection prevalence was low. Future studies will analyze other sources, including water and varying surface samples to obtain a comprehensive view of *C. difficile* in the environment.

Clinical Trial

44613

Plan for a Retrospective Evaluation of a Multi-Modal Weight-centric Prediabetes Intervention

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ABSTRACT IMPACT: By demonstrating the feasibility of a multi-modal, interdisciplinary intervention for prediabetes, the current project aims to provide a template for the prevention of diabetes and associate comorbid conditions. OBJECTIVES/GOALS: To determine if a multi-modal, interdisciplinary intervention delivered to a group of prediabetic patients will result in reduced rates of diabetes progression. This project is a retrospective evaluation that will exam the feasibility and possibly efficacy of this intervention. METHODS/STUDY POPULATION: We will enroll 50 participants for the clinic, aged 21-60 inclusive. Patients will have a Body Mass Index >27kg/m² with a diagnosis of prediabetes. Patients must be non-pregnant, using approved contraception, and agree to not become pregnant for 1 year after enrollment. After enrollment, the initial treatment period is for 1 year and includes a 12 week low calorie diet plan, a 6-month intensive behavioral and lifestyle modification plan followed by a 6 month behavior reinforcement extension. Weight management medications may be used if appropriate for the patient from a clinical perspective during the 6-month intensive behavioral/lifestyle modification. RESULTS/ANTICIPATED RESULTS: It is anticipated that there will be decreased weight with a mean weight loss goal of approximately >10%. Furthermore, it is expect that there will be improvement of other markers of metabolic disease. These include improvement of lipid values (LDL-C, HDL-C, Triglycerides, Total Cholesterol) as well as blood pressure with expected blood pressures of below 130/80 in greater than 50% of participants. Finally, It is expected that 50% or greater participants will have improvement of glycemic control. It is anticipated that greater than 50% of participants will have improvement of glycemic control and achieve normoglycemia. These values will be determined based upon fasting glucose or A1c. DISCUSSION/SIGNIFICANCE OF FINDINGS: The significance of this intervention is enormous. By demonstrating feasibility in this trial, we can work toward both assessing efficacy and possibly dissemination of this model program. If these interventions provide durable changes at scale, this could help slow the epidemic of obesity and obesity related comorbid conditions.