Presentations:

Keynote speaker: *Are brain circuits for emotional behavior organized?*

Anna Wang Roe, PhD

2. Effects of 40 Hz tACS Stimulation on Cognition and Symptoms in Patients with Schizophrenia

Robert C Smith*, Xinyi Cao, Henry Sershen, Yuanyu Lu, Hua Jin, John M Davis, Chunbo Li

Background: Transcranial alternating current stimulation is a technique of brain stimulation to modify neural activity and plasticity by entraining more specifically defined cortical oscillation frequencies. Reviews of tACS effects in schizophrenia suggest that tACS stimulation may affect symptoms and cognition in schizophrenia, from open label trials and case reports, but there have been few RCTs, especially with tACS targeting γ band oscillations. Methods: The current study was a randomized double-blind trial of 10 sessions of active vs. sham tACS 40 HZ gamma band stimulation in 50 patients with schizophrenia conducted in Shanghai China. The primary outcome measure was change in the overall composite score on the MATRICS battery with multiple secondary outcome measures of cognition and symptoms. tACS stimulation was performed using a star-stim stimulator. Placement of stimuli electrodes was: a) active electrode over the left DLPFC (F3), and b) reference electrode over the right parietal region (P2). Comparison to baseline values were done with evaluations 1-2 days after completion of 10 tACS sessions and 2 and 4 weeks later. The main analysis was a mixed model analysis of difference scores from baseline using SAS mixed procedure with three time points (immediately after 10 TACS sessions, 2 weeks later, and 4 weeks later, and also on-line during stimulation session 1). Results: The analyses showed no statistically significant (P<.05) effects on improvement in any of the cognitive measures or PANSS rated positive or negative symptoms. There was a trend (P<.06) for the MATRICs Domain of verbal learning to show greater improvement compared to sham within 1-2 days after the 10 tACS sessions. tACS was well tolerated and side effects were minimal. Results of guess questionnaire showed effective blinding of subjects. Conclusion: The results of this RCT do not support the efficacy of 40 Hz γ tACS stimulation to improve cognition or symptoms in patients with chronic schizophrenia. Three RCTs using alpha or theta band tACS stimulation and other reports of theta band stimulation have shown some more positive effects in patients with schizophrenia and should be investigated further to define the optimal stimulation parameters for tACS trials in patients with schizophrenia.

3. Profiling lamina specific pyramidal neurons using postmortem human formalin fixed paraffin embedded frontal cortex tissue in combination with digital spatial profiling

Aleksandra Stanisavljevic*, Kyrillos W. Ibrahim, Philip H. Stavrides, Christopher Bare, Melissa J. Alldred, Adriana Heguy, Ralph A. Nixon, and Stephen D. Ginsberg

Digital spatial profiling (DSP) is an innovative approach to perform RNA sequencing (RNA-seq) and bioinformatic analysis combined with precise spatial information from individually identified regions or cell types, including neurons. This approach enables expression profiling linking RNA-seq data to spatially characterized samples utilizing tissue bound probes. We employ the GeoMx DSP system to allow spatial characterization of transcriptomic data from lamina specific pyramidal neurons as well as cortical laminar ribbons containing neurons and admixed cell types in human postmortem brain tissue. We established a robust and reproduceable protocol using human postmortem formalin fixed paraffin embedded (FFPE) frontal cortex tissue from nondemented human control brains. Layer III (L3) and Layer V (L5) pyramidal neurons from Brodmann area 9 were identified with the neuronal marker Ca2+/calmodulin-dependent protein kinase II and selected for probe collection. The approach significantly reduced the amount of FFPE tissue needed for

vigorous single population RNA-seq using the GeoMx DSP platform. We demonstrate ~20 identified L3 or L5 pyramidal neurons or one lamina-specific cortical ribbon from a single 5 µm thick section is sufficient to generate robust RNA-seq reads. Bioinformatic analysis of isolated neurons and ribbons showed notable similarities and differences reflective of the single neuron and multiple admixed cell types within the former and latter, respectively. This DSP assay provides high resolution RNA-seq data demonstrating utility and versatility of GeoMx platform for individually characterized neurons as well as isolated cortical ribbons within postmortem FFPE human brain tissue for downstream analyses.

4. Effect of Cognitive Reserve on Plasma Biomarkers of Alzheimer's Disease in Non-Demented Elderly

Chelsea Reichert Plaska*, Davide Bruno, Sang Han Lee, Giovanna Novi, Andrew Orefice, Henrik Zetterberg, Kaj Blennow, Nunzio Pomara

Education, a proxy for cognitive reserve (CR), has long been accepted as a protective factor against cognitive decline related to Alzheimer's disease (AD). In both normal and impaired elderly, higher CR is associated with less decline, even with notable AD pathology, including amyloid and tau. The relationship between CR and plasma AD biomarkers is unknown, so we examined this in nondemented elderly. We hypothesized that higher CR would associate with lower AD-risk blood biomarkers. Cognitively unimpaired participants (n=530; Aged 50-85, MMSE=24+) recruited as part of the Memory Evaluation Research Initiative, completed a cognitive battery (e.g., Memory-AVLT, Executive Function-TMTB). Plasma Aβ42, Aβ40, and Ptau231 concentrations were measured using Simoa assay. Plasma Aβ42/Aβ40 and Ptau/Aβ42 were calculated. Years of education were used as a proxy for CR: Low (CR-L; 12 years or less), Moderate (CR-M; 13-16), High (CR-H; 17 or more). One-way ANOVA examined the effect of CR on plasma indices, which revealed a significant CR effect f =3.54 (p=0.030) on Aβ42/Aβ40. From Bonferroni post-hoc comparison, CR-L had lower Aβ42/Aβ40 than CR-H. A trend with CR-L and higher Ptau/Aβ42, than CR-H, p=0.057 was also found. PROCESS was used for a moderation analysis between plasma indices and cognition, with CR as the moderator. Moderation analyses showed that CR moderated the relationship between Ptau/Aβ42, not Aβ42/Aβ40, and AVLT total (CR-L vs -M: b=1.84, SE=0.58, p=0.001 and CR-M vs -H: b=2.70, SE = 0.58, p< 0.01), and delayed recall (CR-L vs -M: b=5.27, SE=1.71, p=0.002 and CR-M vs -H: b=8.64, SE=1.72, p< 0.01), as well as TMTB (CR-L vs -M: b = -27.82, SE = 9.62, p=0.004 and CR-M vs -H: b = -42.23, SE = 9.59, p< 0.01). Thus, the negative effects of Ptau/Aβ42 on cognitive performance seem to be mitigated by CR. Our results suggest that, in non-demented elderly, lower CR is associated with lower Aβ42/Aβ40 and higher Ptau/Aβ42 consistent with increased brain Aβ and tau burden. In addition, CR also moderated the relationship between plasma Ptau/Aβ42, but not Aβ42/Aβ40, and cognition. Future studies should determine if low CR is associated with an accentuation of brain Aβ and tau pathology in nondemented older adults.

5. Students and Caregivers' Perspectives on Cultural Influences associated with Social Anxiety among Black Youth

Talita Ahmed*, Melissa Escobar*, Hannah Thomas, Samantha Coyle, Carrie Masia

Social anxiety disorder (SAD) has a high prevalence rate among youth, with 9.1% of adolescents meeting the diagnostic criteria (Merikangas et al., 2010). Black adolescents have similar or higher social anxiety levels than their White counterparts (Merikangas et al., 2010). However, Black youth may encounter unique socio-cultural challenges, including experiences of discrimination or community stigma, which can influence their presentation of SAD (Hunter & Schmidt, 2010; Coyle-Eastwick et al., 2024). Despite awareness of cultural aspects influencing SAD, there remains a significant gap in the literature regarding specific cultural factors within Black communities that may affect socially anxious Black youth. Thus, this study sought to understand Black caregivers' perceptions of cultural factors (i.e., barriers to mental health treatment, recommendations for a school-based intervention) that may contribute to the prevalence and expression of SAD among

Black youth. Individual interviews (n = 7; Mage = 44.14; 100% female) and focus groups (n = 6; Mage = 47.67; 100% female) were conducted with Black caregivers recruited from an urban high school in the Northeastern United States. Thematic analysis was used to develop a codebook of overarching themes and subthemes. Qualitative results shed light on perceived cultural factors that may be related to the development of SAD in Black youth. Specifically, parents mentioned that adolescents' social comparison, both in-person and on social media platforms, was a leading contributor to SAD symptoms. Additionally, caregivers described that exposure to community violence, publicized anti-Black violence, discrimination, and internalized racism contribute to a fear of negative evaluation in social interactions. Caregivers also noted that SAD in Black youth may be expressed as anger and that differences in the presentation of mental health symptoms in Black youth have led to misdiagnosis or inappropriate treatment. These findings underscore the critical role of cultural factors in shaping the presentation of SAD in Black youth, highlighting the need to consider cultural factors in assessment and intervention development. Findings from this study will inform the design of a culturally responsive, evidence-based intervention addressing SAD in black youth, which has been implemented in an open pilot and randomized controlled trial in an urban high school.

Posters:

1. Cerebellar mitovesicle secretion is altered in spinocerebellar ataxia and regulated by the activity of the potassium channel Kv3

Pasquale D'Acunzo, Yalan Zhang, Leonard K. Kaczmarek, Efrat Levy

INTRODUCTION: Kv3 voltage-gated potassium channels mediate the rapid repolarization of fast-firing neurons, including Purkinje cells. Dominant negative mutations in the Kv3.3 subunit trigger uncontrolled duration and frequency of action potentials and cause spinocerebellar ataxia type 13 (SCA13), a neurodegenerative disorder characterized by Purkinje cell loss and ataxia of gait. We have previously shown that Purkinje neurons in the cerebellum of a mouse model of SCA13 bearing the disease-causing mutation Kv3.3-G592R are characterized by several endocytic abnormalities, including lysosome impairments, accumulation of undigested endocytic inclusions, and higher number of multivesicular bodies (MVBs). These abnormalities are associated with higher secretion of exosomes, without affecting ectosomes. We hypothesized that in Kv3.3-G592R mice the concomitant presence of ion imbalance caused by the lower activation of Kv3 channels and downstream mitophagy block caused by endolysosomal abnormalities lead to alterations in mitochondria that reverberate to changes in mitovesicles, the mitochondria-derived extracellular vesicles (EVs). Therefore, we investigated changes in mitochondria and mitovesicles in the brain of Kv3.3-G592R and Kv3.3 knock-out (KO) mice, as compared with wild-type (WT) littermate controls. METHODS: EVs were isolated from cerebella and right hemibrains of Kv3.3-G592R, Kv3.3-KO, and WT mice using a high-resolution iodixanol gradient, and analyzed by transmission electron microscopy (TEM), NTA, total protein content, and Western blotting. Mitochondria were studied in the left hemibrains and cerebella by Western blotting and TEM. RESULTS: The number of mitovesicles in the cerebellar extracellular space was inversely correlated with the Kv3 channel activity: lowest in WT, higher in Kv3.3-G592R (impaired activity), and highest in Kv3.3-KO (no activity) mice. Mitochondrial fragmentation markers (phospho-Drp1 Ser616, Ser637) followed the same trends, consistent with a higher production and secretion of mitovesicles. Under TEM, Kv3.3 channels were found to be enriched at the contact sites between plasma membrane and mitochondria, both in WT and Kv3.3-G592R mice, suggesting a direct regulation of the mitochondrial ion metabolism mediated by Kv3.3. CONCLUSIONS: Mitovesicles are a previously unidentified player altered in SCA13 cerebella. Mechanistically, we describe a novel pathway in vivo linking mitochondria and mitovesicle homeostasis to plasma membrane depolarization, shedding new light on the still obscure biology that regulates mitovesicle secretion in the brain.

2. Frontal cortex pyramidal neurons display laminar specific gene expression in Down syndrome

Melissa J. Alldred, Kyrillos Ibrahim, Harshitha Pidikiti, Sang Han Lee, Adriana Heguy, Thomas Wisniewski, Elliott J. Mufson, Grace E. Stutzmann, and Stephen D. Ginsberg

Down syndrome (DS) is caused by triplication of human chromosome 21 (HSA21), resulting in ~1/700 live births per year. Individuals with DS present with altered craniofacial features and deficits in learning, language, memory acquisition and consolidation. By early mid-life (mid-30's-40's), the brain of those with DS display extensive Alzheimer's disease (AD) pathology including amyloid plaques due to the triplication of the amyloid precursor protein (APP) gene, located on HSA21. Additionally, during development individuals with DS have reduced proliferation and disorganization of corticocortical pyramidal projections neurons located in laminar III and V in the frontal cortex {e.g., Brodmann area 9 (BA9)}. BA9 is a hub of the default mode connectome that plays a role in executive memory function. We postulate that trisomy alters unique differentially expressed genes (uDEGs) in layer III compared to layer V, which play a role in executive memory dysfunction in individuals with DS. Here, human frontal cortex (BA9) layer III and V pyramidal neuron gene expression was interrogated via laser capture microdissection combined with single population RNA-sequencing from aged individuals with DS with confirmed AD pathology compared to age- and sex-matched controls. Bioinformatic inquiry revealed that layer III pyramidal neurons displayed ~2,966 uDEGs, while layer V displayed ~1,999 uDEGs ($p<0.05$) in DS compared to controls. These uDEGs revealed multiple pathways implicating functional neuronal differences, including metabolism and production of reactive oxygen species specifically dysregulated layer III DS pyramidal neurons, while layer V DS pyramidal neurons were linked to upregulated pathways including metabolism of phospholipids and sphingolipid metabolism. These findings indicate that trisomy differentially affects gene expression in layers III and V BA9 projection neurons in aged individuals with DS, which may inform the molecular and cellular pathogenesis of DS and AD.

3. Spatio-Temporal Signatures of Cognitive Recovery After Pediatric Arterial Ischemic Stroke

Karl-Heinz Nenning, Florian Ph.S. Fischmeister, Astrid Novak, Rainer Seidl, Ting Xu, Gregor Kasprian, Lisa Bartha-Doering, Kathrin Kollndorfer

Childhood arterial ischemic stroke is a severe disorder that can cause lasting cognitive impairments, particularly in executive functions. Early research assumed an improved outcome due to better neuronal plasticity in childhood stroke patients as compared to adults. However, more recent studies indicate similar rates of disabilities and cognitive impairment, with widespread brain network disruptions underlying these deficits. Here, we used resting-state fMRI to study alterations in functional brain dynamics and their association with cognitive outcome in children and adolescents after childhood stroke. We used co-activation pattern analysis to characterize five recurring brain states and their temporal properties in a cohort of 16 patients and 17 age-matched controls. We found that in pediatric stroke patients a specific brain state characterizing the frontoparietal network was more prevalent and more frequently involved in state transitions. This was paralleled by lower occurrence rates of a brain state that relates to default mode network deactivation. Moreover, our analysis showed that these dynamics relate more to the extent to which functional networks are impacted by the lesion than to lesion size alone. Taken together, our findings suggest that disrupted brain dynamics due to childhood stroke relate to cognitive performance, and that the location of a focal lesion can have wide-ranging implications on brain state dynamics.

4. Insights into early hyperexcitability in an Alzheimer's disease mouse model from PIP-seq

Gabriel Stephens, David Alcantara-Gonzalez, Helen Scharfman

Hyperexcitability is Alzheimer's disease (AD) has captured attention because it appears to occur early in the disease and contribute to cognitive impairment and increased amyloid beta (Abeta). In our prior work with AD mouse models, we found hyperexcitability extremely early in life, just 1 month of age. In vivo, aberrant activity emerged mainly as interictal spikes (IIS) in sleep, with later

onset of rarer seizure events. Silicon probe recordings suggested IIS were generated in the dentate gyrus (DG). In vitro patch-clamp studies identified that there was increased activity in some of the cell types in the DG at the 1 month age, but why this elevated activity occurred was not clear. To gain insight, we examined DG gene expression in wild type (WT) vs. Tg2576 mice, which overexpress a mutant form of the precursor to Abeta (APPSwe). Single-nucleus RNA sequencing (snRNA-seq) was performed using DG from slices of 1 month-old WT and Tg2576 mice (n=3/group). We used Particle-templated Instant Partition Sequencing (PIP-seq) from the NYU Genome Technology Center, which also processed and sequenced the isolated DG nuclei. High quality nuclei were then selected and annotated using validated marker genes that identify 9 major DG cell types. We found similar proportions of the 9 cell types in both Tg2576 and WT mice at yields consistent with prior studies. Notably, differentially expressed gene (DEG) analysis identified thousands of DEGs, many of which may be involved in regulation of excitability, including known risk genes for AD and epilepsy. Surprisingly, a set of shared genes that can regulate excitability were altered across all 9 major cell types. These findings will help develop hypotheses for DG hyperexcitability and could provide new insights into hyperexcitability in clinical AD.

5. Deficiency of calcium-activated potassium channel (KCA3.1) attenuates neonatal ethanol-induced acute neurodegeneration in the mouse cortex and hippocampus

Mariko Saito, Shivakumar Subbanna, Anusha Nalluri, Audrey Hashim, Brandon Marino, John Smiley, Donald Wilson, Bhaskar Das

Ethanol exposure in postnatal day 7 (P7) mice induces acute neurodegeneration and long-lasting neuroanatomical and behavioral abnormalities, providing a third trimester model of FASD. P7 ethanol-induced acute neurodegeneration is accompanied by acute as well as long-lasting glial activation. However, roles of glial activation have not been fully elucidated. Although acute microglial activation seems beneficial by phagocytosing degenerating neurons, previous literature indicates that anti-inflammatory agents suppress microglial activation and exert neuroprotection. The calcium-activated potassium channel KCa3.1, which participates in the regulation of membrane potential by controlling the passage of K+ by the inward flow of Ca2+, has been found in activated microglia and considered one of the key proteins involved in glial activation. In several neurodegenerative diseases and brain injuries, KCa3.1 increases in glia, and the pharmacological or genetic blockade of KCa3.1 ameliorates microglial activation and promotes neuroprotection. Our previous studies indicated that KCa3.1 levels increased in the cortex and hippocampus 24h after ethanol injection into P7 mice. Also, intracerebroventricular (icv) injection of a KCa3.1 inhibitor BT563 (developed by Dr. Das) prior to ethanol injection reduced cleaved caspase-3 (CC3) formation in the cortex and hippocampus. The present studies indicated that not only icv but also intraperitoneal injection of BT563 attenuated CC3 formation 8h and 24h after P7 ethanol treatment when analyzed by immunoblotting. Also, immunohistochemistry showed that densities of Fluoro-Jade positive (degenerating) neurons in the hippocampus were decreased by BT563 treatment when analyzed 24h after ethanol injection. When senicapoc, a known KCa3.1 blocker, was used in place of BT563, P7 ethanol-induced CC3 formation was also reduced in the hippocampus. Furthermore, P7 ethanol induced less numbers of CC3 positive cells in the cortex of KCa3.1 knockout (B6;129S1-Kcnn4tm1Jemn/J) mice compared to those in the wild-type mice. Immunoblot results also indicated reduced CC3 formation by P7 ethanol in the knockout mice. KCa3.1 expression enhanced by P7 ethanol was mainly localized in glia or small apoptotic neurons in the parts of cortex and hippocampus where degenerating neurons were abundant. These results suggest that KCa3.1 may be involved in ethanol-induced neurodegeneration partially through glial activation and may be considered a target for clinical applications for FASD.

6. Single-cell Transcriptomic Maps Identify a Novel Pathway to Regulate Brain Plasticity

Yotam Sagi, Betty Bigio, Shofiul Azam, Amarjyot Singh, Neelu John, Danielle Zelli, Timothy Lau, Paolo de Angelis, Masahiro Okamoto, Hideaki Soya, Carla Nasca

Histone acetylation regulates every physiological function and is dysregulated in multiple human diseases. The source of acetyl groups for histone acetylation is thought to rely on nuclear production. We demonstrate that a novel intracellular mechanism from mitochondria to nuclei maintains neuronal plasticity and is impaired in human diseases. Using calcium imaging, pharmacological probes and Cre-dependent loss of function approaches to perturb this pathway we showed that a specific mitochondrial process in vDG glutamatergic neurons primes histone acetylation to maintain neuronal activity dynamics of the vDG microcircuitry during key behaviors. Chronic stress, a primary risk factor for multiple human diseases, disrupts this mitochondrial pathway. To accelerate the path of this new mechanism to clinical applications, we showed that this pathway in vDG is regulated by exercise and fluoxetine together, but not exercise or fluoxetine alone, and is a key neural substrate for rapid antidepressant response as shown by single-cell transcriptomic maps.

7. Sleep and cognitive performance in individuals with Chronic Schizophrenia participating in Computerized Cognitive Remediation Therapy

Anzalee Khan, Amy Polinsky, Beverly Insel, Jean-Pierre Lindenmayer

Background: Individuals with schizophrenia present with substantial neurocognitive deficits that have been associated with poor functional outcomes. Although prior studies have identified a number of predictors of neurocognitive deficits in schizophrenia, there is limited research on sleep despite sleep disturbances and its relationship to neurocognition. Since sleep and neurocognition change with age, a greater understanding of how the relationship between sleep and cognition changes over time is needed. This study explored the mediating effect of sleep disturbances (i.e., number of hours of sleep, sleep interruption, prn medications for sleep) on the relationship between sleep and cognitive impairment in individuals participating in computerized cognitive remediation on inpatients with schizophrenia. Methods: In the analysis, we included the 51 participants diagnosed with schizophrenia and aggressive behavior and had data on the three sleep-related measures and MCCB domain scores obtained at the first time point. To explore the association between sleep and cognition, linear regression was performed separately for each of the seven MCCB domains as dependent variables and the sleep-related measures as independent variables. Data on sleep (number of hours of sleep prior to cognitive testing, sleep interruptions, whether prn medications were given for sleep) were collected from medical charts. All participants were enrolled in a computerized Cognitive Remediation program. Results: Participants' mean age was 38 years and the majority were male (84%), Black (49%), and received a high school education or less (75%). Moreover, 61% reported having at least 8 hours of sleep, 67% reported having uninterrupted sleep, and 63% requested PRN medication for sleep. Using linear regression to explore the association between the MCCB domains and sleep, reporting ≥ 8 hours of sleep was significantly associated with higher MCCB domain scores, except for Social Cognition. Specifically, sleeping less than 8 hours was associated with lower MCCB Composite score (Ɓ=-12.88; 95% confidence interval [CI]: -20.04, -5.71; p =0.0007). Interrupted sleep was significantly negatively associated with the MCCB domains, except for Attention and Social Cognition. Correspondingly, PRN for sleep was significantly associated with lower MCCB scores, except for Social Cognition. Participants who requested PRN for sleep had significantly lower MCCB Composite score than those who did not request PRN (Ɓ =-18.64; 95% CI: -24.70, -12.58; p <0.0001). Conclusions: Poor sleep quality decreased cognitive performance among the participants. The relationship between sleep and cognition affects response to cognitive remediation therapy. Results of this study indicate that cognitive function should be monitored in individuals with sleep interruption and lower sleep duration. Future studies are needed to examine the mechanisms of the association between sleep disturbances and cognitive performance during clinical trials for cognitive treatments.

8. AI-driven platform to Measure Negative Symptoms of Schizophrenia Through Facial and Acoustic Analysis

Jean-Pierre Lindenmayer, Beverly Insel, Danyah Nadim, Anzalee Khan, Rachael Mishkind, Vikram Ramanarayanan, Kothare Hardik, David Suendermann-Oeft

Background: The digital analysis of facial and acoustic expressions is an evolving field that finds several clinical applications. One of these applications is the study of facial and speech productions in individuals with schizophrenia, which represent negative symptoms of this illness. Features of expressive negative symptoms include the reduction of facial movements and emotional facial expressions. Current methods of assessing negative symptoms depend on verbal report from patients and/or caregivers and a clinical interview. These interviews can be insensitive to change in treatment, subjective, and require extensive training and are subject to cultural effects. Digital assessment platforms can be used to supplement clinical interviews for more objective and precise measurements. This study assesses the psychometrics of a novel artificial intelligence (AI) system analyzing facial and acoustic features when compared to rater administered traditional rating scales of negative symptoms in schizophrenia.
Method: Inpatients with schizophrenia (SZ: n = 70) and healthy controls (N=60) completed a brief 8-minute assessment using the AI-driven platform. Each participant was tested and rated twice by the same clinician within a one week period in order to assess test-retest reliability. For the AI software, participants were each provided a valence-neutral sentence to read; participants then engaged in free speech where they were asked open ended probes by an avatar designed to be emotionally-ambiguous in valence and content (e.g., tell me about yourself?). For the SZ group AI vocal/facial software, PANSS, BNSS, CDSS, CGI-S, AIMS, SAS, BARS were performed. Concurrent, convergent, divergent, and discriminative validity were assessed. For the HC group, individuals received the AI vocal/facial software but did not complete clinician-administered assessments. We initially selected speech and facial metrics that differed significantly between inpatients with SZ and the HC using the Kruskal-Wallis test. Metrics that were highly correlated (>0.90) were eliminated. A factor analysis produced three factors with values loading <0.40 deleted. The remaining metrics were correlated with the corresponding clinical assessments by Spearman's rank correlation. Results: The mean PANSS total score was 71.73 (10.91; range = 50-94). The mean BNSS Total Score was 37.90 (SD = 9.03; range 15-61). The mean Marder Negative Symptom Factor (MNSF) score was 21.28 (SD = 3.98; range – 10-28). A factor analysis resulted in three factors (Speech Factor (speech rate, intonation, articulation, and fluency), Facial Expressivity Factor (facial gestures and movement), Text Factor (in reading)). Factor 1 (Speech) was significantly inversely correlated with the BNSS Alogia Spontaneous (t=-0.32, p=0.009). Factor 3 (Text) was significantly inversely correlated with the PANSS Negative Score (r=-0.34, p=0.005). The Factor 2 (facial expressivity) produced during a reading passage was significantly inversely correlated with MNSF (r=-0.31, p=0.011) and the PANSS Negative subscale (r=-0.42, p=0.004) with both the clinician assessments and AI system showing reduced facial expressivity. Speech metrics when reading a paragraph was significantly correlated with higher PANSS Negative subscale (r=0.33, p=0.006). The PANSS Total Score was significantly inversely correlated with more speech (r=-0.32, p=0.008) and speech speaking time (r=0.33, p=0.006). Additionally, Facial movements during the reading passage task was significantly inversely correlated with the BNSS Asociality Internal (r=--0.32, p=0.008), the BNSS Asociality (r=-0.32, p=0.008), and the BNSS Total Score (r=-0.32, p=0.008). indicating reduced expressiveness in communication identified in clinical assessments and the AI system. Conclusions: We demonstrate convergent validity (associations with PANSS/BNSS Negative symptom score), discriminant validity (no association with PANSS P).

9. Comparative Analysis of Enzymatic Methods for Extracellular Vesicle Isolation from Murine Brain Tissue

Galit Blecher, Logan Jones, Chris Goulbourne, Monika Pawlik, Pasquale D'Acunzo, Efrat Levy

Extracellular vesicles (EVs) are small phospholipid bilayer particles released by all cells, present in biofluids. EVs play crucial roles in intercellular communication and have significant diagnostic and therapeutic potential, including brain disorders like Alzheimer’s disease. The method for isolating brain EVs from the extracellular matrix (ECM) relies on tissue dissociation, risking cellular damage, marker degradation, and contamination with intracellular components. In our lab we have

established a protocol using Papain for releasing EVs from the ECM. Papain is a commonly used enzyme due to its broad substrate specificity and success in generating primary brain cells, including neurons, astrocytes, and microglia. Papain effectively releases cells and interstitial fluid while preserving most cellular integrity. However, Papain's broad proteolytic activity raises concerns that it may degrade crucial surface proteins of EV membranes, potentially hindering the identification of physiologically important EV-associated proteins.
To address this concern, we evaluated and compared EV isolation efficacy and protein integrity using 11 enzymatic treatments of mouse hemibrain tissues: Hyaluronidase, Chondroitinase, Elastase, Dispase (Neutral protease), Trypsin, Collagenase types 1/2/3/4, pure Collagenase, and Papain. Our primary goal was to identify alternative enzymes that release EVs effectively while minimizing membrane protein degradation, thus preserving EVs protein integrity. Preliminary findings indicate that although the brain ECM is enriched in hyaluronic acid and chondroitin, Hyaluronidase treatment yielded very small amounts of EVs depending on concentration used, while Chondroitinase failed to yield detectable EVs. Collagenase treatments, differing in residual nonspecific enzymatic activity, exhibited variability; Collagenase types 1, 2, and 4 produced higher cellular contamination compared to Collagenase type 3 and pure Collagenase. Interestingly, Elastase, targeting elastin primarily found in brain blood vessels rather than ECM, yielded relatively high EV amounts without significant degradation. Dispase showed similarly promising results. Treatment with Trypsin at low concentration produced larger EVs, whereas higher concentration resulted in smaller particles (~20nm), necessitating further characterization. Our preliminary data suggest that Papain, Collagenase type 3, Dispase, and Elastase treatments yield the highest EV numbers with minimal protein degradation compared to Papain, highlighting their potential for isolating physiologically relevant brain-derived EVs for further research.

10. Infra-slow fluctuations in global brain states shape ongoing memory reactivation dynamics during rest

Hyun-Woong Kim, Ting Xu, Karl-Heinz Nenning, Mark D’Esposito, Stanley Colcombe, Michael Milham, Arielle Tambini

Spontaneous reactivation of representations of past experience during offline states such as rest and sleep has been suggested as a key mechanism supporting memory consolidation (Tambini & Davachi, 2019). Here, we examined whether slow fluctuations in global brain states during rest relate to memory reactivation and consolidation. We analyzed an fMRI dataset (n=52), in which the reactivation of incidentally encoded object-face stimuli has been reported (Tambini & D’Esposito, 2020). Reactivation events were defined as time points of post-encoding rest in which patterns of the hippocampus and lateral occipital cortex activity were concurrently similar to those of the encoding phase. To characterize global brain states during reactivation events, we isolated brain states from the pre-encoding rest scans via spatial clustering of the whole brain activation maps and assigned each post-encoding time point to the brain state with the strongest similarity to the current activity pattern. We also examined the relationship between reactivation and fluctuations in global fMRI signal (GS) by computing 1) the GS phase during reactivation events and 2) the rates of reactivation events across time windows with high vs. low GS amplitude (i.e., standard deviation). We identified four distinct brain states with two opposing modes, which captured opposing modes of default vs. dorsal attention networks, activation vs. deactivation of the visual network, the unimodal sensory vs. hetero-modal associative networks, and visual/default vs. somato-motor/ventral attention networks. Reactivation occurred preferentially during one mode of each brain state — default, visual, and sensorimotor networks. Reactivation also occurred more frequently during the peak (i.e., zero) phase of the GS and during high vs. low GS amplitude time windows, both associated with lower arousal (Liu et al., 2018; Wong et al., 2013). Moreover, memory retention performance was positively correlated with the strength of the brain states capturing visual/default network activation as well as the rate of reactivation during high, but not low, GS amplitude time windows. Together, these results show that memory reactivation is coupled with slow fluctuations in brain states and brain-wide activity, which may affect ongoing memory consolidation during rest.

11. Higher Childhood Trauma is Related to BOLD Activation Deficits in Emotion Regulation in Schizophrenia Spectrum Disorder with Suicidal Ideation and Behavior

Matthew J. Hoptman, Kathryn T. Evans, Michael J. Kuhl, Stuti Munjal, Zamfira Parincu, Dan V. Iosifescu

Childhood victimization is a risk factor for suicidal ideation and behavior (SIB) in schizophrenia spectrum disorders (SSD). It is, however, not known whether it is related to another risk factor for SIB, poor emotion regulation (ER), nor whether it is related to abnormalities in its neurocircuitry. Here we examined the relationship between childhood victimization and fMRI activations during a ER task in people with SSD and high levels of SIB. 30 people with SSD were tested on an fMRI ER task in which participants saw neutral and negative pictures preceded by neutral or negative sentences. 16 had low levels of SIB, and 41 had high levels of SIB based on the Columbia-Suicide Severity Rating Scale. We compared activation to negative pictures preceded by neutral sentences vs. neutral pictures preceded by neutral sentences. Group differences in activation were computed, corrected for test multiplicity by a nonparametric test Childhood victimization was measured using the Childhood Trauma Questionnaire (CTQ).
CTQ scores were elevated in the high vs. low SIB group and correlated with both lifetime ideation and attempts. They also showed deficits in activation in several prefrontal and superior temporal regions. Across groups, higher CTQ scores correlated (Spearman correlations) with lower activation in bilateral superior frontal gyrus, right rostral anterior cingulate, DLPFC, and right superior temporal gyrus (rs<-.44, ps<.016).
These data suggest that CTQ and ER effects on SIB share neural circuitry. If there is a causal relationship among them, this would provide important clues for therapeutic strategies to reduce SIB in SSD.

12. Postnatal ethanol vapor exposure triggers Ezh2-dependent neurodegeneration and memory impairments in mice

Shivakumar Subbanna, Balapal S. Basavarajappa

Fetal alcohol spectrum disorders (FASD), resulting from developmental alcohol exposure, are linked to neurodevelopmental and psychiatric dysfunction in humans and animal models. Our prior work has shown that alcohol disrupts epigenetic regulation—including DNA methylation and histone modifications—at synaptic plasticity gene promoters. Preclinical models of postnatal alcohol exposure are essential for investigating underlying mechanisms and potential therapies. Even brief postnatal ethanol exposure in rodents alters critical epigenetic markers, increasing susceptibility to cognitive deficits and adult neuropathologies. Building on these findings, we hypothesized that postnatal ethanol exposure may also enhance specific histone H3 methylation mediated by the polycomb repressive complex 2 (PRC2) protein Ezh2, which executes the trimethylation of lysine 27 on histone H3 (H3K27me3). Here, we establish a mouse model of postnatal ethanol vapor exposure (E-vap), administering 3-hour daily sessions from postnatal days 4–9 (P4–9). We assessed neurodegeneration in neonates and synaptic plasticity and spatial memory in adults. Neonatal E-vap exposure triggered widespread caspase-3 activation (CC3+) in the hippocampus and prefrontal cortex, concurrent with elevated Ezh2 activity, increased H3K27me3, and decreased H3K27ac in NeuN+ neurons. Pharmacological inhibition of Ezh2 with EPZ-6438 (EPZ) restored H3K27me3/H3K27ac balance, preventing neurodegeneration and mitigating adult neurobehavioral deficits. Our findings implicate Ezh2-mediated H3K27me3/H3K27ac dysregulation as a central mechanism in FASD-related synaptic and cognitive impairment, highlighting Ezh2 as a potential therapeutic target.

13. Stereologic analysis of basal forebrain cholinergic neurons (BFCNs) in a mouse model of Down syndrome and Alzheimer's disease at 16-18 months of age

Gabrielle A. Gentile, Melissa J. Alldred, Stephen D. Ginsberg

Down syndrome (DS) is the primary genetic cause of intellectual disability, occurring when human chromosome 21 is triplicated. During early mid-life, the majority of individuals with DS develop the neuropathology associated with Alzheimer's disease (AD), including neurofibrillary tangles and amyloid-beta containing senile plaques with concurrent dementia. The trisomic Ts2 model is a useful model for studying DS/AD, enabling the elucidation of mechanisms and pathways that degenerate as well as those that are preserved in individuals with DS+AD. One age-related alteration mimicking the human condition is degeneration of basal forebrain cholinergic neurons (BFCNs), which is a hallmark pathology associated with both DS and AD. BFCNs degenerate within the Ts2 trisomic mouse model starting at ~6 months of age (MO). A therapeutic intervention, maternal choline supplement (MCS) provides benefits to offspring in terms of learning and memory, including attentional function and spatial cognition. MCS also stimulates hippocampal neurogenesis as well as attenuates loss of BFCNs until ~12 MO. We hypothesize MCS will slow the progression of degeneration in the Ts2 mouse model of DS/AD, with age-associated preservation of BFCNs within the medial septal and ventral diagonal band nuclei. Ts2 dams were fed either a normal choline chow diet (1.1g/kg) or a choline supplemented chow diet (5.0g/kg). Offspring receiving either diet through their dams from embryonic day 0 to postnatal day 21, when they are weaned. Offspring were aged on normal choline chow to ~16-18 MO, where we expect MCS will have a significant benefit on loss of BFCNs. Mice from both maternal diets were accessed and perfused with phosphate buffer to preserve their brains for immunocytochemistry and molecular/cellular analyses. Preliminary immunohistochemistry using an antibody directed against choline acetyltransferase (ChAT) indicates BFCNs are detectable in 16-18 MO disomic control (2N) and Ts2 mice in the medial septal nucleus. We aim to quantify BFCNs in the entire cohort, comparing the impact of maternal diet on Ts2 and 2N offspring to assess potential lifelong benefits of early dietary intervention and how this inexpensive treatment modality may contribute to the rescue of the DS/AD phenotype, which has profound translational implications.

14. Clinical and neural features of sensory urges in individuals with obsessive-compulsive disorder and unaffected siblings

Goi Khia Eng, Nicolette Recchia, Katherine A. Collins, Jeanmarie Harvey, Russell H. Tobe, Emily R. Stern

Introduction: Obsessive compulsive disorder (OCD) is a familial and heterogeneous disorder, yet few studies have investigated the familiality of specific symptom subtypes. In particular, many patients with OCD experience aversive sensory-based urges ("sensory phenomena") that precede or drive compulsions instead of fear. Using an eyeblink suppression task as a model for sensory-based urges, we showed that OCD patients exhibited impaired eyeblink suppression and greater activation in sensorimotor-related brain regions during suppression compared to controls. This study investigates sensory urges in unaffected siblings as a potential OCD endophenotype. Method: 98 individuals with OCD, 32 unaffected siblings, and 71 controls without a family history of OCD performed an fMRI eyeblink-suppression task with eyeblinks measured using eye-tracking. The severity of sensory phenomena was assessed with the Sensory Phenomena Scale. Brain-imaging data were analyzed using SPM12, $p<0.005$ (uncorrected) with $k>50$. Results: OCD patients had more failures of blink suppression than unaffected siblings ($p<0.05$), who did not differ from controls. Unaffected siblings were in-between controls and the OCD group in sensory phenomena severity ($p<0.05$) and showed greater activation than OCD patients in the inferior frontal gyrus during suppression in the task. Both OCD patients and unaffected siblings showed greater activation than controls in sensorimotor regions including the precentral gyrus, postcentral gyrus, rolandic operculum, and insula. Conclusion: Unaffected siblings showed clinical and neural similarities to patients with OCD, suggesting that sensory phenomena and sensorimotor activation may relate to disease vulnerability.

15. Aβ12-28P administration alleviates anxiety in an Alzheimer's disease mouse model

Xutong Guo, Xiaoyi Yang, Shuo Chen

Background: Anxiety is a common symptom of Alzheimer's disease (AD). It has been recently established that anxiety is associated with AD pathology characterized by amyloid-β (Aβ) plaques and neurofibrillary tangles. Our laboratory has demonstrated that acute administration of Aβ12-28P, a synthetic peptide with the potential to competitively inhibit toxic Aβ oligomers, resulted in improved cognitive function in old transgenic AD mice. However, whether inhibiting Aβ toxicity by Aβ12-28P also alleviates anxiety in AD mice remains unknown. Method: Middle-aged (12-15 months old) and old (18-24 months old) APP/PS1 dE9 mice were intraperitoneally injected with Aβ12-28P, while control mice received an equivalent volume of saline. Behavioral tests, including open field test and elevated zero maze, were conducted 2 hours post-injection to assess the acute effect of Aβ12-28P or saline on the anxiety of animals. We further performed in vivo fiber photometry to investigate anxiety-associated neural dynamics across multiple brain regions: ventral CA1 (vCA1), medial prefrontal cortex (mPFC), basal amygdala (BA), and lateral hypothalamic area (LHA). Adeno-associated virus expressing GCaMP8f was injected into the target brain region, above which an optic fiber was implanted. Four weeks later, real-time calcium transients were recorded during anxiety tests. Result: In middle-aged AD mice, Aβ12-28P administration exerted acute anxiolytic effects in the open field test ($n = 8$ drugged versus 8 non-drugged, $p < 0.05$). Furthermore, anxiety was also significantly alleviated by Aβ12-28P in old AD mice in both the open field ($n = 17$ drugged versus 14 non-drugged, $p < 0.0001$) and elevated zero maze ($n = 20$ drugged versus 20 non-drugged, $p < 0.01$) tests. We further compared the neural activity patterns in vCA1, mPFC, BA, and LHA, with or without Aβ12-28P treatment by fiber photometry. Our data suggested significant modification of anxiety-associated neural dynamics in these brain regions by Aβ12-28P administration. Conclusion: These results suggest that Aβ12-28P has an acute anxiolytic effect in both middle-aged and old AD mice. Our finding opens the possibilities to apply the strategy of targeting Aβ pathology for alleviating neuropsychiatric AD symptoms, such as anxiety, and provide insights into a brain region-specific mechanism behind this therapeutic strategy.

16. A preclinical intraneuronal stage of autophagy-lysosomal dysfunction, amyloidosis, and neuron death yields senile plaques in human late-onset Alzheimer's Disease

Ju-Hyun Lee, Philip Stavrides, Sandipkumar Darji, Martin J. Berg, Chris N. Goulbourne, Panaiyur Mohan, Cynthia Bleiwas, James Peddy, Eric B. Dammer, Nicholas T. Seyfried, Ralph A. Nixon

Background: Autophagy-lysosomal pathway (ALP) efficiency declines in Alzheimer's disease (AD). In AD mouse models expressing a fluorescent autophagy and pH probe, autolysosomes pH elevation, resulting from deficient v-ATPase activity, causes autophagy substrates, including Aβ and APP-βCTF, to build up selectively within autolysosomes before extracellular amyloid deposits. In the most compromised but still intact neurons, massive numbers of Aβ-positive autolysosomes pack into huge petal-like blebs bulging out from the perikaryal membrane (PANTHOS). They additionally fuse with the endoplasmic reticulum during its disrupted autophagic turnover, causing plaque-like fibrillar β-amyloid to accumulate in tubular membrane networks. As the neuron dies, the extracellular β-amyloid plaque left behind becomes the major source of senile plaques in early disease, supporting their "inside-out" origin. Here, we show that the same pathological sequence evolves similarly in the late-onset AD human brain. Methods: Our published curated autophagy pipelines were used in a deeper interrogation of earlier unbiased WTA analyses of ROSMAP/Banner transcriptomic and proteomic datasets and additional transcriptomic profiles of individual brain cell types from postmortem AD human brains. Complementary analyses included multiplex immunohistochemistry, biochemical, and ultrastructural approaches. Results: Omic analyses of ALP revealed in AD brains a selective down-regulation of genes and proteins involved in lysosomal efficiency, such as v-ATPase complex, lysosomal acidification, and regulation of lysosomal environments, relative to upstream autophagy pathway components. Notably, excitatory neurons, among brain cell types, exhibited similar lysosomal/acidification findings in the preclinical stage of AD. At the exceptionally early "intraneuronal" pre-plaque stage of AD pathology, susceptible neuronal populations develop progressive ALP pathology leading to intraneuronal β-amyloid aggregate formation, similar to the PANTHOS pattern seen in mice. Close temporal and 1:1 spatial correlation between PANTHOS, intracellular Aβ accumulation, and plaque formation in

early Braak-stage strongly suggests a significant association between PANTHOS and the progression of amyloid pathology in late-onset AD (LOAD). Conclusion: Beginning at a preclinical stage, vulnerable neuron populations in human sporadic AD develop the same PANTHOS pattern of ALP dysfunction and neuronal cell death as observed uniquely in mouse models of AD, which arises from APP-dependent lysosomal acidification deficiency. ALP failure and PANTHOS development result in early selective neuronal death that initiates the emergence of amyloid plaques.

17. Pharmacological reacidification of lysosomes attenuates intraneuronal amyloidosis, early neuron death, and amyloid plaque formation in 5xFAD mice

Sandeep Malampati, Philip H. Stavrides, Panaiyur S. Mohan, Martin J. Berg, Eunju Im, Ohno Masuo, Chris N. Goulbourne, Basavaraj S. Balapal, Subbanna Shivakumar, Bhaskar C. Das, Ju-Hyun Lee, Ralph A. Nixon

Background: Autophagy-lysosomal pathway (ALP) dysfunction emerges early in Alzheimer's disease(AD). In mouse AD models, lysosomal acidification deficits impair ALP in neurons, in some cases inducing massive autolysosome accumulations, intraneuronal amyloid plaque, and early neuronal death, yielding an extracellular amyloid plaque. This distinctive neurodegenerative pattern (PANTHOS) emerges in early-stage AD and is recapitulated in human late-onset AD (accompanying poster). Restoring lysosomal acidity via β2-adrenergic receptor (β2-AR) activation with Isoproterenol (ISO) reverses ALP dysfunction in PSEN1-FAD patient fibroblasts. Methods: Candidate compounds compared to ISO underwent screening in AD model cell lines and PSEN1-FAD fibroblasts. After toxicity and PK/PD studies in vivo, a lead compound achieving an effective 11.63 micromolar brain concentration was administered IP for 2 or 4 months in 2-month 5xFAD mice expressing a ratiometric autophagy/pH LC3 probe (5xFAD/TRGL) or in 2-month 5xFAD versus vehicle-treated 5xFAD/TRGL or 5xFAD controls respectively. Effective ISO sustained release pellets were implanted SC in the matched cohorts for 2 months. Outcomes were assessed using multiplex immunocytochemistry, computer-aided ALP and pH analysis, EM, and behavioral tests (fear conditioning, novel object recognition). Results: Incubating AD cell lines with the lead compound, restoring lysosome pH by acting through β2-AR mediated, PKA-dependent pathway, efficiently rescued autophagy. 5xFAD mice treatment with compound or isoproterenol restored neuronal lysosomal acidification and markedly attenuated ALP deficits. Lysosomal re-acidification strikingly reduced ALP-related intracellular amyloid/APP-βCTF/Aβ (PANTHOS) and the death of these neurons. Moreover, extracellular plaque burden reduction (>50%) commensurate with reduced neuronal death frequency. Notably, compound induced pH restoration alleviated memory impairments in 5xFAD mice. Conclusion: These studies establish that lysosomal acidification deficit in the 5xFAD mouse is upstream of, and the likely basis for ALP failure, neuronal death, and amyloid pathology, consistent with the pathological sequence previously documented in multiple mouse AD models. The mechanism of action of β2-AR activators via correcting PKA-regulated Cl- and H+ flux in vATPase-deficient lysosomes as established in PSEN1-FAD fibroblasts, likely accounts for autolysosome pH correction in 5xFAD neurons. These findings demonstrate the potential of lysosomal remediation as a therapeutic approach for AD.

18. Orexins as anxiety modulators in instrumental safety-seeking

Cristina Siller-Perez, Erika C. Andrade, John Smiley, Christopher K. Cain, Robert M. Sears

Understanding the neuromodulatory systems that regulate adaptive coping behaviors in threatening scenarios is essential for addressing human anxiety. A central orchestrator in this context is the hypothalamic orexin (hypocretin) system, which modulates behaviors critical to survival in large part via projections to the dopaminergic ventral tegmental area (VTA). The VTA plays a crucial role in reinforcing behaviors that lead to desirable outcomes, but its involvement in reinforcing threat-motivated instrumental behaviors, such as signaled active avoidance (SigAA), remains unclear. Previous research in our lab, utilizing a SigAA shuttling task, indicates that avoidance is positively reinforced by safety signals. Based on this, we hypothesized that the LH-

orexin to VTA projection is necessary for safety-seeking behavior, with safety acting as a rewarding outcome. To examine this, we used an orexin-specific viral vector in adult Sprague-Dawley rats to express an inhibitory opsin (AAV1-Ple112-Arch3.0-eYFP) in perifornical/lateral hypothalamus neurons. Then, after a 6-8-week incubation, optic fibers were implanted in orexin axon fields in the VTA, and rats were trained in the SigAA paradigm. During SigAA training, rats learned to shuttle during a white noise warning stimulus (WS) to prevent mild foot shocks. Explicit feedback (FB) cues (pure tones) were delivered immediately after successful avoidance responses. Prior studies show that avoidance FB cues rapidly become safety signals, due to their negative correlation with the threatening WS and aversive shock. Rats received 15 trials per day until they reached a pre-defined avoidance criterion. In shock-free test sessions, inhibition of orexin to VTA axon terminals during FB cue presentations gradually impaired avoidance. This finding suggests that orexinergic modulation of VTA is crucial for processing safety signals as valued instrumental goals during threats. It also provides additional evidence that active avoidance is positively reinforced by safety. Future research will further explore the orexin system's role in adaptive coping behaviors and may pave the way for innovative treatments targeting maladaptive coping strategies beyond anxiety.

19. The Influence of Short-form Videos on Behavior and Brain Activity

Jenna Lembo, Teddy Hoppe, HyunWoong Kim, Samuel Louviot, Eduardo Gonzalez-Moreira, Karl Heinz-Nenning, Alexandre Franco, Michael Milham, Arielle Tambini

Media platforms aim to captivate users with short-form videos using sophisticated recommendation algorithms. Given the widespread use of such platforms, understanding the impact of short-form video consumption on brain activity and behavior is critical. Moreover, recommender algorithms promote highly engaging videos (Shani & Gunawardana, 2011), making it crucial to understand how such content affects brain activity and behavior. Since users scroll through videos at will, the impact of active engagement should also be considered, as active decision-making influences brain activity and behavior (Murty et al., 2015). To characterize short-form video consumption effects on brain activity and behavior, here, healthy participants watched short-form videos during fMRI while collecting physiological and behavioral measures. TikTok videos were selected based on viewer engagement metrics to create high and low-engagement video categories. Video watching was blocked in a 3 (engagement level: high, low, mixed) × 2 (viewing condition: active-ability to skip videos, passive-no skipping) design, with participants completing six blocks per session. After each block, participants rated their preference for each video, confirming a significant preference for high-engagement videos ($t = –2.86$, $p = .006$). To assess how video-watching impacts subsequent behavior, participants completed the Psychomotor Vigilance Task (PVT), a measure of sustained attention (Baumann et al, 2014), before and after video-watching. We addressed how video-watching influences attention by examining the effects of engagement level and viewing condition on PVT response times (RT) after video-watching using a two-way ANOVA. Preliminary analyses revealed an overall effect of video-watching on RT, such that RT was slower after video-watching, reflecting a time-on-task effect or attentional fatigue. However, post-video-watching RT was modulated by our design, indicated by a significant interaction between engagement level and viewing condition. The smallest time-on-task effect was observed after active, high-engagement blocks, with the largest after low-engagement blocks, suggesting differential modulation of attention or vigilance by distinct video-watching conditions. In conclusion, our preliminary findings suggest that watching short-form videos influences subsequent attention, such that active watching of high-engagement videos may induce a state of heightened vigilance that persists over time. Future analyses will continue characterizing how short-form video watching impacts behavior, physiological arousal, and brain activity.

20. Neuronal responses to omitted tones in the NHP auditory thalamocortical system.

M.N. O'Connell, A. Barczak, C.A. Mackey, T. McGinnis, K. Mackin, C.E. Schroeder, D.C. Javitt

EEG studies in humans have demonstrated that the brain generates neural responses when expected stimuli are omitted from rhythmic streams of identical auditory tones. These omission-

related responses are thought to reflect the brain's encoding of prediction errors. In individuals with schizophrenia, these responses are reduced compared to healthy controls, which is thought to indicate a disruption of predictive sensory processing in this disease. We hypothesize that omission-related responses are related to the entrainment of neural activity to rhythmically presented standard stimuli, which creates a temporal expectation for upcoming sounds. When an expected stimulus is omitted from a rhythmic stream, the mismatch between the expectation and the absence of the stimulus leads to a measurable neural response. If stimuli are non-rhythmic or less predictable, we expect a reduction in, or even absence of, an omission-related response. To test this, we recorded neuroelectric activity from the auditory cortex (core and belt regions) and the auditory thalamus in awake macaque monkeys using a linear array multielectrode. During recording, monkeys were presented with streams of pure tones delivered at a delta frequency rate, with occasional (~10 %) tone omissions. Two conditions were tested: (1) isochronous stimuli presented at a fixed SOA of 624 ms, and (2) non-isochronous stimuli with temporally jittered tone onsets that maintain the same mean delta presentation rate. Both conditions included random tone omissions to assess neural responses when temporal expectations were violated. Results so far indicate that, in Condition 1, time-frequency analysis of local field potentials reveal significant delta-band inter-trial coherence (ITC) at the expected onset time of both presented standard tones and omitted stimuli. This indicates phase-locking of neural activity to the temporal structure of the stimulus stream and, when compared to baseline amplitudes, was coupled with a late enhancement of multiunit activity (MUA) following omissions. As expected, in Condition 2, ITC values were markedly reduced for both standard tones and omissions, suggesting a breakdown in temporal entrainment. Correspondingly, omission-related MUA enhancement was significantly diminished or altogether absent compared to that observed during Condition 1.

21. MicroRNAs exhibit diverse profiles and interactions in three key hippocampal neuron populations in a mouse model of Down syndrome and Alzheimer's disease

Kyrillos W. Ibrahim, Sang Han Lee, Melissa J. Alldred, and Stephen D. Ginsberg

Individuals with Down syndrome (DS) develop Alzheimer's disease (AD) pathology, including neurofibrillary tangles and amyloid plaques early in mid-life, which results in early onset AD dementia. Hippocampal neurons exhibit dysregulation in postmortem human tissue and mouse models of DS/AD, which have been linked to learning and memory impairments. MicroRNAs (miRNAs) play a key role in the regulation of gene expression by modulating RNA transcription and translation. Recent results from RNA-sequencing of three hippocampal excitatory neuron populations from the Ts65Dn mouse model of DS/AD at ~6 months of age indicate each neuron population has unique gene expression in trisomic mice compared to normal disomic controls. We postulate the miRNA signature within hippocampal neurons is also dysregulated in the DS/AD mouse model and unique miRNAs play a key driving role in the dysregulation of downstream genes and pathways. Utilizing newly developed bioinformatic inquiry methodologies, miRNA expression profiles for each neuronal subtype were examined in CA1 pyramidal neurons, CA3 pyramidal neurons, and dentate gyrus granule cells. Individual hippocampal neuron populations exhibited unique miRNA expression profiles that likely alter mRNA expression in a cell-type specific manner. Profiling miRNAs at the single population level elucidates miRNA-mRNA interactions and enhances discovery of unique mechanistic drivers of DS/AD. These cell-type specific vulnerabilities, including dysregulated miRNA-mRNA interactions, may be contributing to neurodegenerative endophenotypes that can be targeted at the single vulnerable neuronal population level through precision medicine therapeutics.

22. Integrating Single-Cell and Spatial Transcriptomics to Map Cellular and Regional Alterations in an Alzheimer's Disease Mouse Model

YunJuan Chang, Philip Stavrides, Christopher Bare, Ju-Hyun Lee, Ralph Nixon

Understanding how individual cell types are organized within the spatial architecture of the brain is crucial for studying neurodegenerative diseases such as Alzheimer's. In this study, we demonstrate an integrative approach to decode the spatial context of Alzheimer's disease (AD) in mouse cortex tissue. Publicly available single-nucleus RNA-seq datasets from wild-type and AD model mouse cortex samples were downloaded and processed, followed by single-cell analysis, annotation, and characterization. This analysis revealed a significantly increased proportion of astrocytes and oligodendrocytes in the AD model. In contrast, certain layer-specific neuronal subtypes in cortical layers 2/3, 4/5, 5, and 6 were significantly reduced, while the major subtype population structure of the cortical layers was maintained compared to the wild type. These data can subsequently used to construct a reference for deconvolving and annotating spatial transcriptomic profiles generated using the Curio Seeker platform. By anchoring spatial data to a curated matching single-cell reference, the cellular composition and microenvironment across spatial locations in the cortex can be reconstructed. This approach enables visualization of disease-associated shifts in cell-type organization and local cellular interactions. Additionally, spatially resolved differential expression analysis reveals region-specific transcriptomic alterations between diseased and normal conditions. Our integrative strategy provides a framework for multiscale spatial analysis, facilitating the discovery of disease-related cellular changes within their native anatomical context.

23. Estrogen regulates BDNF expression in dentate gyrus mossy cells of adult mice

Chiara Criscuolo, Meghan Kennedy, Helen E Scharfman

Mossy cells (MCs) of the dentate gyrus (DG) play a key role in DG-dependent functions, including memory formation. MCs form an intrinsic excitatory loop with granule cells (GCs) that exhibits a form of synaptic plasticity—MC-GC long-term potentiation (LTP)—which requires brain-derived neurotrophic factor (BDNF)/TrkB signaling and presynaptic cAMP/PKA activation. Unlike other DG neurons, MCs are uniquely positioned to influence hippocampal function due to their extensive axonal projections along the septotemporal axis, allowing communication between functionally distinct regions involved in spatial (dorsal) and emotional (ventral) memory. However, while BDNF protein is known to be highly expressed in GCs, its expression and regulation in MCs has remained poorly characterized. Here, we investigated whether MCs express BDNF and whether this expression is modulated by estrogen. Using double immunofluorescence for BDNF and MC markers (GluR2/3, SATB1, Calretinin), we confirmed BDNF protein expression in MC somata and axonal processes, including the inner molecular layer (IML)—the primary target of MC axons—with particularly strong labeling in the ventral IML. Tracking the estrous cycle in adult female mice (3–5 months), we found significantly increased BDNF immunoreactivity in the dorsal IML during high-estrogen phases (proestrus, estrus) compared to low-estrogen phases (metestrus, diestrus). Males exhibited higher variability. Since estrogen may regulate BDNF via either activity-dependent or genomic mechanisms, we assessed the expression of the neuronal activity marker c-Fos and observed no changes across sexes or cycle phases. Instead, supporting a hormonal mechanism where estrogen activates the BDNF gene, we found strong expression of the G-protein coupled estrogen receptor 1 (GPER1), co-localized with MC markers SATB1 and Calretinin in the hilus, along with modest estrogen receptor alpha (Erα) expression. GPER1 activation is particularly relevant as it stimulates both cAMP production and intracellular signaling cascades implicated in BDNF regulation and MC-GC LTP. Our data suggests that estrogen may modulate BDNF expression in MCs via receptor-mediated, activity-independent mechanisms. Together, these findings reveal a hormone-sensitive, activity-independent mechanism regulating BDNF in MCs, which may shape dentate gyrus plasticity, memory processes, and sex- and region-specific hippocampal function.

24. Silent Endosomes, Lost Signals: Defective NGF endocytosis and signaling lead to Cholinergic Neurodegeneration in a Tauopathy Model of Frontotemporal Dementia

Kuldeep Sachdeva, Martin J. Berg, Cynthia Bleiwas, Philip H. Stavrides, Sandipkumar Darji, Chris Goulbourne, John F. Smiley, Mala V. Rao, Ralph A. Nixon

Endosomal dysfunction, or endosomopathy, has emerged as a key early pathological feature across multiple neurodegenerative diseases. In Alzheimer's disease (AD), extensive research over the past two decades has demonstrated that β-secretase-derived C-terminal fragment of APP (βCTF) hyperactivates the small GTPase Rab5, resulting in aberrant early endosome enlargement and disruption of retrograde neurotrophic signaling. Dysregulation of endocytic trafficking has also been genetically linked to AD risk through variants in BIN1, PICALM, and SORL1, among others. In contrast, the role of endosomal pathology in Frontotemporal Dementia (FTD), particularly tauopathy-associated forms of the disease, remains unexplored. FTD is the second most common cause of early-onset dementia and encompasses a range of clinical syndromes often associated with tau, TDP-43, or FUS pathology. In this study, we investigated early endosomal alterations in the PS19 mouse model expressing human P301S mutant tau, a well-established model of FTD-tau. Strikingly, unlike the endosomal enlargement observed in AD models, cortical and hippocampal neurons of PS19 mice exhibited smaller and fewer early endosomes. Biochemical and immunocytochemical analyses revealed impaired RabGEF1-dependent activation and membrane recruitment of Rab5, a key regulator of early endosome fusion and maturation. These deficits were also apparent in hippocampal synaptosome preparations, indicating a contribution of endosomopathy to synaptic dysfunction. Notably, this pattern of endosomal hypo-activity is in stark contrast to the hyper-active endosomal phenotype in AD, underscoring disease-specific mechanisms of vesicular trafficking dysregulation. We further demonstrated that hypo-activation of Rab5 compromises the endocytosis of nerve growth factor (NGF) at the synapse, which leads to reduced neurotrophic signaling and the progressive degeneration of cholinergic neurons, a cell population that is strongly correlated with memory deficits. Altogether, our findings identify defective Rab5 signaling as a novel early feature of FTD-tau pathology and propose a mechanistic link between impaired endocytosis, disrupted neurotrophic support, and selective cholinergic vulnerability in FTD-tau. These results not only highlight fundamental differences in endosomal pathobiology between AD and FTD but also point to Rab5 activation as a potential therapeutic strategy to restore endosomal function and neurotrophic support in FTD-tau.

25. Investigation of EEG and pupillometry correlates of fMRI brain state dynamics

Teddy Hoppe, Hyun Woong Kim, Samuel Louviot, Jenna Lembo, Eduardo Gonzalez-Moreira, Michael P. Milham, Alexandre R. Franco, Karl-Heinz Henning, Arielle Tambini

Previous work has identified patterns of network activations using functional magnetic resonance imaging (fMRI), referred to as brain states, and related these to behavior, cognition, and psychiatric disorders. Given limitations to large-scale fMRI data acquisition, one challenge is to identify signatures of fMRI-defined brain states in scalable modalities. Here, we investigate links between concurrent fMRI, electroencephalography (EEG), and pupillometry signals, focusing on data while participants viewed a flashing checkerboard and during eyes-open rest. We defined eight co-activation patterns across participants using k-means clustering [5] and calculated their strength over time. Similar to prior work, we identified four pairs of anti-correlated fMRI brain states. To investigate EEG correlates of these states, we examined power in delta, theta, alpha, beta, and gamma frequency bands. Similarly, we examined fluctuations in pupil diameter and its first and second derivatives. To characterize correlates of identified brain states, we calculated cross-correlations between the strength of each brain state and power in each EEG frequency band or pupillometry feature. Significance of the cross-correlations was evaluated using permutation tests. We validated this approach with data from the checkerboard task, revealing pupil and EEG correlates of brain state 1, a state we found to respond to visual stimulation. We then focused on periods of rest to eliminate the possibility of confounding stimulus-driven fluctuations. We found changes in the second derivative of the pupil diameter preceded changes in all brain states, indicating spontaneous events during rest manifest as coupled changes in brain states and pupil diameter. We found increases in EEG delta power preceded decreased visual activation (reflected by state 1) and increased default network activation (state 7). Lastly, we found increases in EEG alpha power preceded decreases in dorsal attention network (DAN) activity and increases in DMN activity, indicated by state 3. This suggests a role of alpha power fluctuations in shifting from externally to internally-oriented attention, given antagonism between externally and internally-

oriented networks in state 3 (DMN and DAN). By showing relationships between pupillometry, EEG power, and fMRI brain states, these results further bridge the gap between fMRI and more accessible modalities.

26. Integrating Cultural and Structural Humility in New York State Systems of Care: Qualitative Insights from a Statewide Health Habitus Motivational Interviewing Training Initiative

Zoe Bertone, Kerstin Pahl, Crystal Lewis, Sebrena Tate, Daniela Galvez, Helen-Maria Lekas

Introduction: Youth and families experiencing serious behavioral health challenges frequently encounter cultural, structural, and social barriers that impede access to and continuity of care. In response, the Center for Research on Cultural and Structural Equity in Behavioral Health (CCASE) at the Nathan S. Kline Institute implemented an adapted version of the Health Habitus + Motivational Interviewing (HHMI) training for Single Point of Access (SPOA) coordinators of behavioral health services across New York State over the past seven months. Description of the Training: The HHMI training included a self-guided virtual module (sent in advance) and an in-person, 5-hour training. The training consisted of two main parts: the first, focused on cultural and structural humility, health equity, bias and how to integrate into care approaches the construct of health habitus (HH; theory- and evidence-supported) as a means of contextualizing the youth and families' experiences. The second part combined health habitus with the communication approach of motivational interviewing (MI; evidence-based). Through didactics, guided discussions and experiential learning the training aimed to foster self-reflection, cultural and structural awareness and humility, and impart the communication skills for meaningful, equity-informed engagement of youth and families in diverse communities. Trainings were conducted in five regions: New York City, Syracuse, Batavia, Elizabethtown, and Albany. A total of 59 participants took part, representing varied systems of care roles including Single Point of Access (SPOA) coordinators, peer advocates, clinicians, and case managers. Post-training data collection included 47 surveys and 17 in-depth qualitative interviews lasting about 30 minutes each. Analytic Insights: Thematic analysis of qualitative interviews revealed several core domains of impact. Participants consistently characterized the HHMI training as distinct from previous cultural competency initiatives and trainings, citing its emphasis on practical integration, self-reflection, and the facilitation of open, nonjudgmental discourse. The interdisciplinary facilitation team, reflective of diverse professional and cultural backgrounds, was credited with fostering an atmosphere of psychological safety, mutual respect, and critical dialogue. The multimodal training structure was described as essential in deepening participants' engagement and comprehension of the cultural and structural determinants of their clients' lives. Participants reported enhanced critical consciousness regarding the influence of stigma, structural inequities, and provider positionality on service delivery. Many described a shift toward more intentional, strengths-based clinical practices, de-pathologizing language, and client-centered framing. Several identified a growing capacity to align services with family-defined goals, even when those goals diverged from institutional norms or systemic expectations. Conclusion: These preliminary findings suggest that HHMI is a promising and scalable model for integrating cultural and structural humility into systems of behavioral health care. By supporting providers in critically reflecting on their identities, biases, and power, while also providing the tools to enhance their awareness of the cultural and structural determinants of their clints' lives, the HHMI training may strengthen client-centered practices and promote more equitable behavioral health outcomes for youth and families navigating complex care systems.

27. AI-driven platform (Openwillis) to Measure Negative Symptoms of Schizophrenia Through Facial Analysis

Rachael Mishkind, Jean-Pierre Lindenmayer, Beverly Insel, Danyah Nadim, Anzalee Khan

Background: The development of digital phenotyping technology has broadened the potential applications of facial and speech metrics. These digital metrics offer a potential supplement to traditional assessments of reduced facial and speech metrics indicators of negative symptoms in

individuals with schizophrenia. Currently, the assessment of negative symptoms relies on verbal reports from patients and or caregivers along with clinical interviews conducted by trained raters. However, these interviews can be insensitive to changes in treatment, subjective, require extensive training, and may be influenced by cultural factors. This study assessed the psychometrics properties of a novel artificial intelligence (AI) open-source digital platform, Open Willis, which records facial and acoustic features of patients with negative symptoms. We compared the results to rater-administered rating scales for negative symptoms in schizophrenia. Method: Inpatients with schizophrenia (SZ: n = 70) and healthy controls (HC: n=60) completed a brief 8-minute assessment using an AI-driven platform on two occasions within a one-week period. each participant was assessed and rated twice by the same clinician using several instruments including PANSS and BNSS, CDSS, CGI-S, AIMS, SAS, and BARS. During the digital assessment, each participant was provided with a neutral valence sentence to read. Following this, they engaged in free speech, prompted by open-ended questions posed by an avatar designed to be emotionally ambiguous (e.g., "Tell me about yourself?"). All assessments were recorded, and the videos analyzed using the Open Willis platform. This platform utilizes functions to measure framewise displacement in facial coordinates, which allows for the quantification of facial expressivity. The data generated included several metrics related to facial movement, such as movements of the overall face, lower face, upper face, lips, eyebrows, and mouth openness. The metrics were then correlated with corresponding clinically rated assessments such as PANSS Marder Factors. Concurrent, convergent, divergent, and discriminative validity were assessed. For the HC group, individuals received the digital assessment but did not undergo clinician-administered assessments. Results: The mean age for patients was 42.53 (±11.68). The mean age for controls was 36.66 (±11.60). The mean PANSS total score was 73.83 (±10.89) The mean PANSS Negative Symptom Score was 23.26 (±2.87). The Kruskal-Wallis Test performed on data from the initial session found that patients with SZ and HC significantly differed on the following Open Willis variables: mouth openness mean, overall_std, lower_face_std, upper_face_std, and eyebrows std. Additionally, Spearman's correlation coefficient was produced between Open Willis variables and clinical assessment variables (PANSS and BNSS). While the PANSS was not significantly correlated with any of the Open Willis variables, the BNSS alogia was significantly negatively correlated with several of the Openwillis variables. The mean of the lips was correlated with the BNSS Alogia quantity (corr=-0.269, p value=0.010) and BNSS Alogia (corr=-0.248, p value=0.017). The std of the lower face was significantly correlated with the BNSS Alogia quantity (corr=-0.298, p value=0.004), BNSS Alogia Spontaneous (corr=-0.263, p value=0.011), and BNSS Alogia (corr=-0.304, p value=0.003). The std of the lips was significantly correlated with the BNSS Alogia quantity (corr=-0.342, p value=0.001), BNSS Alogia Spontaneous (corr=-0.285, p value=0.006), BNSS Alogia (corr=-0.337, p value=0.001), and BNSS Total score (corr=-0.262, p value=0.012). The std of the openness of the mouth was correlated with BNSS Alogia quantity (corr=-0.227, p value=0.029), BNSS Alogia Spontaneous (corr=-0.233, p value=0.025), BNSS Alogia (corr=-0.241, p value=0.021). The mean of the mouth openness was correlated with the BNSS Alogia Quantity (corr=-0.231, p=0.027) and BNSS Alogia (corr=-0.216, p value=0.039). The lower face mean was correlated with the BNSS Alogia Quantity (corr=-0.221, p value=0.034). The PANSS Positive Total Symptom score was significantly positively correlated with the mean of the lips (r=0.220, p=0.035) and std of the lips (r=0.205, p=0.050). Conclusion: This study demonstrates the potential of the Open Willis digital phenotyping platform to objectively assess facial and acoustic metrics in individuals with SZ with negative symptoms. Reduced Open Willis lip, openness of the mouth and reduced lower face movements were all significantly correlated with the BNSS Alogia quantity and spontaneous items supporting good confirmatory and concurrent validity of the Open Willis measures. Discriminant validity of the Open Willis measures was shown by the positive correlation of lips with PANSS total positive symptoms. Our findings suggest that these digital metrics may serve as valuable supplementary tools in evaluating specific aspects of negative symptoms. These findings highlight the importance of integrating digital technologies into research practice to enhance the assessment of SZ, Further research is warranted to explore the full capabilities of digital phenotyping in diverse clinical settings.

28. Precise Functional Localization of the Foveolar Representation in Anesthetized Macaque Visual Cortex

Meizhen Qian, Meixuan Chen, Anna Wang Roe

Background: The primate foveola, which represents the central ~1° of the visual field, is critical for high-acuity vision, color perception, and gaze-directed attention. In a cortical region previously called the foveal confluence, Qian (2025 Nature Neurosci) using ultrahigh field 7T MRI in awake fixating macaques discovered multiple (8) foveolar loci encircling a large area we termed the “foveolar core”, suggesting novel hierarchy for central vision. To study this foveolar area in anesthetized monkeys to determine exactly where the foveola is directed on a monitor, we introduce a systematic approach for foveolar localization (with 0.1º precision) using high-resolution fMRI. Methods: Functional EPI data were acquired at 0.8 mm³ isotropic resolution in a 7T MRI using a custom 16-channel RF coil. Data acquisition was monocular. We used a progressive method of imaging with large to narrow stimuli to triangulate the center of gaze. Initially, wide (4.5°) vertical and horizontal bars containing drifting gratings were presented, at each of 3 different positions; the bar that induced the strongest and largest response was chosen as the most central. Successively narrower bars (1.5° wide, then 0.5° wide) were presented at 3 positions within the centralmost location. 0.2° spots were presented at the estimated foveal coordinate to further refine the centeralmost location. Results: Retinotopic shifts in activation corresponded well with stimulus position. The most central vertical bar stimulus activated the vertical meridian at the V1/V2 border; off-center bars produced parallel activations on either side of the border. When stimuli crossed into the ipsilateral hemifield, activation shifted to the other hemisphere as predicted. For horizontal meridian, the centralmost stimulus produced the largest cortical magnification factor and strongest BOLD amplitudes. Foveolar spot stimuli exactly at the center produced bilateral foveolar activations. Summary & Significance: These results confirm the feasibility of precise foveolar localization in anesthetized macaques and provide a foundation for further studies of foveolar cortical organization using fMRI coupled with other recording and stimulation methodologies.

29. Meeting People Where They Are: Field Note Analysis of Participant-Led Goal Setting Amidst Compounding Structural Barriers to Substance Use Treatment and Resource Access in Two Bronx Pharmacies

Zoe Bertone, Helen-Maria Lekas, Crystal Lewis

Innovative, low-barrier models are urgently needed to expand access to addiction treatment and supportive services for people who use opioids (PWUO). This study examines the implementation of Pharm-Link/VBC+, a pharmacy-based intervention offering on-site patient navigation, resource referrals, and telehealth addiction and HIV-related care. Conducted in two community pharmacies in the Bronx, a borough characterized by disproportionate rates of opioid use and overdose events, the intervention aims to reduce barriers through in-pharmacy support and linkages to health care services including buprenorphine initiation, naloxone access, HIV prevention or treatment, assistance with public assistance program navigation (e.g., SNAP, cash assistance), and referrals to local community-based organizations. As of this analysis, 20 of a proposed sample of 30 participants have enrolled in the study. Eligible participants are individuals currently using opioids, not engaged in medication for opioid use disorder (MOUD), and open to initiating telehealth treatment. At baseline, pharmacy and research staff conduct a semi-structured interview to better understand each participant’s life context, including structural vulnerabilities, care goals, and resource needs. Field notes recorded by staff during and after these interviews form the basis of this qualitative, exploratory analysis. Preliminary findings indicate that engagement in addiction treatment is often delayed or complicated by more immediate and compounding life challenges such as housing instability, experiences of domestic violence, lack of access to public assistance, and untreated mental health conditions. Participants frequently discussed mistrust toward formal healthcare systems, often rooted in past experiences of stigma or discrimination. Many shared stories of prior attempts to access care that were obstructed by complex bureaucratic systems, long waitlists, or rigid program requirements, such as being required to demonstrate sobriety before receiving services, which left them feeling dismissed or undeserving of support. Many described the relief of being met with empathy, patience, and genuine interest by the research staff,

emphasizing the significance of feeling heard and not judged. Field notes further reveal how pharmacy and research staff support participant-led goal setting, documenting not only logistical barriers but also how participants determine which needs to prioritize, such as housing, food insecurity, or mental health, before engaging with services like MOUD. These decisions reflect their own assessments of readiness, urgency, and what feels most manageable, allowing care to move at a pace that aligns with their lived realities and self-defined goals. These notes also provide insight into how frontline staff interpret and respond to participants' needs, shaped by their own habitus, identities, and lived experiences. Staff reflections reveal the values and assumptions they bring to care, and how these intersect with the goals and priorities defined by participants themselves; insights that can inform future staff training. These early insights point to the importance of person-centered navigation embedded in familiar, low-barrier settings. Ongoing data collection will inform refinement of the intervention and offer broader implications for the role of pharmacies as accessible, equity-oriented points of entry for harm reduction and behavioral health care.