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ABSTRACTS COLLECTION

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Panel

1. Dissecting the Contributions of Dopamine D1 and D2 Receptor-Expressing Neurons in Behaviors Dysregulated in Neuropsychiatric Illness

1.1 Dichotomous Structural Adaptations in Nucleus Accumbens Neuron Subtypes Underlie Stress Susceptibility

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Background: Ventral striatum (nucleus accumbens-NAc) medium spiny neurons (MSNs) undergo structural plasticity after stress. However, there is little information into these adaptations, and the underlying molecular mechanisms, in the two NAc MSN subtypes, those enriched in dopamine receptor 1 and 2 (D1-MSNs vs. D2-MSNs). Previously we showed that enhanced levels of the transcription factor, early growth response 3 (Egr3), caused dendritic atrophy and depression-like behavior after 10-day chronic social defeat stress (CSDS). We further showed that Egr3 transcriptionally regulates RhoA, a negative regulator of dendritic complexity. However, it is unclear if RhoA or other structural molecules regulate these morphological adaptations and whether these changes also occur in D2-MSNs.

Methods: Using D1-Cre and A2A-Cre lines we examined dendritic complexity in NAc D1-MSNs vs. D2-MSNs of male mice after CSDS. We used D1-Cre and A2A-Cre mice crossed to the Cre-inducible RiboTag line to isolate and examine mRNAs of RhoA pathway molecules in MSN subtypes of stress susceptible mice and controls. We then infused Cre-inducible wildtype (WT)-RhoA, dominate negative (DN)-RhoA, or eYFP adeno-associated virus (AAVs) into NAc of D1-Cre mice and subjected them to 1-day subthreshold (S)SDS or CSDS. To examine sex specific depression-like effects of RhoA in D1-MSNs, unstressed male and female D1-Cre mice receiving RhoA AAVs into NAc underwent the splash test, sucrose preference, and forced swim test. Finally, to determine if Egr3, the upstream transcriptional regulator of RhoA, plays a role in D2-MSNs in CSDS behavior and dendritic morphology we infused Cre-inducible Egr3 AAV into NAc of A2A-Cre mice. Samples sizes are 6-12 or 4-6 (pooled 4 mice; RiboTag experiments). Statistical analysis was performed with Two-way-ANOVAs. All experiments were performed in 8-10-week-old male or female mice on a C57 background.

Results: We observed dendritic atrophy in NAc D1-MSNs but not D2-MSNs in CSDS susceptible mice ($P < 0.001$). mRNAs of RhoA pathway molecules were significantly altered in D1-MSNs of CSDS susceptible mice ($P < 0.05$). Genetic overexpression of WT-RhoA in D1-MSNs induced dendritic atrophy and a susceptible outcome to SSDS ($P < 0.01$), while DN-RhoA in D1-MSNs restored dendritic complexity and caused a resilient outcome to CSDS ($P < 0.05$) compared to eYFP controls. RhoA (WT) in D1-MSNs caused reduced time grooming in splash test of female mice, reduced sucrose preference in male mice, and enhanced time immobile in forced swim test of both sexes ($P < 0.05$). Increased Egr3, the RhoA transcriptional regulator, in D2-MSNs promotes stress resiliency ($P < 0.05$) by preventing D2-MSN enhanced density of mushroom spines that occurs in stress susceptible mice ($P < 0.01$), without altering dendritic arbor.

Conclusions: D1-MSNs display dendritic atrophy and D2-MSNs have enhanced dendritic spines in CSDS susceptible mice. Enhanced levels of the transcription factor Egr3 and its target RhoA mediate D1-MSN dendritic atrophy and corresponding CSDS susceptible behavior. In contrast, enhanced Egr3 in D2-MSNs blocks dendritic spine formation and causes stress resiliency. Overall, we demonstrate dichotomous structural, molecular, and behavioral outcomes to social defeat stress through NAc D1-MSNs vs. D2-MSNs.

Disclosure: Nothing to disclose.

1.2 Cooperative Synaptic and Intrinsic Plasticity in Nucleus Accumbens D1 Medium-Sized Spiny Neurons Promotes Stress-Induced Anhedonia and Behavioral Despair

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Background: Stress is a powerful trigger of symptoms in psychiatric disorders. Anhedonia and behavioral despair are hallmark symptoms in a plethora of psychiatric disorders, including depression. The nucleus accumbens (NAcc) is a brain region that has been widely implicated in mediating these symptoms. However, it is currently not clear how stress impacts

synaptic plasticity and intrinsic excitability in the NAcc, and how they promote anhedonia and behavioral despair.

Methods: Utilizing a combination of ex-vivo electrophysiology, male and female transgenic mice, optogenetics, and viral-mediated gene transfer, we determined the impact of footshock stress on anhedonia and behavioral despair, synaptic plasticity of excitatory inputs to D1 and D2 MSNs, as well as changes in intrinsic excitability. Furthermore, we determined whether synaptic and intrinsic excitability changes in D1 MSNs were sufficient and necessary to drive stress-induced depressive behavior and established that both synaptic and intrinsic changes cooperate to promote this maladaptive behavior.

Results: In both male and female mice, footshock stress induces anhedonia and behavioral despair as assessed by the sucrose preference ($n = 8$ per group; t-test; $t = 2.516(14)$; $p = 0.0247$) and forced swim tests ($n = 7$-8 per group; t-test; $t = 3.488(13)$; $p = 0.004$;), respectively. Ventral hippocampus (VH) inputs were potentiated onto NAcc D1 MSNs ($n = 9$-13 per group; t-test; $t(20) = 3.47$, $p = 0.0024$), but not D2 MSNs. Depotentiating VH inputs to the NAcc rescued both stress-induced anhedonia ($n = 9$-13 per group; t-test; $t(20) = 2.154$, $p = 0.0436$) and behavioral despair ($n = 9$-13 per group; t-test; $t(20) = 3.47$, $p = 0.0024$), demonstrating that potentiation of VH inputs to D1 MSNs is necessary for these effects. Intrinsic excitability was robustly enhanced in D1 MSNs from stressed mice ($n = 22$-32 per group; Two-way ANOVA; $F(20, 730) = 6.39$, $p < 0.0001$), while a modest hypoexcitability was observed in D2 MSNs ($n = 12$-20 per group; Two-way ANOVA; $F(20, 470) = 2.03$, $p = 0.0056$). Increased excitability of D1 MSNs was mediated by a decrease in inwardly-rectifying potassium currents mediated by KIR channels ($n = 7$-13 per group; t-test; $t(17) = 2.18$, $p = 0.043$), which normally limit excitability of MSNs. Overexpression of KIR channels selectively in D1 MSNs rescued both stress-induced anhedonia ($n = 6$ per group; t-test; $t(10) = 6.542$, $p < 0.0001$) and behavioral despair (Two-way ANOVA; Treatment x Time Interaction; $F(5, 70) = 2.701$, $p = 0.0273$). Furthermore, expression of dominant negative KIR channels, which interfere with endogenous KIR function, was sufficient to promote anhedonia and behavioral despair ($n = 9$ per group; t-test; $t(16) = 2.707$, $p < 0.0001$) in naïve animals. Lastly, we utilized novel disconnection procedures to demonstrate that stress-induced depressive behaviors require both potentiation of VH inputs to D1 MSNs and increased D1 MSN excitability.

Conclusions: Here we demonstrate that stress promotes anhedonia and behavioral despair via cooperative increases in excitatory synaptic strength from VH inputs to D1 MSNs and intrinsic excitability in this cell population. These data reveal an unexpected role for NAcc D1 MSNs in promoting negative affective states relevant to depression and other psychiatric disorders. This provides a novel therapeutic target for the treatment of depression and psychiatric disorders characterized by anhedonia and behavioral despair.

Disclosure: Nothing to disclose.

1.3 Induction of Stress-Like Effects on Sleep Architecture by Selective Alterations in the Activity of Dopamine D1 Receptor-Expressing Medium Spiny Neurons Within the Nucleus Accumbens

William Carlezon

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Background: Stress plays a critical role in the neurobiology of mood and anxiety disorders. Sleep is commonly dysregulated in these conditions: however, some people sleep more, whereas others sleep less. We recently showed that chronic social defeat stress (CSDS) in mice causes persistent alterations in sleep architecture, producing increases in time spent in paradoxical sleep (PS) as well as increases in the number of PS bouts that persisted after cessation of the stressor. Previous work shows that stress activates the transcription factor CREB and elevates target gene expression within the nucleus accumbens (NAc), and that non-selective elevations in NAc CREB function produce depressive-like effects whereas disruptions in CREB function produce antidepressant- and anxiolytic-like effects. Elevated NAc CREB function is associated with increased expression of dynorphin, an endogenous agonist at kappa-opioid receptors (KORs) that is co-expressed with GABA in dopamine D1 receptor-expressing medium spiny neurons (MSNs). Dynorphin, in turn, produces feedback inhibition via KORs expressed on the cell bodies and terminals of ventral tegmental area (VTA) dopamine neurons. Implicating dynorphin in the effects of CSDS on sleep, administration of the KOR antagonist JDTic (10 mg/kg, IP) mitigated CSDS-induced alterations in PS. Several lines of evidence suggest that susceptibility to CSDS is accompanied by reduced activity of D1-MSNs in the NAc; however, the ways in which this neural population contributes to the persistent effects of stress on sleep have not been thoroughly explored. Here we examined how selective manipulation of D1-MSNs affects sleep architecture, and the degree to which it can recapitulate effects of CSDS on sleep-related endpoints.

Methods: We used a wireless EEG system that enables continuous data collection in freely-moving male mice over a period of weeks. To examine mechanisms of stress-induced sleep changes, we used viral vectors to express excitatory (hM3Dq) or inhibitory (hM4Di) DREADDs (or mCherry control) in the NAc of mice expressing cre-recombinase in D1-MSNs (GENSAT FK-150). Mimicking the design of our previous CSDS study, after a 5-day baseline, all mice received clozapine (0.3 mg/kg/day) in their drinking water for 10 days, followed by a 5-day washout.

Results: Chronic inhibition of D1-MSNs (via hM4Di) produced CSDS-like increases in PS time ($F(2,24) = 12.87$, $p = 0.0002$) without affecting slow wave sleep (SWS) or wakefulness (W) times. In contrast, chronic activation of D1-MSNs (via hM3Dq) produced decreases in PS time ($F(2,20) = 4.662$, $p = 0.02$), also without affecting SWS or W times. These effects persisted following a 5-day DREADD ligand washout, suggesting that even transient activation or inhibition of this neuronal population can produce long-lasting effects on sleep.

Conclusions: Alterations in the function of NAc D1-MSNs produces complex effects on sleep and wakefulness. Chronic inhibition of D1-MSNs is sufficient to mimic effects of CSDS on key sleep-related endpoints. When considered together, our findings suggest a circuit model whereby stress effects on the NAc lead to KOR-mediated reductions in the function of midbrain dopamine systems, decreased activity of D1-MSNs, and disruptions in sleep architecture that resemble those seen in people with mood and anxiety disorders.

Disclosure: Psy Therapeutics, Consultant, Biogen, Employee (Spouse)

1.4 Investigating the Role of Striatal Neuron Subtypes in Compulsive Behavior and Treatment Response

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Background: OCD functional neuroimaging studies have consistently demonstrated symptom-associated striatal hyperactivity

that is resolved by effective treatment. However, it is unknown how the two major opposing cell-types of the striatum, D1 and D2-spiny projection neurons (SPNS), contribute to striatal hyperactivity during compulsive behaviors, and how their activity is impacted by pharmacologic treatments. Although a prevailing theory suggests compulsive behaviors result from either excessive activation of the D1-associated direct pathway or decreased inhibition of the D2-associated indirect pathway, there is little direct evidence to support this idea. To dissect contributions of SPN subtypes to striatal hyperactivity and associated compulsive behaviors, we used an animal model system that displays hyperactivity in central striatum (CS) and OCD-relevant behaviors including compulsive grooming: SAPAP3-KO mice.

Methods: Cohort 1: Male and female SAPAP3 KOs ($n = 5$) and WT littermate controls ($n = 5$) were injected with AAV-GCaMP6m and implanted with GRIN lenses in CS to visualize striatal calcium activity during grooming in both D1 and D2-SPNs. Cohort 2: D1-cre/SAPAP3-KOs ($n = 12$) and WTs ($n = 11$) were injected with cre-dependent AAV-GCaMP6m to selectively image D1-SPN activity during grooming. After baseline assessments, both cohorts received 7 days fluoxetine (5 mg/kg), with imaging and grooming assessments on treatment days 3, 5, and 7. Calcium signals were extracted using CNMF-e algorithm, converted to ΔF/F, and aligned to grooming onset. To determine if cells were activated/inhibited by grooming onset, $Ca2+$ event rates were shuffled (1000 iterations) to create a null-distribution, and event rate difference > 1 SD from null distribution was considered significant.

Results: SAPAP3-KOs displayed increased grooming ($p < .01$) and behavior transitions ($p < .01$) that were normalized by fluoxetine. Cohort 1: When all SPNs were examined together, SAPAP3-KOs showed increased grooming-associated calcium activity relative to WTs ($p < .01$) that was specifically localized to grooming onset. Fluoxetine 1) significantly reduced the grooming-associated increases in striatal SPN activity and 2) normalized the peri-grooming elevation in fluorescence observed in KOs. Cohort 2: In contrast, when D1-SPNs were examined in isolation, grooming-associated event rates were lower in SAPAP3-KOs, and peri-grooming calcium activity was significantly less varied. KOs also had significantly fewer D1-SPNs activated by grooming ($p < .05$). After fluoxetine, significantly more D1-SPNs were activated in response to grooming. Preliminary ex vivo data suggest that fluoxetine may be modulating striatal fast spiking interneurons to normalize striatal activity.

Conclusions: Using in vivo microscopy in freely moving animals, we demonstrated that SAPAP3-KOs have increased baseline and grooming-associated striatal firing rates, consistent with previously published work. Surprisingly, when we selectively examined D1-SPNs, contrary to expectations we saw decreased activity compared to WT at initiation of compulsive grooming events. This activity pattern was normalized by fluoxetine. These data suggest a novel model in which decreased activity in D1-SPNs and excessive activity in D2-SPNs promotes compulsive behaviors. Ongoing work is testing this model by examining the contribution of D2-SPNs to compulsive grooming and treatment response. Understanding cell-type specific effects of successful and unsuccessful SSRI treatment may help develop treatments with improved efficacy and fewer side effects.

Disclosure: Nothing to disclose.

Panel

2. Neurobiological Predictors of Treatment Response in Pediatric Mood and Anxiety Disorders

2.1 Neural Networks for Cognitive Control May Underlie Response to Cognitive Behavioral Therapy in Clinically Anxious Youth

Kate Fitzgerald

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Background: Cognitive control enables the flexible adjustment of behavior by constraining attention to task-relevant stimuli and may be critically impaired in anxiety. Fronto-parietal and cingulo-opercular regions comprise networks for task control, as shown by activation of these regions during a range of cognitive tasks, including conflict between competing cognitive stimuli. Abnormal engagement of these networks has been demonstrated in anxiety disordered youth, suggesting impaired ability to engage cognitive control. Unknown is whether treatment-induced changes in task control network activation associate with treatment response.

Methods: Conflict was induced during fMRI scanning with the Multi-Source Interference Task (MSIT) and in 16 clinically anxious (12.8 ± 3.1 years) and 9 low anxious youth (13.8 ± 3.2 years). Anxious youth were re-assessed after 12 weeks of cognitive behavioral therapy (CBT) and low anxious youth after a similar time interval, using the MSIT and the Pediatric Anxiety Rating Scale (PARS). Activation maps for high relative to low conflict trials, at both first and second time points, were generated in SPM8 and displayed at punc < .001. For each scan, contrast estimates were extracted from task control regions, defined by 10 mm spheres placed at peak coordinates. Changes in activation were tested for associations with change in anxiety using partial correlations, controlling for between-subject variations in age and performance.

Results: Across subjects and at both time points, whole brain analyses revealed conflict-activation of the dorsal anterior cingulate cortex (-6, 5, 50), left superior parietal cortex (-24 -61 44) and bilateral anterior insula (right aI 33, 20, 5; left aI -27, 23, 5); coordinates shown for first scan only. Between time points, CBT-treated youth exhibited significant reduction in anxiety on the PARS (pre-CBT:18.8 ± 3.5: post-CBT:10.9 ± 4.1, $p < .001$). There were group differences in ROI activation between first and second scans for clinically anxious compared to low anxious youth. However, in clinically anxious youth, greater pre-CBT activation of right ($r = -.56$, $p = .05$) and left aI ($r = -.58$, $p = .04$) predicted better CBT response (i.e., lower post-CBT anxiety, controlling for pre-CBT anxiety on PARS). In addition, increase in activation of the left superior parietal cortex ($r = -.55$, $p = .04$), but trend-level decrease in activation of the right aI ($r = .51$, $p = .063$) associated with greater reduction in PARS, from pre- to post-CBT.

Conclusions: Results implicate neural networks for cognitive control as potentially relevant to mechanisms of CBT effect in clinically anxious youth. Increases in frontoparietal activation and decreases in cingulo-opercular activation were associated treatment-related reduction in anxiety, suggesting that these networks are differentially involved in producing CBT response.

Disclosure: Nothing to disclose.

2.2 Task Control Circuit-Based Predictors of Response to Cognitive Behavioral Therapy in Pediatric Obsessive-Compulsive Disorder

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Background: Neuroimaging data suggest functional and structural abnormalities in task control circuits in OCD, and data from adults suggest that patterns of resting-state connectivity within these circuits predicts response to cognitive behavioral therapy (CBT). Much less is known about these circuits in pediatric OCD and identifying circuits or cognitive processes that predict treatment response in children could pave the way for the development of novel circuit-targeted treatments and prevention strategies.

Methods: Multi-modal MRI data was collected from 27 unmedicated children and adolescents with OCD (12.3 + /-3.2 years) and 27 healthy controls (HC, 11.3 + /-3.2 years). OCD participants then received 16-20 weeks of CBT with exposure and response prevention. Multi-band fMRI data was collected at rest (two 7 min runs) and during performance of the Simon Spatial Incompatibility task. Data were preprocessed with the HCP pipeline; subsequent processing and analyses were conducted on CIFTI 32k grayordinate surface space. Head motion, outlier frames, and global signal were regressed from the resting state data. Mean timeseries during resting state and task were extracted from cortical parcellations identified by Gordon et al., (2014). Parcel-to-parcel Fisher-Z transformed correlations during resting state were averaged within and between networks. Standard task GLM analyses were run on parcel timeseries to identify effects of conflict. Linear regression analyses were used to assess resting state and task-related group differences and associations with CBT-related symptom change (CYBOCS) in the OCD group.

Results: Group differences in baseline resting state connectivity were detected within dorsal attention (DA) and cingulo-opercular (CO) networks and between these task control and default mode (DM) networks, with greater within and reduced between network connectivity in OCD vs HC (p's < 0.05, corrected). Further, reduced connectivity between frontoparietal and DM networks predicted better CBT response in the OCD group ($p = 0.03$). Group differences in conflict-related activations were detected in the DA (OCD > HC), and activation in CO regions predicted better CBT response in the OCD group.

Conclusions: These new findings suggest that the functioning of task control networks predicts response to CBT in pediatric OCD. The OCD participants with the more 'normal' patterns of connectivity between task control and DM networks and better engagement of CO regions during a cognitive control task responded better to CBT. These data point to the potential utility of using cognitive control training enhance the functioning of task control circuits early in development to prevent the onset or enhance the remission of OCD in children and adolescents.

Disclosure: Nothing to disclose.

2.3 Resting-State Functional Connectivity Predictors of Response to N-Acetylcysteine in Adolescents With Non-Suicidal Self-Injury

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Background: Non-suicidal self-injury (NSSI) is a common problem in adolescents and is associated with future suicide attempts. We recently conducted an open-label pilot study examining the clinical of oral N-acetylcysteine (NAC), a nutritional supplement with antioxidant properties, in 35 adolescents with NSSI (Cullen et al. 2018). While we found a reduction in NSSI frequency and depression symptoms, there was also significant variability in response. A subset of these adolescents underwent resting-state functional magnetic resonance imaging. Here we explored whether baseline patterns of resting-state functional connectivity (RSFC) could serve to predict which adolescents with NSSI would benefit from NAC. Since both negative and positive valence systems are potentially implicated in NSSI (Westlund Schreiner et al. 2015), we focused our analyses on neural circuits centered around the amygdala and the nucleus accumbens (NAcc).

Methods: We conducted whole-brain, seed-based analyses using anatomically-defined bilateral amygdala and NAcc seeds in adolescents aged 13-21 with NSSI prior to starting an 8-week course of NAC. Eighteen of adolescent participants had usable RSFC data for analysis. We then correlated clinical response (defined as [a] a decrease in NSSI frequency after 8 weeks of NAC treatment, and [b] a decrease in depression symptoms as measured by the Beck Depression Inventory [BDI]) with baseline amygdala and NAcc connectivity maps. To reduce the number of comparisons, we examined the association between clinical response and connectivity between (1) amygdala seeds and salience and default mode network regions and (2) NAcc seeds and other regions implicated in reward and addiction. Significant clusters were identified using a Monte Carlo permutations approach as implemented in AFNI's 3dClustSim, using a voxel-wise p threshold of 0.001 and alpha = 0.05.

Results: Greater reduction in NSSI frequency, was associated with a baseline pattern of RSFC including (1) greater RSFC between left amygdala and left anterior cingulate cortex, (2) lower RSFC between left amygdala and right lateral occipital cortex; and (3) lower RSFC between right NAcc and right superior frontal gyrus and paracingulate cortex. Greater reduction in BDI scores was associated with lower RSFC at baseline between left amygdala and right middle frontal cortex.

Conclusions: We provide preliminary evidence that baseline patterns of amygdala and NAcc RSFC may provide useful information to guide whether NAC should be selected as a treatment for a given adolescent presenting with NSSI and depressive symptoms. Our data suggest that the RSFC patterns predicting reduction in NSSI versus depression symptoms are distinct. These findings may shed light on the mixed findings in prior studies of NAC for depression and suggest an opportunity for future research using fMRI to confirm whether our preliminary neuroimaging-based predictive patterns could be replicated. Limitations of the study include the small sample size, lack of randomization and lack of a placebo arm to differentiate true response from expectation effects or regression to the mean. While the results here show preliminary evidence of predictors of NAC response, it is not clear whether these predictors are specific to NAC or if similar RSFC patterns would predict a positive response to other treatments. Research investigating this question will be critical for advancing precision medicine.

Disclosure: Nothing to disclose.

2.4 The Role of the Nucleus Accumbens as a Treatment Marker in Pediatric Depression and Obesity

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Background: Youth with depressive disorders and obesity have shared anatomical and functional abnormalities in frontostriatal neural circuits important for reward processing (Singh et al., Hormones and Behavior, 2018). Few studies have evaluated these structural and functional abnormalities in youth prospectively and in the context of standard therapies to target depressive symptoms. Even less is known about how the degree of reward circuit dysfunction in pediatric depression and obesity predicts

treatment response. Identifying baseline neural characteristics that predict treatment response could accelerate the development of novel and selective treatments.

Methods: Multi-modal MRI data was collected from 41 children and adolescents with depression (MDD, 14.7 + /-1.9 years) and 39 healthy controls (HC, 13.9 + /-2.5 years). Some of the MDD participants then received either an antidepressant alone (n = 3), psychotherapy alone (n = 20), or a combination of antidepressant or psychotherapy (n = 29) between baseline and 6 months follow up. Nucleus accumbens (NAcc) structural MRI and seed-based intrinsic connectivity were analyzed. Structural data were free-surfed, and head motion, outlier frames, and global signal were regressed from the resting state data. We assessed baseline group differences between MDD and HC groups, and examined treatment-related symptom change (CDRS-R depression severity) associations between nucleus accumbens structure and function over six months and by treatment within the MDD group.

Results: As expected, MDD participants had significantly higher CDRS-R scores (M = 53.341, SD = 10.64) than did HC (M = 19.103, SD = 2.49) participants. MDD youth had weaker NAcc-bilateral insula connectivity ($p = 0.001$) and stronger NAcc-superior frontal gyrus (SFG) connectivity ($p < 0.001$) than did HC youth. Stronger NAcc-SFG connectivity ($r = .35$, $p = 0.026$), smaller left NAcc area ($r = .26$, $p = .08$), and cortical thinning ($r = 0.46$, $p < 0.001$) predicted greater depression symptom severity. Compared to untreated youth, MDD youth who sought treatment showed reductions from baseline to 6 months in NAcc-SFG connectivity ($p < 0.02$) approaching normalized values seen in HC at baseline, and a trend for smaller left nucleus accumbens area ($p = 0.05$).

Conclusions: These new findings suggest that nucleus accumbens structure and intrinsic connectivity may be an important predictor of response to depression treatment in pediatric MDD and obesity. The MDD youth with the more 'normalized' patterns of frontostriatal connectivity may be conceptualized as treatment responders. These data suggest the potential utility of targeting frontostriatal networks to enhance the functioning of this circuit early in development to prevent the progression of MDD and insulin resistance into adulthood.

Disclosure: Nothing to disclose.

Panel

3. Neural Computations as Markers of Stress, Anxiety and Trauma

3.1 Stress Modulates Learning and Decision-Making Under Uncertainty

Abstract not included.

3.2 Decision Making Under Uncertainty in Post-Trauma Psychopathology

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Background: Traumatic events are often associated with high levels of uncertainty, yet the role of individual uncertainty attitudes in the development of trauma-related psychopathology has hardly been examined. We have recently used a paradigm inspired by behavioral economics and identified variations in decision making under uncertainty that were associated with posttraumatic stress disorder (PTSD; Ruderman et al., 2016). Here, we use the same task to explore neural markers of trauma-related symptoms.

Methods: We used a monetary task to assess uncertainty attitudes of 78 male combat veterans. Subjects chose between a certain gain (or loss) and playing a lottery which offered a larger gain (or loss) but also chance of zero outcome. Outcome probabilities for half of the lotteries were precisely known ("risk") and were ambiguous for the other half ("ambiguity"). Functional MRI was used to track neural activation while subjects completed 240 decisions. One choice was randomly picked for payment. The choice behavior of each subject was used to fit a computational model, which provided estimates of individual risk and ambiguity attitudes in the gain and loss domains. We evaluated PTSD symptoms with the Clinician-Administered PTSD Scale (CAPS) and used additional measures to assess trauma exposure and other psychiatric symptoms, including depression, anxiety and addiction.

Results: Using a dimensional approach, we replicated our recent behavioral results, and found that veterans suffering from more severe trauma-related symptoms (higher CAPS) were more averse to ambiguous losses (Pearson's correlation $r = 0.25$, $p < 0.05$), but not to ambiguous gains. In addition, this approach revealed increased risk aversion in veterans with higher CAPS scores, when these veterans were making choices between gains ($r = 0.3$, $p < 0.05$), but not between losses. A whole-brain analysis highlighted the ventromedial prefrontal cortex (vmPFC), an area involved in both value-based decision making and fear learning. General activation in this area during the decision phase was negatively correlated with CAPS ($p < 0.05$). Interestingly, when controlling for correlations between symptom clusters, emotional numbing remained the only significant cluster. Severity of this symptom cluster predicted activation when making choices between gains, but not losses ($p < 0.05$), emphasizing the significance of studying reward, in addition to punishment, processing in post-trauma psychopathology.

Conclusions: Our results demonstrate the potential of neuroeconomics techniques for studying the neural basis of psychopathology in a transdiagnostic manner, and for devising objective diagnostic tools that compensate the categorical diagnoses of the DSM.

Disclosure: Nothing to disclose.

3.3 Neural Computations of Threat Value After Combat Trauma

Daniela Schiller

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Background: Although abnormal threat conditioning features prominently in theoretical accounts of post-traumatic stress disorder (PTSD), the manner in which learning becomes dysfunctional is less clear. Moreover, it is possible that PTSD abnormalities are not observable in overt behavior but rather reside in latent parameters of learning, such as learning rate and expected value, which could only be estimated from observable behavior. Another key issue in the field is the specific role that the amygdala plays in PTSD: we do not know what is the relationship between amygdala structure and function in relation to PTSD symptoms, and we do not know what exactly is computed in the amygdala in response to negative stimulation. To address these issues here, we used the Rescorla-Wagner (RW) learning model to estimate learning rate and expected value in a threat conditioning and reversal paradigm, in a population of 54 combat veterans with varying degrees of psychopathology.

Methods: The experiment began with an acquisition stage, in which participants were presented with two visual stimuli, one of which co-terminated with an aversive outcome on some of the trials, and the other was never paired with the shock. The acquisition phase was immediately followed by an unsignaled reversal stage, in which the contingencies were flipped. Skin conductance response (SCR) served as the index of conditioned defensive responses. To estimate the learning rate, the free parameter in the RW model, we fitted a Hierarchical Bayesian RW model to the recorded SCR data. Using the RW model and the estimated learning rate, we created parametric predictors comprised of the expected value in each trial for each participant. We then used these parametric predictors to examine the degree of value tracking in the amygdala, and to what extent amygdala value-dependent activity corresponds to PTSD symptoms (CAPS).

Results: We found a structure-function relationship with CAPS in the right amygdala, where both volume ($\beta = -0.50$, $t(46) = -2.73$, $P = 0.009$) and neural activity ($\beta = -0.33$, $t(46) = -2.52$, $P = 0.015$) independently predicted the total CAPS score. No other factors, including learning rate, showed a significant relationship with PTSD symptoms.

Conclusions: This study provides evidence for independent structural and neurocomputational contributions of the amygdala to combat-related PTSD symptoms. Higher levels of PTSD symptoms in combat veterans related to lower fidelity of value representation in the amygdala during threat learning, and smaller amygdala volume. Thus, the combined power of computational, morphological, and functional analytic tools enables us to relate latent markers of learning and morphological indices to overt symptoms, as specific targets for investigating trauma related psychopathology and its potential treatment.

Disclosure: Nothing to disclose.

3.4 Neurocomputational Correlates of Exploratory Decision in Anxiety

Rony Paz

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Background: An appropriate balance between exploiting what is known and exploring what is largely unknown is relevant for everyday-life decision making. The extent of exploration biases may be governed by how an individual processes uncertainty. Yet, how these biases relate to anxiety trait is not completely known. Moreover, the effects of gain vs. loss on uncertainty processing was not examined. It is therefore unclear if anxious individuals would confront short-term uncertainty and perform exploratory decision in order to reduce uncertainty in the environment, and how it differs across loss or gain contexts.

Methods: Subjects ($n = 28$) participated in a three-armed bandit task. Exploration was promoted by varying the outcome in each machine across trials. Each participant performed both a Gain condition, where all outcomes were positive and a loss condition where all outcomes were negative and participants had to avoid. Sine-wave functions described the outcomes associated with each slot machine. The magnitude and mean of each sine-wave was always 20 and 50 points respectively. The period lengths of the three sine-waves were different in each session, and the sine-waves were phase-shifted. Individual trait anxiety scores were estimated via Spielberger's State-Trait Anxiety Inventory (STAI).

The tasks were performed while subjects underwent model-based fMRI scans. We used computational modeling to determine whether a decision was exploratory or exploitation. A second model examined the contribution of the expected value, outcome uncertainty, change uncertainty (time-dependent), and random switching. Models were fitted to each individual.

Results: Participants were more likely to explore when trying to avoid losses as compared to acquiring gains, yet more anxious individuals showed increased exploration independent of the Gain/Loss condition (repeated measures ANOVA). The computational modelling (see methods) revealed that anxious individuals show i) a reduced motivation to exploit the best option, and ii) increased intolerance to uncertainty in the decision-making environment. Both of these tendencies contribute and increase the proportion of exploratory decisions.

The same model was used to examine neural activations, with separate repeated measures ANOVAs conducted for each of ROIs derived from exploration-exploitation contrasts, with within-subject factor Condition (Gain, Loss) and Trait anxiety as continuous covariate, to identify how decision-related BOLD signal is modulated by differences in expected outcome and change uncertainty (the main factors revealed by behavior). The main findings were: (1.) Neural activations for difference in expected outcomes was significantly and positively correlated with Trait anxiety in the dACC. (2.) Neural activations for change-uncertainty were significant and negatively correlated with Trait anxiety bilaterally in the anterior-insula.

Conclusions: Individuals with higher levels of trait anxiety showed increased exploration in both Gain and Loss conditions, associated with two distinct mechanisms. First, decisions made by more anxious individuals were less influenced by expected outcomes and associated with a less efficient recruitment of the dACC. Second, trait anxiety increased the motivation to reduce change uncertainty, associated with a more rapid engagement of the anterior-insula as uncertainty increased. Thus, high levels of trait anxiety boosted exploration via a combination of a reduced focus on value-based decision making and an increased intolerance of uncertainty in the environment.

Disclosure: Nothing to disclose.

Mini Panel

4. Novel Technological Strategies to Assess Suicidality and Develop Suicidal Phenotypes

4.1 Digital Phenotyping and Predicting Suicidal Thoughts and Behaviors Using Smartphones and Wearable Biosensors

Matthew Nock

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Background: Although suicide is a leading cause of death worldwide, suicidal thoughts and behaviors (STBs) are not well understood. A major limitation is that because we cannot ethically induce STBs in laboratory or clinical settings, we have not been able to empirically observe their occurrence. This is in contrast to virtually all other topics of scientific study in which we understand phenomenon by observing their natural occurrence (animal behavior, chemical reactions, exoplanets, etc.). We propose to help bridge this gap by using new digital monitoring methods (smartphones, biosensors) to monitor STBs while they unfold in nature and to use these technologies to learn to predict their occurrence in the near-term.

Methods: We have been monitoring samples of adult ($n > 70$) and adolescent ($n > 50$) inpatients with a recent history of STBs using smartphones (active monitoring via $6\times$/day surveys; passive monitoring of GPS, accelerometer, incoming and outgoing calls/

texts) and biosensors (e.g., accelerometer, EDA, skin temperature, heart rate) during hospitalization and after hospital discharge.

Results: Results reveal that we identify 5 different phenotypes in our data that differ regarding the average severity and variability of suicidal thoughts. These 5 subtypes are reliably observed across samples (k-1LRT: sample 1: 58.95; sample 2: 34.37, Ps < .001). Cross-validated results also reveal that using passive monitoring data, we can predict next-day presence of suicidal thoughts with a fair degree of accuracy (AUC = .75).

Conclusions: The results of this study suggest that there are reliable phenotypes of suicidal thinking that are observed across samples and that real-time monitoring data can be used to improve the short-term prediction of suicidal thoughts. These data have implications for advancing the understanding, prediction, and ultimate prevention of STBs.

Disclosure: Nothing to disclose.

4.2 Suicidal Phenotypes: Patterns of Suicidal Ideation in Mood Disorders Based on Ecological Momentary Assessment

Maria Oquendo

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Background: Suicidal ideation (SI) is often a harbinger of suicide attempts or death. We hypothesize that a propensity for SI variability, wherein SI can rise quickly from low to high scores, may be a phenotype predisposing to stress induced suicidal behavior. SI variability may be a consequence of childhood abuse and relate to poorer ability to harness orbital prefrontal cortex during emotion regulation resulting in affective lability and greater responsivity of the hypothalamic pituitary adrenal (HPA) axis to psychosocial stress. In this preliminary study, we sought to test parts of this hypothesis and evaluated the stability of SI variability over time.

Methods: We followed 51 depressed subjects, 33% with a past suicide attempt, for 2 years. Assessed with the Hamilton Depression Rating Scale (HDRS), Beck Depression Inventory (BDI), Affective Lability Scale (ALS), and Childhood Trauma Questionnaire (CTQ), the sample was 59% female and aged 37.3 ± 11.2. Subjects provided SI data using Ecological Momentary Assessment (EMA), up to 6 times a day, for 7 days at baseline and 3, 6, 12, 18 and 24 months after baseline. For each of the 6 time-points, within-subject SI severity was defined as the average SI score and within-subject SI variability as the (square root of the) Mean Successive Squared Deviations of the total SI score at each prompt. Mixed effect models tested the differences in both SI severity and variability across time points, and the effect of baseline variables on SI severity and variability, with time point and baseline variables as fixed effects, and subject-specific random intercepts.

Results: Momentary SI measured after 6001 prompts (average = 118 per subject) yielded a SI severity score of 6.05 (SD = 5.95, range: 0-36). While SI severity decreased over time (baseline: m = 7.62, SE = 0.70; 3 month: m = 5.40, SE = 0.80; F = 4.03, df = 5,137, p = 0.002), within-subject SI variability did not differ over time (F = 1.48, df = 5,137, p = 0.2004). Baseline predictors of SI severity were depression severity (HDRS: b = 0.25, SE = 0.10, t = 2.46, df = 135, p = 0.015; BDI: b = 0.16, SE = 0.06, t = 2.51, df = 133, p = 0.013), and affective lability (b = 0.07, SE = 0.02, t = 2.94, df = 116, p = 0.004) with a trend observed for CTQ total score (b = 0.07, SE = 0.04, df = 123, t = 1.97, p = 0.052. Baseline predictors of greater SI variability included childhood physical abuse (b = 0.07, SE = 0.03, t = 2.06, df = 127, p = 0.042) with trends observed for total CTQ score (b = 0.02, SE = 0.01, df = 123, t = 1.72; p = 0.089) and affective lability (b = 0.01, SE = 0.01, t = 1.85, df = 116, p = 0.067). Across time points, suicide attempters and non-attempters had comparable SI severity (time * attempt history: p = 0.179, attempt main effect: p = 0.834) and variability (time*attempt: p = 0.826, attempt main effect: p = 0.843).

Conclusions: The propensity for SI variability among depressed individuals appears to be a stable trait, at least over 2 years. Consistent with our hypothesis, more severe childhood abuse, especially physical abuse and difficulties with emotion regulation expressed as affective lability predicted highly variable SI. Ongoing studies are assessing HPA responsivity and orbital prefrontal cortex function during emotion regulation and their relationship to SI variability.

Disclosure: Bristol Myers Squibb, Stock / Equity, (Spouse) Research Foundation for Mental Hygiene, Royalties

4.3 Suicidal Ideation Variability Using Momentary Assessments and Stress Responsivity as Methods for Developing Suicidal Phenotypes in Borderline Personality Disorder

Barbara Stanley

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Background: There is a limited understanding of short term indicators of suicide risk. Possible reasons are: 1. Our prior methods to study suicidality are not robust enough to detect subtle changes in risk and 2. We have treated suicidal individuals as a unitary group and have not adequately considered that there may be distinct suicidal phenotypes. Suicidal ideation (SI) can either be static or fluctuate dramatically over the course of the day and, for some individuals, suicide attempts (SA) occur within one hour from initial ideation while for others planning an attempt is a lengthy process. Capturing moment-to-moment fluctuations in mood, thought processes, and environmental triggers may be critical to understanding underlying patterns of SI and short-term predictors of SA and may help us better identify suicidal phenotypes. Unlike traditional techniques, ecological momentary assessment (EMA) allows for this real-time assessment, outside of the clinical setting. Using EMA, we examined individual patterns of SI, how clinical and environmental factors influence SI severity and variability, and which factors predict SA. In this study, we examine patterns of SI and their relationship to stress responsivity and clinical factors.

Methods: Methods: Our sample (n = 50) consisted of co-morbid mood disorder and borderline personality disorder (BPD) patients with current SI and a history of suicidal behavior. Using EMA, participants were prompted with questions about SI and daily stressors at 6 time points daily for a 1-week period. We examined the relationship of baseline clinical variables and 9 daily stressors with respect to EMA SI severity, EMA SI variability, and SA. Participants were also administered the Trier Social Stress Test (TSST) to determine cortisol responsivity to a psychosocial stress.

Results: Results: Using mean square successive differences (MSSD) analyses we find three patterns of SI variability exist within suicide attempters with borderline personality disorder: high variability SI, moderate variability SI and chronic, unremitting SI. The Beck Depression Inventory (BDI) (p < 0.0001), Beck Hopelessness Scale (BHS) (p < 0.0001), Affective Lability Scale (ALS) (p = .04), and Zanarini Rating Scale for BPD (ZAN-BPD) (p = .023) correlated with EMA SI severity, while the BHS (p = .003), ALS (p = .027), and ZAN-BPD (p = .0198) correlated with EMA SI variability. Furthermore, we find that individuals high SI variability are more likely to respond to daily stressors with increased SI indicating that those high in SI variability may represent a stress

responsive suicidal phenotype. In order to examine this further, we examined the relationship between the TSST cortisol response and SI variability and found that SI variability is positively related to cortisol response as measured by the area under the logarithmic transformed curve. ($t = 2.2816$, $df = 41$, $p = 0.0277$).

Conclusions: Conclusion: Our findings indicate the EMA is valuable tool for studying suicidal states and suggest distinct patterns of SI variability may exist. Results also provide insight into the clinical variables and stressors that contribute to SI and predict SA. Future research using EMA is needed to better understand SI patterns, short-term predictors of SA, and suicidal phenotypes.

Disclosure: Abbvie, Consultant; Merck, Consultant; Research Foundation for Mental Hygiene (CSSRS), Royalties

Study Group

5. Dueling Computational Approaches to Big Biomarker Data in Psychosis: Four Experts Reveal Gems in the B-Snip Database

Carol Tamminga, Brett Clementz, Martin Paulus, Albert Powers, Robert Gibbons, Brett Clementz, Vince Calhoun, Joshua Gordon*

University of Texas Southwestern Medical Center, United States

Study Group Summary: Clinical psychosis syndromes are "poor mirrors of nature". There is considerable variance within and unacceptable overlap between clinical syndromes at essentially ever level of measurement used in psychosis research. Clinical syndromes have yet to progress past their initial identifications to include physiological and/or brain functional information of any kind.

One solution is to leverage a large data resource across multiple levels of analysis using sophisticated analytic strategies. In the present context, big data refers to a large number of participants with a variety of data types across multiple levels of analysis available on most participants (from brain structure to symptoms). The Bipolar-Schizophrenia Network for Intermediate Phenotypes (B-SNIP), a multisite deep phenotyping study whose target is neurobiological heterogeneity in psychosis, provides such a resource.

One influence on B-SNIP work was the RDoC initiative, which formalizes a multi-level, diagnosis-independent approach to understanding basic to complex disease features. The logical distance from genes to symptoms is considerable. Theories need to be integrated from genes to molecules to circuits to physiology to behavior. Work at only the symptomatic level independent of physiology may lead to incomplete, perhaps incorrect, answers to pressing questions. NIMH Director Gordon has commented on the challenge of deconstructing such syndrome, like psychosis, into subcomponents – the difficulty and analytical sophistication required when using data-driven approaches to derive domains that capture the most important features of these syndromes, and then using this information in translational models to develop treatments targeting specific functional/physiological domains. Deriving a thesis from the bottom-up and from the top-down must match.

The B-SNIP consortium has published multiple papers using different analytical approaches with the general theme of deriving more data-driven approaches to understanding neurobiological heterogeneity in the psychoses. To foster additional discoveries in the B-SNIP dataset, we invited four analytical experts to discover features in our data that we, as a consortium, have yet to consider. Drs. Vince Calhoun, Robert Gibbons, Martin Paulus, and Al Powers, all known for their analytical rigor, creativity, and sophistication, used the same large, multivariate B-SNIP dataset. They used the analytical method of their choice, with no intervention or suggestion on the part of the B-SNIP PIs. Dr. Calhoun used a combination of ICA and deep learning on multi-level brain structure and function data, Dr. Gibbons used his item response theory approach to integrating different levels of analysis, Dr. Powers used a hierarchical predictive coding model to integrate across levels of analysis, and Dr. Paulus used group factor analysis to identify latent variables in the multivariate B-SNIP data.

Given his interest in using numerical approaches with big data to help solve pressing questions related to borders and differentiations of syndromes, and for enhancing understanding of neural mechanisms that can be translated across animal-human research platforms, Dr. Josh Gordon, NIMH Director, will discuss the significance of this work presented by the four analytical experts and future directions for similar projects.

Disclosure: APA, Board Member; AStellas, Consultant; Sunovion, Consultant; Merck, Consultant; Autifony, Consultant; Intracellular Therapies, Board Member

Panel

6. Behavioral Neuroscience in Translation: From Animals to Humans

6.1 Skewed by Cues? Reward-Concurrent Cues Modulate Risky Decision Making in Rats and Humans

Abstract not included.

6.2 The Neural Basis of Instrumental Learning in Monkeys and Humans: Contingency Degradation as a Test of the Habit Hypothesis of Obsessive-Compulsive Disorder (OCD)

Trevor Robbins

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Background: The concept of distinct cortico-striatal systems of goal-directed and stimulus-response (S-R) habitual learning that jointly influence behavioral output has been applied to drug addiction and obsessive-compulsive disorder (OCD) to suggest biases in these disorders towards habitual control. Effects on behavior of goal devaluation have often been used to infer the relative contributions of goal-directed and S-R habit-based control; however there are some practical problems in applying goal devaluation in a comparative manner across species. Hence, we have used another method for inferring habit-based responding that depends on degrading the causal contingency between action and environmental outcome (thus affecting beliefs about control). We describe the mapping of cortico-striatal determinants of instrumental control over performance in marmoset monkeys on a contingency degradation procedure using intra-cerebral infusions to induce reversible inactivation or activation of cingulate, orbitofrontal and striatal regions. In parallel, we have determined effects of degrading instrumental contingencies in patients with OCD and healthy age- and IQ-matched controls using an analogous test procedure.

Methods: Marmoset monkeys ($n = 8$, 2 m and 6 f) received intracerebral cannulae into several regions of the cingulate cortex, including Brodmann areas (BA) 24 and 32, as well as BA 11 and 14 of the orbitofrontal cortex (OFC) and caudate nucleus. They were trained on a novel contingency degradation procedure whereby different actions were associated with differently flavored rewards on different days. On contingency degradation or non-

degradation probe days they received free (i.e. non-contingent) presentations of the different rewards to increase the probability of an outcome given no action ((P) O I ~A) and assess the persistence of habit-based responding. We compared effects of muscimol ('inactivation') or the glutamate reuptake blocker, dihydrokainate, DHQ; 'over-activation') infused into several distinct regions of the cingulate cortex and the orbitofrontal cortex. In a human analogue of the procedure, we systematically varied the probability of delivering reward outcomes following actions, again by increasing ((P) O I ~A) in 27 (13 m, 14 f) medicated OCD patients and 27 (14 m, 13 f) healthy control volunteers. We measured both behavioral output and subjective appraisal of control exerted over outcomes. All data were subjected to mixed-model analysis of variance.

Results: In marmoset monkeys, both inactivation and over-activation of the perigenual cingulate cortex (BA 24) significantly ($P < 0.01$) and selectively attenuated effects of contingency degradation, leading to persistent responding. Inactivation of OFC BA 11 produced significantly greater contingency degradation, whereas over-activation impaired it ($P < 0.01$), there being no significant effects in OFC BA 14. Caudate effects are still being determined. In patients with OCD, behavior persisted after contingency degradation for certain values of positive contingencies ($P < 0.05$), despite intact and accurately reported action-outcome knowledge of the causal effect of their actions.

Conclusions: Integrity of the perigenual cingulate cortex and certain regions of the orbitofrontal cortex is essential for instrumental control over goal-directed behavior in primates. These regions may underpin parallel deficits in the control of goal-directed behavior, relevant to the egodystonia of compulsive behavior in OCD and supporting the 'habit' hypothesis.

Disclosure: Cambridge Cognition, Consultant, Royalties (Self & Spouse); Mundipharma, Consultant, (Self & Spouse); Shionogi, Grant; SmallPharma, Grant; Unilever, Advisory Board; Springer Verlag, Honoraria; Elsevier, Honoraria

6.3 Capturing the Propensity to Attribute Incentive Salience to Reward Cues in Human Children: Insights From the Sign-Tracker/Goal-Tracker Animal Model

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Background: The way an individual responds to cues in the environment may be a key determinant of psychopathology. For example, in patients with addiction, relapse is most often triggered by exposure to cues (e.g. paraphernalia) previously associated with the drug-taking experience. Such cues acquire the ability to trigger complex motivational states when they are attributed with incentive salience. In rats, when the presentation of a Pavlovian lever-cue is followed by the delivery of a food reward, some individuals, termed sign-trackers (ST), approach the lever-cue; whereas others, termed goal-trackers (GT), approach the location of impending food delivery. The lever-cue is a predictor for both ST and GT, but for ST it also becomes an incentive stimulus. Relative to GT, ST are also more impulsive on tests of impulsive action, show deficits on tests of sustained attention, and show a greater propensity for reinstatement of drug-seeking behavior following brief periods of cocaine exposure and abstinence. Thus, ST exhibit a number of behavioral traits that have been associated with psychopathology in humans. To determine whether comparable individual variation exists in humans, we have to assess sign- and goal-tracking behavior and associated traits in children.

Methods: Twenty-eight (16 f, 11 m) 5 to 7-year olds were exposed to a Pavlovian conditioned approach paradigm adapted from that used in rodents. The paradigm consists of 4 blocks of 10 trials, with each trial comprised of the 8-sec presentation of a lever-cue followed by the delivery of candy into an adjacent food cup (ITI, 8-32 sec). Output measures include the number, latency and probability of contacts with the lever-cue or food cup. A composite Pavlovian conditioned approach score is calculated based on these metrics, and ST are defined as those with a score > 0.5, and GT < -0.5. Subjects were also tested on a Go/No-Go task and a peg-tapping task to assess inhibitory control. Linear mixed effects models were used to assess the effects of sex and/or age on sign- and goal-tracking behaviors across blocks. The relationship between various behavioral measures were assessed using Pearson correlation coefficients.

Results: Using the composite index score, only one individual showed a tendency to goal-track. All others had an index score > 0, with a tendency to sign-track. Although there were no apparent age-related effects, there was considerable variation in the distribution of scores between 0 and 1, and this seemed to be dependent on sex. Approximately 38% (6) females and 63% (7) males had index scores between 0 and 0.5, and ~62% (10) females and ~36% (4) males between 0.5 and 1. Thus, a larger percentage of females showed a greater propensity towards sign-tracking; yet, sex differences were not apparent in the behavioral outcome measures assessed across training blocks ($P > 0.05$). These data are consistent with our rodent findings.

Conclusions: It is perhaps not that surprising that the majority of children between the ages of 5 and 7 show a tendency to sign-track. The rodent literature suggests that goal-tracking behavior is a function of "top-down" cortical control; whereas sign-tracking behavior results from enhanced subcortical drive overriding that cortical control. Thus, given the trajectory of cortical development, younger children may be more likely to exhibit sign-tracking behavior and associated impulse control deficits. Ongoing analyses are being conducted to determine the relationship between sign- and goal-tracking measures and other indices of impulse control in these children.

Disclosure: Nothing to disclose.

6.4 Substance-Specific Influences of Setting on the Neural and Affective Response to Opiates Versus Psychostimulants: Translating a Rat Model to Humans With Substance Use Disorder

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Background: Humans with substance use disorder (SUD) prefer distinct settings for using heroin versus cocaine. Regardless of the route of administration, individuals addicted to both heroin and cocaine report to prefer using heroin at home and cocaine outside the home. Based on this finding, as well as on similar findings in the rat, an arousal state mismatch hypothesis was proposed. According to this hypothesis heroin and cocaine produce distinct spectra of interoceptive effects that may be at odds with certain settings, but not with others. The central and peripheral arousal produced by cocaine, for example, would be at odds with a quiet domestic setting but not with an exciting non-home setting; whereas the sedative effects of heroin would be at odds with exciting settings but not at home. Thus, we hypothesized that the positive affective valence of the drug experience would decrease in the presence of a mismatch.

Methods: To test this hypothesis, we used a within-subject design to compare the subjective response to heroin and cocaine

in co-abusers. Specifically, we asked 51 individuals (12 females and 39 males) with SUD and a long history of both heroin (15.1 yrs, SD = 9.4) and cocaine (14.1 yrs, SD = 7.9) abuse, to rate central and peripheral effects (including state of arousal, heart rate, respiratory rate, muscular tension), as perceived when under the influence of the two drugs. The scores for each effect were compared with a pre-specified null hypothesis using the one-sample Mann-Whitney test. The two-tailed Wilcoxon signed rank test for paired data was used to assess differences between heroin and cocaine scores for each effect. We also calculated the Pearson's correlation as a measure of effect size. In a separate group of individuals with SUD we investigated how the affective valence of the drug experience changed as a function of the setting. We also used emotional drug imagery procedures combined with fMRI to investigate the activity of brain reward areas as a function of drug-setting interaction.

Results: The participants reported that under the influence of cocaine they experienced a significant increase in arousal, heart rate ($p < 0.001$), respiratory rate ($p < 0.001$), and muscular tension ($p < 0.001$), as well as a decrease in salivation ($p < 0.001$); whereas, when under the influence of heroin, the same individuals experienced sedation, and a reduction in heart rate ($p < 0.01$), respiratory rate ($p < 0.01$), and muscular tension ($p < 0.001$). When the participants were asked to rate the affective valence of the drug experience, those who experienced cocaine-induced arousal rated cocaine more pleasant outside the home than at home (McNemar's test, $p = 0.0015$); whereas those who experienced heroin-induced sedation rated heroin more pleasant at home than outside the home ($p < 0.001$). The fMRI data indicated that setting of drug use exerts a substance-specific influence not only on the affective response to heroin and cocaine, but also on the activity of brain regions, including those involved in processing drug reward and contextual information: the prefrontal cortex, caudate and cerebellum. In these regions we observed in fact a double dissociation ($p < 0.0001$) in activity as a function of drug and setting.

Conclusions: Drug addiction research should pay more attention to the distinctive effects of different classes of drugs and to the settings of drug use. Most important, our study has potential therapeutic implications, especially for the prevention of relapse in real world settings.

Disclosure: Nothing to disclose.

Panel

7. Innovative Approaches to Advance CNS Drug Discovery

7.1 Integrated Pipeline for Probe Discovery Targeting Protein: Channel Interactions in the CNS

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Background: The nexus of protein:protein interactions (PPI) organized around the voltage-gated Na + (Nav) channels is the molecular determinant of neuronal excitability and a converging node in the etiology of psychiatric disorders. PPI interfaces are highly specific and flexible, and could be ideal scaffolds for probe and drug development. Here, we present initial phases of a drug discovery campaign based on an integrated in vitro-to-ex vivo pipeline targeting the PPI interface at the level of the Nav1.6 channel and its regulatory protein, fibroblast growth factor 14 (FGF14). The FGF14:Nav1.6 complex regulates firing rate of medium spiny neurons in the nucleus accumbens. Thus, compounds from this campaign could lead to a new class of anti-depressants and/or mood stabilizers.

Methods: We employed medicinal chemistry, chemoinformatics, the split-luciferase assay (LCA), surface plasmon resonance (SPR), patch-clamp electrophysiology in heterologous expression systems and ex vivo brain slices to synthesize and validate the activity of hits in in vitro, in-cell and ex vivo preparations.

Results: We conducted an in-cell high-throughput screening against the FGF14:Nav1.6 complex. Following development of a double-stable HEK293 cell line expressing LCA constructs and assay optimization in 384-well plates, we screened ~50,000 small molecules, peptides and rationally-designed drug-like analogues. Compounds were ranked by a combination of %maximal luminescence and individual Z-scores, which were calculated based on the mean and SD of its respective plate controls (0.3% DMSO). Using cut-offs of $Z \leq -5$ and $\geq 60\%$ reduction in complex formation for inhibitors and $Z \geq 3$ and $\geq 150\%$ increase in complex formation for enhancers, we identified 960 primary hits. Of these, 640 compounds failed to achieve significance during triplicate validation screening, and counter-screening against full-length luciferase in transiently transfected HEK293 cells resulted in the exclusion of an additional 149 compounds due to significant inhibitory effects ($Z \leq -3$). Thus, the primary set of hits was reduced to 151 inhibitors and 20 enhancers, which were then stratified by structural and chemical parameters including predicted permeability (logP). Orthogonal screenings of these hits included cell-free surface plasmon resonance (SPR) that was used to determine drug binding affinity. One top hit included ZL177, which inhibited the FGF14:Nav1.6 complex formation ($19.5 \pm 2\%$, $n = 6$, $p < 0.001$, Student's t test) compared to control (0.5% DMSO, used as a vehicle) with an estimated $IC50 = 11$ μM. ZL177 and other small molecules from this campaign are currently being functionally evaluated as modulators of Nav1.6 currents and firing of MSNs in the nucleus accumbens slice preparation.

Conclusions: Using an integrated platform targeting protein: channel interactions we have identified new compounds that might lead to novel classes of therapeutics in neuropsychopharmacology.

Disclosure: Nothing to disclose.

7.2 PRESTO-Tango Screening at Novel Striatum GPCRs Identified by Transcriptomic Profiling

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Background: The striatum performs essential brain functions including movement control and reward encoding while striatal dysfunction occurs in many psychiatric diseases including addiction and psychosis. Although G protein-coupled receptors (GPCRs) have emerged as essential drug targets in human medicine, roughly 120 are orphan receptors with untapped potential for therapeutics. A previous challenge has been the appropriate design of reliable screens for orphan receptors, since their endogenous ligands and G protein signaling are largely unknown. But a recent, groundbreaking advance in the PRESTO-Tango assay now allows a measurement of receptor activation for more than 300 GPCRs. This innovative assay employs the universal readout of GPCR/beta-arrestin coupling and a highly sensitive luciferase gene reporter-based activity. Here we pursue the goal of identifying druggable GPCRs selectively expressed in the human striatum, combine genome-wide human tissue transcriptomics and the Presto-Tango platform to discover ligands for striatal orphan GPCRs.

Methods: We conducted a comprehensive genome-wide survey of human tissue-selective gene expression using 1640 high-quality RNA-seq samples from the Genotype Tissue Expression (GTEx) project. We developed a weighted tissue-selectivity scoring method that measures the similarity and differences of gene expression in all tissues and variability across donor samples. To identify receptor function and screen compounds, human clones of receptors were expressed in HEK293 cells and signaling was assessed using the Glo-sensor cAMP assay and Tango beta-arrestin assay. As a ligand discovery proof-of-concept, the PRESTO-Tango assay was used to screen the LOPAC library, in parallel, against five striatal orphan GPCRs.

Results: Human gene expression analyses identified a total of 123 protein-coding genes and 76 non-coding RNAs selectively expressed in the human striatum, including striatal-selective expression of 18 GPCRs. 11 of these receptors have known ligands including many established therapeutic or investigational drug targets (e.g. dopamine D1, D2, D3 receptors). The remaining 7 human striatal GPCRs, namely, GPR6, GPR52, GPR55, GPR88, GPR101, GPR139, GPR149, were all identified as class A orphan receptors. Biochemical and CRISPR/Cas9 knockout studies confirmed GPR6, GPR52 and GPR101 increased cAMP via Gs/olf G proteins, while GPR88 decreased cAMP levels via Gi/o G proteins. Results from the PRESTO-Tango screen showed approximately 60 compounds selectively induced a 2-fold activity above or below vehicle control treated cells for one of the five receptors. Dose response assays confirmed two compounds with agonist activity for GPR88 (carbachol and clozapine) and one with agonist activity for GPR149 (PDTC). Interestingly, both carbachol and clozapine are approved drugs for CNS indications. Clozapine is a classic atypical antipsychotic medication and inverse agonist at serotonin and DA receptors; however, clozapine was unexpectedly an agonist for GPR88/beta-arrestin signaling activity. PDTC agonist activity for GPR149 represents the first described agonist compound for this orphan striatal receptor.

Conclusions: This research provides a template for how brain region specific druggable targets may be identified using recently available human tissue transcriptomic datasets. These findings also demonstrate the flexibility and universality of the Presto Tango platform to advance CNS GPCR drug discovery, including for poorly defined and understudied brain orphan GPCRs.

Disclosure: Nothing to disclose.

7.3 Using Stem Cell Derived Neurons for Drug Discovery in Neuroscience

Ricardo Dolmetsch

Novartis Institutes for Biomedical Research, Cambridge, Massachusetts, United States

Background: Drug development in neuroscience has been particularly challenging over the last two decades. A major limitation has been the lack of predictive preclinical models that can be used at early stages of drug discovery to select and optimize drug candidates. iPSC-derived neurons have the potential to improve our drug discovery pipeline by providing human cellular models of neurological and psychiatric disease.

Methods: We have built a platform for generating iPSC derived neurons, engineering them using CRISPR Cas9 and using them to conduct both high throughput screens and secondary hit selection and optimization assays.

Results: I will discuss our efforts to optimize this system and some of the learnings that we have made along the way. I will also discuss our ongoing programs to identify targets and develop drugs for neurodevelopmental and psychiatric diseases using human induced pluripotent stem derived neurons. I will focus on efforts to identify targets in schizophrenia using iPSC cells and to develop drugs that address synaptic defects and the activity of inhibitory neurons.

Conclusions: More than a decade after the initial discovery of induced pluripotent stem cells, we are now using neurons derived from these cells for target discovery and drug development. Patient-derived neurons offer a tool for drug development that can provide useful preclinical human data. This is complementary to more established approaches like animal models and cancer cell lines which continue to be useful in the development of drugs for neuropsychiatric indications.

Disclosure: Novartis Pharmaceuticals, Employee

7.4 Novel Phenotypic In Vivo Approaches to CNS Drug Discovery

Taleen Hanania

PsychoGenics, Inc., Paramus, New Jersey, United States

Background: Most neuropsychiatric disorders are polygenic and involve multiple neuronal circuits. Target-based approaches to CNS drug discovery have largely failed to deliver meaningful treatments. The SmartCube® System is a fully automated, mouse-based, behavioral platform that is used to phenotypically screen libraries of novel or repurposed compounds with known or unknown mechanisms of action. Compound testing is unbiased and using proprietary machine learning algorithms, the system delivers complex behavioral signatures that are compared to a database of FDA-approved drugs.

Methods: C57Bl/6 male mice are used. Test compound(s) are administered acutely prior to test. The SmartCube® system is designed to measure over 2000 spontaneous and challenge-induced behavioral features in the same testing environment. During the test, mice are exposed to a sequence of challenges. High-resolution cameras provide a three-dimensional view of the mouse behavior in the SmartCube.

Data are processed using machine learning algorithms that consider thousands of measures including frequency and duration of behavioral states, such as grooming, rearing, mobility, behavioral transitions, and many more features during the test session, and reveal a test compound's behavioral signature.

Test compound signatures are compared to reference compound data sets which include hundreds of marketed drugs and tool compounds tested at multiple drug doses. By comparing the behavioral features obtained from the test compounds to signatures of reference compounds in the database, the therapeutic potential of a test compound is revealed.

Results: Thousands of novel compounds have been tested in SmartCube from several libraries of known and unknown compounds and five programs are currently in clinical trials with several more in advanced preclinical development. One such program is a compound known as eltoprazine, a serotonin 5HT1A and 5HT1B partial agonist. When tested in SmartCube eltoprazine showed a signature consisting of antidepressant and psychostimulant which was very different from other 5HT1A agonists that mostly show an anxiolytic profile. This unique signature resembled the profile of methylphenidate, atomoxetine and amphetamine, which are approved treatments for Attention Deficit Hyperactivity Disorder (ADHD). We confirmed efficacy in preclinical models and showed that eltoprazine significantly normalized the hyperactivity and reduced impulsivity. In addition, we saw improvement in learning and memory when tested in the novel object recognition and radial arm maze tests. In a phase 2 clinical trial involving adult ADHD subjects, eltoprazine (5 and 10 mg/day) achieved its

primary objective on the ADHD-RS-IV with a > 40% drop from baseline. The compound also achieved statistically significant improvement on the inattention and impulsivity sub-scores.

Conclusions: The unbiased and high throughput nature of the SmartCube system has already delivered numerous clinical and advanced preclinical programs and offers a new approach to neuropsychiatric drug discovery. However, there are numerous ways to interrogate the complex behavioral data from SmartCube to reveal putative treatments for a variety of CNS disorders including for many as yet untreated or poorly treated disorders such as ALS, Alzheimers disease and Autism spectrum disorders. One such approach is to contrast the behavioral profiles of the thousands of test compounds to the profiles of disease models tested in SmartCube in search for symptomatic treatments that reverse a disease phenotype in silico. We expect this approach to deliver many more novel treatments for CNS disorders

Disclosure: Psychogenics Inc, Employee

Panel

8. Neurosteroids and GABA: Role in Postpartum Depression, Mood and Anxiety Disorders

8.1 Neurosteroids as Psychotropic Drugs: GABA Receptors and Beyond

Abstract not included.

8.2 The Promise of Neurosteroids for the Treatment of Postpartum Depression: Lessons From Preclinical Models

Jamie Maguire

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Background: Altered sensitivity to neurosteroids has been proposed to play a role in the underlying neurobiology of postpartum depression. Consistent with this notion, we discovered that the expression of GABAA receptors (GABAARs) incorporating the δ subunit, which confer greater neurosteroid sensitivity, are altered throughout the peripartum period which we proposed is a homeostatic mechanism which if disrupted could increase vulnerability to mood disorders during the postpartum period. Supporting this hypothesis, mice lacking the δ subunit (Gabrd-/- mice) exhibit depression-like behaviors restricted to the postpartum period, deficits in maternal care, and excessive corticosterone levels commonly found associated with depression. Based on these findings, we generated a mouse model mimicking the excessive corticosterone levels associated with abnormal postpartum behaviors, accomplished by eliminating the K + /Cl- co-transporter, KCC2, specifically in corticotropin-releasing hormone (CRH) neurons (KCC2/Crh mice). KCC2/Crh mice phenocopy Gabrd-/- mice, exhibiting postpartum depression-like behaviors and deficits in maternal care. These studies established two unique mouse models of postpartum depression (PPD) which serve as useful tools for testing novel therapeutic compounds.

Methods: Depression-like behavior was assessed using the forced swim test at 48 h postpartum in Gabrd-/- mice, KCC2/Crh mice and wild type littermate controls in maintained on either standard chow or treated with a synthetic neurosteroid, SAGE-516 (450 mg/kg), or the benzodiazepine, clobazam (250 mg/kg) from day 14-21 of pregnancy. Maternal care was also assessed in standard-, SGE-516-, or clobazam-treated Gabrd-/-, KCC2/Crh and wild type dams using the Maternal Approach test.

Results: SGE-516 treatment was sufficient to decrease depression-like behaviors in Gabrd-/- and KCC2/Crh mice, evident from the increased latency to immobility and decreased total time immobile in the forced swim test. However, clobazam treatment was ineffective at altering depression-like behaviors in postpartum Gabrd-/- and KCC2/Crh dams. SGE-516 also improved maternal care, evident from a decrease in the latency to approach and increased total interaction time with their pups in the Maternal Approach test; whereas, clobazam, was ineffective at altering the deficits in maternal care observed in the preclinical PPD models.

Conclusions: These findings demonstrate that SGE-516 is effective at ameliorating postpartum depression-like behaviors and improving maternal care in two independent preclinical models of PPD, consistent with the positive clinical trial using brexanolone IV, a proprietary formulation of allopregnanolone, in patients suffering from postpartum depression. These studies also validate the use of these preclinical models for investigation into the underlying neurobiology as well as testing potential novel treatments for PPD.

Disclosure: Nothing to disclose.

8.3 Pooled Efficacy and Safety Analyses From Placebo-Controlled Trials of the GABAA Receptor Modulator Brexanolone Iv in Postpartum Depression

Samantha Meltzer-Brody

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Background: Postpartum depression (PPD) is the most common medical complication of childbirth. In the U.S., prevalence of PPD varies by state with an overall average of 11.5%. Although the etiology of PPD is likely multifactorial, disruptions in gamma-aminobutyric acid (GABA) signaling may play a key role, supporting the investigation and development of brexanolone iv (USAN; formerly SAGE-547 Injection), a proprietary formulation of the GABAA receptor positive allosteric modulator allopregnanolone, as a potential PPD pharmacotherapy. The efficacy and safety of brexanolone iv (BRX) was examined in three double-blind, randomized, placebo (PBO)-controlled studies in women with PPD across a range of disease severities. A pre-planned analysis was conducted using the integrated study population to allow the assessment of safety and efficacy in the largest PBO-controlled PPD patient population possible.

Methods: Data from three pivotal studies (Study A: NCT02614547; B: NCT02942004; C: NCT02942017) in women aged 18-45 years, ≤ 6 months postpartum, with a diagnosis of PPD (defined as a major depressive episode no earlier than third trimester and ≤ 4 weeks following delivery) by the 17-item Hamilton Rating Scale for Depression (HAM-D; ≥ 26 in Studies A and B; HAM-D 20-25 in Study C) were pooled for analysis. Subjects were randomized 1:1 to receive PBO or brexanolone iv 90 µg/kg/hour (BRX90) in Studies A and C. In Study B, randomization was 1:1:1 to PBO, BRX90, or 60 µg/kg/hour (BRX60). Study drug was administered as a 60-hour infusion. Efficacy measures included the HAM-D (primary) and Clinical Global Impression-Improvement (CGI-I). Safety was assessed throughout the study and subjects were followed to day 30.

Results: In these pivotal studies, subjects were dosed over a 60-hour infusion as follows: BRX90, N = 102; BRX60, N = 38; and PBO, N = 107. Across a range of disease severities, analysis of pooled results showed significantly larger mean reductions from baseline in HAM-D total scores with BRX90 (-17.0; $p < 0.001$) and BRX60

(-19.1; $p < 0.001$) versus PBO (-12.8) at Hour 60 (primary time point). The significant reductions from baseline in HAM-D with BRX were maintained through Day 30 (BRX90 -16.9, $p = 0.021$; BRX60 -19.0, $p = 0.003$; PBO -14.3). At Hour 60, BRX90 and BRX60 groups had statistically significant higher rates of HAM-D responders (≥ 50% reduction in total score) than PBO (BRX90 $p = 0.0003$; BRX60 $p = 0.0007$). BRX groups also had significantly higher rates of CGI-I responses (rating of "very much improved" or "much improved") versus PBO at Hour 60 (both BRX90 and BRX60 $p < 0.001$). BRX was generally well tolerated relative to PBO. Most common (≥ 10% total BRX population) adverse events for BRX included headache, dizziness, and somnolence.

Conclusions: Brexanolone iv treatment resulted in rapid (by Hour 60) HAM-D reductions from baseline, and these reductions were sustained over the 30-day study period. The primary efficacy endpoint results were supported by secondary measures, and brexanolone iv was generally well tolerated relative to PBO. If regulatory authority approval is obtained, brexanolone has the potential to improve treatment options for women suffering from PPD.

Disclosure: Sage Therapeutics, Grant; Janssen, Grant; Medscape, Consultant; Global Medical Education, Honoraria

8.4 SAGE-217: Results From a Phase 2, Double-Blind, Randomized, Placebo-Controlled Study of a Novel, Orally-Bioavailable Neuroactive Steroid GABAA-Pam in Major Depressive Disorder

Stephen Kanes

Sage Therapeutics, Cambridge, Massachusetts, United States

Background: Major depressive disorder (MDD) is a common, episodic, disabling, and potentially life-threatening condition estimated to affect > 300 million people worldwide. Decreased ɣ-aminobutyric acid (GABA) signaling has been linked to MDD, presenting a potential new approach to therapy. This Phase 2 study is the first double-blind, randomized, placebo-controlled study to evaluate SAGE-217, an investigational, orally-bioavailable positive allosteric modulator of synaptic and extrasynaptic GABAA receptors (GABAAR), in MDD.

Methods: The study enrolled 89 subjects (34 male, 55 female), ages 18-65, with a 17-item Hamilton Rating Scale for Depression (HAM-D) total score ≥ 22, at 7 US centers. Subjects were randomized 1:1 to receive a nightly dose of SAGE-217 Capsule 30 mg or placebo on Days 1-14, with follow up for 4 weeks. The reduction in depressive symptoms compared to placebo, assessed by HAM-D total score change from baseline at Day 15, was the primary endpoint. Secondary efficacy endpoints included HAM-D total score change from baseline at other assessment points, Montgomery–Åsberg Depression Rating Scale (MADRS) total score change from baseline at Day 15, HAM-D response rate (≥ 50% reduction from baseline in HAM-D), HAM-D remission rate (HAM-D ≤ 7), and the Hamilton Rating Scale for Anxiety (HAM-A) total score change from baseline at Day 15. Health-related quality of life (HRQoL) was assessed by the Medical Outcomes Study Short Form-36 (SF-36v2) acute version. Safety and tolerability were assessed by standard safety parameters.

Results: SAGE-217 achieved the primary endpoint with a greater LS mean reduction from baseline in HAM-D total score versus placebo (-17.4 versus -10.3; $p < 0.0001$) at Day 15. Significant mean differences ($p < 0.05$) in HAM-D from placebo were observed at Day 2 and were maintained through Day 28. At Day 15, Improvements for SAGE-217 versus placebo were also observed for HAM-D response (78.6% versus 40.5%; $p = 0.0002$) and remission (64.3% versus 26.2%; $p = 0.0005$). These measures remained statistically significant through Day 28. Statistically significant improvement in MADRS total score compared to placebo was consistent with the HAM-D at Day 15. Statistically significant improvements in anxiety were observed by HAM-A total score. At Day 15, SAGE-217 group SF36v2 scores approached general population normative levels for several health domains, exceeding the placebo group by more than the minimally important difference for general health (8.6 vs. 4.7; $p = 0.025$) vitality (15.7 vs. 7.9; $p = 0.002$), social function (15.6 vs. 12.1) role emotional (16.5 vs. 12.2), mental health (17.3 vs. 10.4; $p = 0.004$), and mental component summary (20.2 vs. 12.8; $p = 0.008$) scores. SAGE-217 improvements versus baseline were maintained through Day 42. There were no deaths, serious or severe AEs. Common AEs (≥ 5%) in the SAGE-217 group included headache, dizziness, nausea, and somnolence.

Conclusions: SAGE-217 administration for 14 days resulted in rapid, robust, and sustained (over the study period) reductions in depressive symptoms that were associated with improvements in multiple domains of HRQoL and was generally well tolerated. This first double-blind, placebo-controlled study supports further development of SAGE-217 as a potential therapeutic for MDD.

Disclosure: Sage Therapeutics, Employee, Stock / Equity

Mini Panel

9. Empathy and Addiction

9.1 Relapse to Opioid-Seeking in Mice in Response to Witnessing Another's Distress

Abstract not included.

9.2 Attenuation of Opiate-Induced Deficits in Social Function by Chemogenetic Activation of the Anterior Insula

M. Foster Olive

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Background: Diagnostic criteria for opiate use disorder include impaired social functioning as a result of excessive drug use. We recently established a rodent model of deficits in prosocial behavior induced by heroin self-administration. This model utilizes a "rescuing" paradigm in which rats are allowed to free their cagemate from a plastic restrainer. Prior studies have implicated the anterior insular cortex in mediating prosocial, addictive, and empathy-related behaviors. The present study sought to utilize designer receptors exclusively activated by designer drugs (DREADDs) to examine the effects of chemogenetic activation or inhibition of the anterior insular cortex on heroin-induced deficits in social functioning.

Methods: Male Sprague-Dawley rats (250-300 g) were pair-housed upon arrival and underwent daily handling for 14 days. One rat in each pair was randomly selected to be the "trapped" rat, and the other to be the "rescuer" rat. Rescuer rats were given the opportunity to release their trapped cagemate from a modified plastic restrainer in 21 daily sessions. After baseline rescuing behavior was established, rats underwent stereotaxic infusion of an AAV encoding either an excitatory DREADD (AAV-CaMKIIa-hM3Dq-mCherry, $n = 8$), inhibitory DREADD (AAV-CaMKIIa-hM4Di-mCherry, $n = 7$) or a control vector (AAV-CaMKIIa-GFP, $n = 11$) into the anterior insula (0.5 ul/side). The CaMKIIa promoter was utilized to allow selective expression in cortical excitatory neurons. Rats were also implanted with intravenous catheters, and

then allowed to self-administer heroin (0.06 mg/kg/infusion, i.v.) in 6-hr daily sessions for 14 days. Next, clozapine-N-oxide (CNO, 1.5 mg/kg i.p.) was administered 20 minutes prior to re-assessment of rescuing behavior and/or concurrent heroin self-administration in 6 daily sessions. DREADD functionality was verified by patch clamp electrophysiology in separate groups of animals. All experimental procedures were approved by an institutional IACUC and conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

Results: Prior to heroin self-administration, the proportion of rats showing rescuing behavior ranged from 64-100% across experimental groups. During post self-administration test sessions, rats infused with the control virus or AAV-CaMKIIa-hM4Di-mCherry showed ~62% reductions in rescuing behavior, indicating heroin-induced social deficits that were unaltered by chemogenetic inhibition of the anterior insula. There were also no differences in heroin self-administration between these groups of animals during the test sessions. However, rats infused with AAV-CaMKIIa-hM3Dq-mCherry showed partial reversal (~28%) of heroin-induced reductions in prosocial behavior.

Conclusions: Heroin-induced deficits in social functioning in a rodent model of opiate addiction can be attenuated by chemogenetic activation of excitatory neurons in the anterior insular cortex. Thus, activation of this region may promote recovery of social functioning and aid in the treatment of opiate addiction.

Disclosure: Nothing to disclose.

9.3 Prosocial Behavior Independent of Social Reward

Carmela Reichel

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Background: Prosocial behaviors, such as social interaction and empathy, are imperative for an adaptive social structure by the allowance for personal understanding of the perceived valence of others. Animal species will behave prosocially in the hopes of receiving social reward, but it has been intimated that some animals are also capable of behaving empathically. Empathy can be broadly defined as the capacity for one to experience the valence of another which, in turn, generates a response more appropriate to another's emotional situation than one's own, independent of personal gain. Further, these behaviors may play a role in the underlying pathology of some neuropsychiatric disorders, such as substance use disorder (SUD). The neurological underpinnings of empathy are poorly understood, due to a paucity of animal behavioral models designed to study it. Some research has demonstrated that rats will perform tasks to reduce the distress of a conspecific, and this prosocial behavior is subject to past experience. These data suggest an understanding of the valence of a conspecific and a motivation to reduce their perceived distress.

Methods: In these series of experiments we developed a rat model of helping behavior that requires the animal to pull a chain to release a distressed conspecific from a pool of water via an automated guillotine door. In the first experiments we used male rats in a two-chamber apparatus in which one side was a 100 mm pool of water with the only escape being a guillotine door. The door was controlled by a chain pull in the opposite compartment. One rat was placed in the pool (wet rat) and one in the escape chamber (dry rat). Dry rats readily learned to chain pull to release their partner in twice daily five minutes trials. In the second set of experiments a three-chamber apparatus was used in which the wet rat was released into a separate compartment than the dry rat to eliminate social interaction as a motivation for the chain pull response.

Results: In the first experiment, rats ($n = 8$) learned to pull a chain to release a conspecific from a pool of water indexed as decreased over trials [$F(9,54) = 6.8$, $p < 0.0001$] with trials 6-10 significantly faster than trial one. Rats ($n = 8$) with a previous experience of the distress learned to release a conspecific faster than experience-naïve rats [$F(9,54) = 16.74$, $p < 0.0001$] with faster latencies on trials 2-10 significantly than trial one. Using a three-compartment apparatus to eliminate social interaction as a motivator, rats ($n = 8$) learned to release a conspecific from water into a dry area separate from their own in a three-chamber apparatus [$F(15,105) = 5.99$, $p < 0.0001$]. Again, in this model, rats with previous experience of the distress showed faster latency to chain pull than those without [$F(9,54) = 16.74$, $p < 0.0001$]. This behavior is specific to helping a distressed rat, because latency to chain pull is significantly increased if the conspecific is removed or replaced with an 'imposter rat' (a fake rat) or if the pool is empty.

Conclusions: We have demonstrated that rats will work to release a distressed conspecific from an adverse situation and that this prosocial outcome is faster and more readily executed if rats have prior experience with the distress. We assert that the three-compartment apparatus allows for an evaluation of empathic behavior in rats that will help elucidate the neural underpinnings of empathy and how they are affected by substance use disorders. Future studies include and assessment of empathic responding between animals in a withdrawal state from heroin and/or methamphetamine self-administration compared to controls.

Disclosure: Nothing to disclose.

Panel

10. Homeostatic Regulation of Sleep: Roles for Adenosine and Cortical nNOS/NK1R Neurons?

10.1 Adenosine Mediation of the Homeostatic Sleep Response

Abstract not included.

10.2 Cortical nNOS/NK1R Neurons: Orchestrators of EEG Slow Wave Activity?

Thomas Kilduff

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Background: Type I cortical nNOS/NK1R interneurons are a rare and unique cortical cell type. These cells express Somatostatin (SOM) and NPY but, in contrast to other GABAergic local circuit neurons, many of these cells have long-range projections. We previously showed that these cells are sleep-active in three different rodent species: they accumulate c-FOS during sleep in relationship to the magnitude of EEG slow wave activity (SWA). Moreover, the proportion of FOS + /nNOS neurons is related to prior wake duration. nNOS knockout mice have disrupted sleep and are objectively sleepier yet, in the absence of NO, these mice fail to respond to a sleep debt with an increase in EEG SWA. Consequently, we have proposed that cortical nNOS/NK1R neurons integrate homeostatic sleep drive arising from subcortical regions and coordinate EEG SWA through their long-range projections and release of GABA, SOM, NPY and/or NO. Here, we report on three experiments intended to further test this hypothesis.

Methods: Expt 1: To determine whether c-FOS expression in cortical nNOS/NK1 neurons is more closely related to the amount of NREM sleep or to the sleep pressure that accrued during the prior waking period, we used sleep deprivation (SD) to vary sleep pressure and zolpidem (ZOL) or almorexant (ALM) to control the amount of NREM sleep in rats (6 groups; N = 6-7/group). Expt 2: The effects of in vivo optogenetic stimulation of cortical nNOS cells on EEG activity were determined in Nos1-CreERT2;Sst-FlpO; Ai80 mice that were instrumented for EEG/EMG recording. After thinning the bone at 5 locations, blue (470 nm) LEDs were placed onto the skull of each mouse. While EEG/EMG was recorded, LED activation delivered 1 s pulses at 0.5 Hz for 1 h during the inactive phase to mice that were treated with tamoxifen (TMX; 300 mg/Kg, p.o.) 8 wks prior to experiments (N = 3) vs. a TMX(-) group (N = 3). Expt 3: In a pilot study, Cynomolgus macaques were sacrificed in one of 4 conditions: (1) SD for 8 h into the dark period before sacrifice at ZT20 (ZT12 = lights off); (2) SD until ZT20 but then allowed a 2 h recovery sleep (RS) opportunity before sacrifice at ZT22; (3) ad libitum sleep before sacrifice at ZT20; or (4) ad libitum sleep before sacrifice at ZT22. All animals were perfused, the brains sectioned and stained for both c-FOS and nNOS, and the percentage of FOS + /nNOS neurons calculated.

Results: Expt 1: The proportion of Fos + cortical nNOS/NK1R neurons was minimal when sleep pressure was low irrespective of the amount of time spent in NREM sleep, but high when sleep was preceded by SD. Consequently, the % Fos + /nNOS cells distinguished between the low vs high sleep pressure conditions following VEH ($p < 0.001$), ZOL ($p < 0.001$) and ALM ($p < 0.001$). Expt 2: When the average EEG power spectra during NREM sleep during 1 h optogenetic stimulation was compared to NREM EEG power during the preceding hour, there was a 77% increase in EEG power (0.5-4 Hz) for the TMX(+) group ($p = 0.0048$, paired t-test); stimulation was ineffective in the TMX(-) group. Expt 3: The proportion of Fos + /nNOS neurons in the 3 sleep groups exceeded that of the Cyno kept awake (Group 1).

Conclusions: (1) Although sleep is necessary for cortical nNOS/NK1R neuron activation, the proportion of cells activated is dependent upon the buildup of sleep pressure during prior wakefulness. (2) Optogenetic stimulation of cortical nNOS/NK1R neurons can increase EEG delta power during NREM sleep. (3) Cortical nNOS/NK1R neurons may be activated during sleep not only in nocturnal rodents, but also in diurnal primates that exhibit a consolidated sleep pattern that is similar to human sleep.

Disclosure: Nothing to disclose.

10.3 Regulation of Type I Cortical nNOS/NK1R Neurons by Sleep/Wake Signalling Molecules

Abstract not included.

10.4 A Mini-Review of Cortical Type I and Type II NOS + Neurons, and Their Role in a Sleep-Relevant Network

Kathleen Rockland

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Background: NOS + neurons are a small, multifunctional GABAergic subpopulation, implicated in neurovascular control and sleep homeostasis. They are phylogenetically conserved and have been reported to show changes in neuronal density and/or molecular expression in some cases with schizophrenia (e.g., Connor et al., 2011). Type I neurons (more intensely stained for the enzyme NADPHd and generally larger than Type II) are abundant in the subgriseal white matter (WM) and scattered, in an area- and species-dependent manner, in the cortical gray matter. Type II neurons are abundant in layer 2 and often in the deeper layers as well. Subdivisions are reported for both types. Type I neurons – the more fully visualized and better investigated - have several features of interest in the context of SWA: they have cholinergic, 5HT3, neurotensin, and hypocretin/orexin receptors; they have gap junction coupling, and they have widespread local and global connectivity. Aspects of the broader network and microcircuitry have been identified, but basic questions remain (see Results).

Methods: Standard histo-(NADPHd) and immunohistochemistry processing (parvalbumin; PV), in perfused macaque tissue sections (50 μm thick). Tracer injections in vivo (Tomioka and Rockland, 2007; n = 7). In nonhuman primate (NHP) macaque monkeys: N = 3 for NADPHd; N = 3 for PV, with reference to the literature (NHP, rodent, and human).

Results: 1) Small PV + cells occur in layer (L.) 2 and at the deep L. 6/WM zone. Does this population co-localize with the Type II NOS neurons? 2) Is there a potential complementary action between Type I and Type II/NPY/somatostatin neurons? 3) In vitro studies show that axons of intensely-stained Type I WM neurons (WMNs) often invade the overlying gray matter and extend for long distances (0.2-2.0 mm), spanning and potentially linking large gray matter and WM sectors. 4) The deep L. 6/WM border is a specialized region where descending dendrites from L. 5 and 6 intermingle with dendrites of NOS + WMNs, and with synaptic terminations from thalamocortical and feedback cortical projections, among others. How does this circuitry play out in the context of sleep regulation? 5) L. 1 has a dense band of NADPHd + fibers. The source is not clear, but this system could converge in L. 1 with cholinergic, thalamic, and cortical feedback fibers presynaptic to distal dendrites of pyramidal cells. This might serve as an additional level of excitation/inhibition regulation. 6) In vitro studies report that GABAergic WMNs (putatively NOS +) collateralize to multiple, functionally non-related areas (i.e., in rodents: overlying and distant cortex, striatum, and hippocampus). This architecture likely achieves an intricate interplay of local/global circuitry, which prompts speculation about specific functional implications - in the sleep cycle and/or neurovascular regulation.

Conclusions: NOS + neurons have been proposed as orchestrators of EEG SWA. At the network level, the long-range GABAergic NOS + in cortical layers and in WM are well-positioned to participate in temporal coordination of distributed neuronal activity, including SWA. An intricate network is likely to involve cooperative/competitive interactions of NOS + with other GABAergic and nonGABAergic subpopulations, including multiple WM-WM, and WM-gray matter interactions.

Disclosure: Nothing to disclose.

Panel

11. Larger-Scale Transcriptome and Epigenome Mappings, Modeling and Analyses in Developing and Diseased Human Brain

11.1 Widespread Dysregulation of Cortical Splicing, Isoform and Noncoding Gene Regulatory Networks Across Autism, Schizophrenia, and Bipolar Disorder

Michael Gandal

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Background: Non-coding regions harbor the majority of psychiatric disease-associated genetic variation, implicating dysregulation of gene expression in disease pathogenesis. However, comprehensive large-scale characterization of non-coding RNAs, alternative splicing and isoform-level gene regulation are lacking in primary human brain tissue from subjects with psychiatric disorders and controls.

Methods: To survey the disease-relevant transcriptome, we generated an unprecedented resource of > 2000 brain samples from 1695 subjects with ASD, SCZ and BD and controls, integrating genotype and RNA-sequencing data as a core component of the PsychENCODE consortium.

Results: Over 25% of the transcriptome exhibits differential splicing or expression in at least one disorder, including 944 non-coding RNAs. Using gene and isoform co-expression networks, we isolate precise cellular and molecular processes altered in each disease, several enriched for causal genetic factors. We identify disease-specific alterations in microglial, astrocyte, interferon-response, and NFkB modules, defining distinct trajectories of neural-immune mechanisms. A transcriptome-wide association study further prioritizes numerous novel disease loci likely mediated by cis-effects on brain expression. Convergent evidence points to genetically-mediated downregulation of NPY as a regulator of synaptic dysfunction in SCZ.

Conclusions: These results represent the largest transcriptome-wide characterization across three major psychiatric disorders, providing an unparalleled resource for mechanistic insight and therapeutic development.

Disclosure: Nothing to disclose.

11.2 Integrative Analyses of Human Brain Development and Neuropsychiatric Risk

Abstract not included.

11.3 Histone Acetylation Mapping in Neuronal Chromatin From Prefrontal Cortex of Subjects With Schizophrenia

Kiran Girdhar

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Background: Genomic dysregulation is likely to contribute to neuronal dysfunction in prefrontal cortex (PFC) and other brain regions affected in schizophrenia (SCZ), but genome-scale mapping of neuronal transcriptomes and epigenomes has not been conducted in larger cohorts. We have shown recently that the genomic landscape of open chromatin-associated histone modifications, including histone H3-acetyl-lysine 27 (H3K27ac), show in neurons significant associations with the genetic risk architecture of schizophrenia.

Methods: We conducted H3K4me3 (H3K27ac) chromatin immunoprecipitation sequencing (ChIP-seq) on fluorescence-activated cell sorted (FACS) nuclei from dorsolateral PFC of 250 postmortem postmortem samples from SCZ and 280 matched control brains, generating altogether ~7(11) billion reads representing H3K4me3 (H3K27ac)-tagged nucleosomal DNA from PFC neurons. In addition, genotyping for the entire cohort of 530 brains was performed.

Results: Integrating H3K27ac ChIP-seq with WGS, we identified thousands of new histone quantitative trait loci (hQTLs) and various neuron-specific gene categories affected by dysregulated histone acetylation in PFC neurons from subjects with schizophrenia.

Conclusions: Our dataset will provide unique insights into non-coding variants associated with neuronal dysfunction in schizophrenia. Our PsychENCODE sponsored resource highlights the critical role of cell-type specific signatures at regulatory and disease-associated non-coding sequences in the human frontal lobe.

Disclosure: Nothing to disclose.

11.4 Comprehensive Functional Genomic Resource and Integrative Model for the Adult Brain

Daifeng Wang

State University of New York at Stony Brook, School of Medicine, Stony Brook, New York, United States

Background: Disorders of the brain affect nearly one fifth of the world's population. Our molecular-level understanding of how genomic variants relate to brain disorders is still limited. To address this challenge, our capstone project of PsychENCODE consortium has developed a comprehensive functional genomic resource and integrative model for the adult brain (manuscript under review).

Methods: We uniformly processed the transcriptomic and epigenomic datasets of PsychENCODE consortium and other large studies including GTEx and CommonMind for 1,866 adult brains and systematically identified the functional genomic elements for the adult brain using the statistical analysis and deep learning model.

Results: We deconvolved the gene expression of bulk tissue using single-cell data, finding that differences in the proportions of cell types explain > 85% of the cross-population variation observed. Moreover, using chromatin and Hi-C data from reference prefrontal-cortex samples, we found ~79,000 brain-active enhancers and linked them to genes and transcription factors in an extended regulatory network. We identified ~2.5 M eQTLs (comprising ~238 K linkage-disequilibrium-independent SNPs) and many additional QTLs associated with chromatin, splicing and cell-type-proportion changes. We, also, leveraged our QTLs, Hi-C data and regulatory network to connect more genes to GWAS variants for psychiatric disorders than possible before (e.g., 304 for schizophrenia). Finally, we developed a deep-learning model embedding the regulatory network in a framework connecting genotype to observed traits. Our model achieves a ~6X improvement in disease prediction over an additive model, highlights key genes for disorders, and allows imputation of missing transcriptome information from genotype data alone.

Conclusions: In summary, our integrative analyses on adult brain demonstrate that functional annotation of gene regulatory elements is useful for unraveling molecular mechanisms in the brain.

Disclosure: Nothing to disclose.

Panel

12. Quo Vadis: Psychotropic Drug Development in the 21st Century - The NIMH Fast-Fail Initiative

12.1 Failure IS an Option…as Long as it Results in Scientific Progress

Mi Hillefors

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Background: Although promising novel therapeutic targets for treating psychiatric disorders have been discovered in recent years, translation into effective treatments has not followed. Large companies have curtailed their investment in novel treatments for psychiatric conditions and in hopes of filling this gap NIMH is now focused on an experimental medicine approach to early stage drug trials. This presentation describes how NIMH implemented an Experimental Medicine approach to validate (or reject) putative novel molecular targets, how specific targets were selected, and how the studies were designed to set the stage for detailed presentations of the findings. Finally, the way in which the NIMH effort has supported industry investment in further development will be highlighted.

Methods: A unique target selection scheme was selection leading to the design of contracted studies under a FAST-PS project, focused on Psychosis Spectrum Disorders, and a FAST-MAS project, focused on Mood and Anxiety Spectrum Disorders. To qualify to be studied, a compound was judged against the following criteria: 1) specific and testable hypothesis, 2) PET ligand for receptor occupancy (RO), 3) functional measure of target engagement, 4) at least IND ready compound, and 5) Research Domain Criteria (RDoC).

Results: A FAST-PS project was designed and implemented involving: 1) a validation of ketamine induced glutamatergic brain effects comparing imaging-based biomarkers (pharmacoBOLD-fMRI, 1H-MRS, task-based fMRI) and 2) assessment of mGluR2/3 agonist activity in brain, aimed at assessing whether the mGluR2/3 agonist Pomaglumetad (LY2140023, used at higher doses than those that had failed in a previous Phase 3 trial in schizophrenia) could inhibit ketamine-induced glutamate effects. A FAST-MAS study was designed to assess whether antagonism of the kappa opioid receptor (KOR) engages key neural circuitry related to the hedonic response in subjects with mood and/or anxiety disorders using the KOR antagonist JNJ-67953964. Protocol details of both will be presented to set the stage of presentation of results from the studies themselves.

Conclusions: NIMH has successfully implemented studies under a Fast-Fail Trials (FAST) Initiative that have provided a platform for assessing and validating biomarkers of target engagement and testing new or repurposed compounds for their potential as novel psychiatric therapeutic agents. These results are informing important decisions by both NIMH and industry whether to move forward into costly efficacy trials and provide a model of collaboration to address the translational gap between target identification and validation in humans. Ideally, experimental medicine trials will test hypotheses about whether and how a molecular target is linked to specific brain functions thought to underpin domains of psychiatric dysfunction such as anhedonia and cognitive function. The goal is to expeditiously evaluate the viability of putative novel molecular targets for further development.

Disclosure: Nothing to disclose.

12.2 Neuroimaging Biomarkers for Neuropsychiatric Drug Discovery

Jia Guo

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Background: Critical for drug discovery, biomarkers are needed for target engagement (for dose finding and for efficacy tracking), and for reducing heterogeneity in patient enrollment. Neuroimaging biomarkers are particularly well suited for neuropsychiatric illnesses. Recent studies have suggested that glutamate and its receptors might be therapeutic targets for schizophrenia and related psychotic disorders and that opioid and its receptors might be therapeutic targets for mood and related disorders.

Methods: A range of studies has suggested that abnormal elevations in brain glutamate is a therapeutic target in schizophrenia and related psychotic disorders. Studies have shown that chronic elevations in hippocampal glutamate can secondarily cause regional hippocampal hypermetabolism and atrophy. Accordingly, we have recently optimized MRI-based techniques designed to image these three hippocampal pathologies. Proton magnetic resonance spectroscopy (1H-MRS) to measure hippocampal glutamate, cerebral blood volumes (CBV) fMRI to measure hippocampal hypermetabolism and structural MRI (sMRI) to measure atrophy. We have recently applied these three neuroimaging techniques to a large number of patients with attenuated psychotic symptoms who were then longitudinally followed.

Results: We validated the three techniques showing that 1H-MRS can detect elevations in glutamate as the primary hippocampal pathology, that CBV-fMRI can measure regional hippocampal hypermetabolism, and that sMRI can detect regional hippocampal atrophy in patients with prodromal psychosis.

Conclusions: Our results support the use of these MRI-based tools as biomarkers in schizophrenia drug discovery. We are currently using these techniques in a NIHM sponsored clinical trial, testing the glutamate-reducing agent pomaglumetad methionil (POMA) in patients with prodromal psychosis.

Disclosure: Nothing to disclose.

12.3 NIMH FAST-MAS Phase IIa Study Establishing Proof of Mechanism for κ Opioid Antagonism by Demonstrating That the Selective κ Opioid Antagonist JNJ-67953964 Engages Neural Circuitry Related to the Hedonic Response in Anhedonic Patients With Mood and Anxiety Spectrum Disorders

Andrew Krystal

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Background: A compelling body of preclinical research suggests that κ opioid receptor (KOR) antagonism has promise as treatment for anhedonia. However, we lack data on the effects of selective KOR antagonists in humans. We carried out this Phase IIa study under the NIMH New Experimental Medicine Studies: Fast-Fail Trials in Mood and Anxiety Spectrum Disorders (FAST-MAS) program to test the hypothesis that engaging this target with a highly selective KOR antagonist, JNJ-67953964 (formerly CERC-501 and LY2456302) modulates ventral striatal reward circuitry. This was intended to attempt to establish proof of mechanism (POM) that KOR antagonism has potential therapeutic effects on anhedonia and to serve as a model for using biomarker-based outcomes to establish POM in early phase drug development.

Methods: Subjects 21-65 years of age meeting DSM-5 criteria for either a mood ($N = 70$) or anxiety ($N = 19$) disorder who had anhedonia (Snaith-Hamilton Pleasure Scale score ≥ 20), were randomized to 8 weeks of double-blind treatment with JNJ-67953964 10 mg ($N = 45$) or placebo ($N = 44$). A dose of 10 mg was selected based on prior positron emission tomography receptor occupancy data showing robust KOR antagonism. Primary outcome was assessed with fMRI in conjunction with a monetary incentive delay (MID) task administered at baseline and after 8 weeks of treatment. Analysis examined group activation differences during anticipation of monetary gain, contrasted with non-incentive trials. Mixed-model ANOVA queried average activation in an a priori bilateral accumbens area mask defined by the Harvard-Oxford Subcortical Atlas.

Results: Mixed-model analysis carried out with the Intent to Treat sample revealed a significant Group x Time interaction in monetary gain anticipation ($p < 0.01$) (a priori primary outcome), consistent with relatively greater increase from baseline in ventral striatal activation during anticipation of gain with Study Drug vs placebo. JNJ-67953964 was not associated with any serious adverse events and was generally well tolerated. Side-effects of more than mild severity occurring more than 5% more frequently with JNJ-67953964 than placebo were pruritis (11.1%), depression exacerbation (6.7%), and rash (6.7%).

Conclusions: The results of this study establish that KOR antagonism has the hypothesized effect on neural function, thereby establishing POM that engaging this target is a promising means of treating anhedonia. The findings also specifically suggest the promise of JNJ-67953964 as an anhedonia therapy. This study provides the basis for carrying out larger trials with KOR antagonists powered for the use of clinical endpoints to determine the clinical impact of engaging this target. It also serves as a model for novel, RDoC-based, early phase drug development methodology incorporating rigorous testing of whether engaging a target has a hypothesized effect on neural function as a means of establishing POM before proceeding to trials with downstream clinical endpoints.

Disclosure: Merck, Advisory Board; Jazz, Advisory Board, Grant; Janssen, Advisory Board, Grant; NIH, Grant; Ferring, Advisory Board; Galderma, Advisory Board; Takeda, Advisory Board; Neurocrine, Advisory Board; Pernix, Advisory Board; Adare, Advisory Board

12.4 Biomarker Assessment of Dose Dependent Target Engagement of mGluR-2,3 Partial Agonist for Schizophrenia Treatment

Jeffrey Lieberman

Columbia University, New York, New York, United States

Background: There is a desperate need for novel drugs for treatment of schizophrenia. Glutamate is a prioritized target for drug development. Attempts to develop mGlu-R partial agonists were unsuccessful. Upon reviewing the data, we concluded that doses to achieve adequate target engagement were tested. This study tested the ability to an mGluR-2,3 partial agonist to inhibit the effects of a pharmacologic model of schizophrenia as reflected by fMRI.

Methods: To evaluate the effects of the mGluR2/3 partial agonist LY2140023 (Pomaglumetad Methionil, "POMA") at selected doses on ketamine-stimulated glutamate release in prefrontal cortex, as measured by pharmacoBOLD fMRI (also termed resting BOLD fMRI). Ketamine-induced glutamate release is hypothesized to simulate the synaptic dysregulation that occurs endogenously in the pathogenesis of schizophrenia. This study will determine the degree to which the POMA dose utilized in recent failed clinical trials (40 mg BID) is sufficient to engage the primary target (mGlu2/3 receptors) and whether a higher dose (160 mg BID) successfully engages the mGluR2/3 receptor. Both treatment arms will be compared only to placebo.

Study population: 100 healthy volunteers (100 randomized, for 81 completers)

Design: 3-arm randomized (1:1:1) double blind administration of 1) low dose (40 mg BID) POMA, 2) high dose (160 mg BID) POMA, or 3) placebo

Outcome measures: Ketamine-induced prefrontal glutamate activity as measured by PharmacoBOLD (primary).

Results: Data collection has been completed and data cleaning is being finalized. When completed the data base will be locked, the blink broken, and analysis carried out. We expect this process to be completed and results available by mid-June. This attests to the new and uniqueness of the data from this study.

Conclusions: We hypothesize that POMA will suppress ketamine induced increases in fMRI bold activity compared to placebo indicating its glutamate target engagement.

Disclosure: Nothing to disclose.

Mini Panel

13. Prenatal Folic Acid Exposure, Neurodevelopment, and Severe Mental Illness in Youth

13.1 Periconceptional Folic Acid and Risk of Neurodevelopmental Disorders: Results: From the Norwegian Mother and Child Cohort Study

Christine Roth

Lovisenberg Hospital, Oslo, Norway

Background: We, and others, have previously reported perinatal use of folic acid supplements to be associated with a reduced risk of language delay and Autism Spectrum Disorder (ASD). Here we extend our previous work using a much larger sample and more rigorous statistical methods, with four times as many ASD cases and many more language delay cases than our previous report.

Methods: The Norwegian Mother and Child Cohort Study (MoBa) is an ongoing prospective pregnancy cohort conducted by the Norwegian Institute of Public Health (NIPH). The data collection during pregnancy and at birth included self-report questionnaires and biological samples from the mother, father, and child. Follow-up after birth includes questionnaires periodically sent to mothers for the entire study sample (n=114,000). On a language grammar rating scale in the age three questionnaire children were rated as having severe language delay, moderate language delay or no language delay. Cases of ASD were identified through linkages to the Norwegian Patient Registry (NPR). Information about folic acid use was derived from the first pregnancy questionnaire with detailed question about use of vitamins, minerals, and dietary supplements in four-week time windows from before start of pregnancy. Unlike the US, Norway does not fortify foods with folic acid, increasing the contrast in relative folate status between women who do and do not take folic acid supplements. For our most recent analysis, there were 2 024 children with moderate or severe language delay and 808 children with registry diagnoses of ASD. Both the measures of language delay and the diagnoses of ASD were validated in sub studies. In this much larger study sample, we have applied multiple imputation for missing data, and adjusted for propensity scores.

Results: Of the 106 109 MoBa children 808 (0.7%) had an ASD diagnosis. Children whose mothers took no folic acid supplements in the period four weeks before start of pregnancy to eight weeks after were the reference group. For risk of ASD the adjusted OR was 0.69 (95% CI, 0.57-0.84) for use of folic acid supplements compared to no use. Of the 54 101 children with available three-year data 279 (0.5%) had severe language delay and 1 745 (3.2%) had moderate language delay. For risk of severe language delay the adjusted OR was 0.63 (95% CI, 0.47-0.85) for use of folic acid supplements compared to no use. For risk of moderate language delay the adjusted OR was 0.76 (95% CI, 0.68-0.86) for use of folic acid supplements compared to no use.

Conclusions: Using a much larger study sample and more rigorous statistical methods we replicated our previous findings

that use of folic acid in the period around conception is associated with a reduced risk of language delay and a diagnosis of ASD. We are currently conducting further analysis including; comparison of sibs discordant for maternal folic acid intake, five-year data on dimensions of language, overlap between language delay and ASD, Mendelian randomization to further check for confounding, and gene-environment interaction. Some of these results will be presented at the meeting.

Disclosure: Nothing to disclose.

13.2 U.S. Studies of Prenatal Folic Acid and Autism Development: Links and Potential Mechanisms of Prevention

Abstract not included.

13.3 Complementary Effects of Fetal Folic Acid Exposure and Gene Expression on Postnatal Cortical Development

Joshua Roffman

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Background: Autism, schizophrenia, and other serious mental illness (SMI) entail subtle alterations in cerebral cortical development that likely begin in the womb. Recent epidemiologic studies have associated periconceptional folic acid exposure with reduced risk of autism spectrum disorders and other SMI in youth. While robust and well-replicated, these results are limited in that they stem from observational cohorts. Here, we sought to obtain biological evidence for effects of fetal folate exposure on subsequent brain development, and to frame these findings within the context of the developmental transcriptome.

Methods: Using MRI, we evaluated effects of doubled prenatal folic acid exposure on cortical thickness across three large cohorts of youth age 8-18 (Massachusetts General Hospital, Philadelphia Neurodevelopmental Cohort, NIH MRI Study of Normal Brain Development; total $N = 1{,}370$) leveraging the U.S. population-wide rollout of grain product fortification in the late 1990s as a natural experiment. Exposure groups gestated just before, during, or just after the fortification rollout in 1996-97, and were contrasted for mean cortical thickness and age-related cortical thinning, after accounting for potential demographic, socio-economic, nutritional, and scanner-based confounds. Using a separate MRI + GWAS dataset (Brain Genomics Superstruct Project/GSP, $N = 1{,}318$ unrelated individuals of European decent, age 18-35) in conjunction with BrainCloud gene expression data from postmortem dorsolateral prefrontal cortex (DLPFC), we determined the relative impact of genes expressed in fetal life versus early adulthood on cortical thickness variation.

Results: Fetal exposure to folic acid fortification associated with replicated changes in cortical development, characterized by increased cortical thickness in bilateral frontal and temporal regions (9.9-11.6%, p-corrected < .001 to .03) and delayed onset of age-related cortical thinning in frontal, temporal, and parietal regions (p-corrected < .001 to .02). Genome-wide polygenic scoring indicated uniquely strong effects of fetal-expressed genes on DLPFC thickness, when compared to genes with predominantly postnatal expression (p-corrected < .05).

Conclusions: Convergent data from folate exposure and gene expression studies herein implicate the fetal milieu in subsequent cortical development. This work presents novel evidence to support neurodevelopmental theories of SMI and takes an initial step toward resolving molecular mechanisms for neuroprotective effects of fetal folic acid exposure. Ongoing work to be presented is identifying specific biological pathways wherein effects of genetic variation on cortical thickness are conditioned on fetal folate exposure.

Disclosure: Nothing to disclose.

Study Group

14. What is the Importance of Animal Models to Neuropsychiatric Disease?

Tracy Bale, Kerry Ressler, Eric Nestler, Scott Thompson, Ted Abel, Bita Moghaddam, Huda Akil, William Carlezon, Joshua Gordon*

University of Maryland School of Medicine

Study Group Summary: Current changes to our thinking about neuropsychiatric disease mechanisms and classifications have prompted a dynamic discourse as to the importance and reliability of animal models. At a time when most major pharmaceutical companies are disbanding their R&D in neuroscience and mental health programs, academia is the remaining bastion in the development of novel drug targets and validation. ACNP is the ideal audience for this discussion, providing a professional setting in which all sides to this topic can be presented, and allowing voices from all arenas of basic and translational neuroscience to be heard. There is a growing concern in the field as to translational ability of outcomes in animal models matching that of human clinical trials. While it is clear we cannot completely model complex neuropsychiatric and neurodevelopmental disorders in most animals, there is certainly agreement across domain criteria and endophenotypes, those factors that integrate many levels of outcomes, including cellular, circuits, genomics/epigenomics, and behavior, of disease that have proven informative. While the current 'omics generation continues to provide novel and informative big data sets, the critical importance of how these results relate to disease risk and resilience requires validated measures. Similarly, while GWAS studies have given us valuable clues into the genes and pathways that are associated with disease risk, how these loci interact with the environment and their importance across developmental and life stages requires focused and pointed studies. How do we go from findings in rodents to complex diseases in humans? How do we control for the variables across labs and between species that make interpretation or translation difficult? At the cellular level, a recent push toward the utilization of human iPSC cells for phenotypic characterization, including organoids, migration assays and electrophysiology, have been suggested as an alternative to animal models. How these cells resemble the human disease condition and respond in a controlled environment is a critical question for this important area with great potential to provide molecular insight. Key factors and variables are still necessary, including sex as a biological variable and appropriate developmental stages, to draw reliable conclusions. There are key questions that remain as to the importance of animal models and require discussion and consensus across the field. What can we learn from animal models regarding the underlying causes and relevant interventions and treatments of mental health disorders? Can we map novel circuits and identify important gene x environment interactions? How does stress in animals influence these points, promote relevant dysregulation of physiological and behavioral measures, and are these analogous to the impact of stress in diseases such as depression and PTSD? Are some of the more translational behavioral measures, including fear conditioning, PPI, and sociality well validated, and can they be applied across species, sexes, and the lifespan of animals? Confidence and

reliance on animal models have been a cornerstone of neuroscience and mental health research for decades. Can we determine a path forward in improving mental health?

Disclosure: Nothing to disclose.

Panel

15. Feeding Behavior in Health and Disease: Preclinical and Clinical Approaches to Dissecting Maladaptive Food Intake and Neurohormonal Biomarkers of Treatment Response

15.1 Focus on the Positive: Pathways for Biasing Responses to Mixed-Valence Food Cues

Mark Andermann

Beth Israel Deaconess Medical Center, Brookline, Massachusetts, United States

Background: Fasting increases positive reactions to food signals and decreases defensive reactions to danger signals that inhibit food-seeking. Patients with anorexia nervosa (AN) exhibit a selective behavioral bias to danger signals, and an associated neural response bias in insular cortex (InsCtx), an area essential for food-seeking under threat. We hypothesize that habitual fasting in AN patients serves to partially attenuate the flow of anxiety-promoting signals to the basolateral amygdala (BLA), and that the behavioral and InsCtx hypersensitivity to danger signals in AN is mediated by excessive flow of anxiety-promoting signals from the paraventricular thalamus (PVT) to the BLA, thereby modifying the perceived valence and salience of learned cues.

Methods: To test these predictions, we will use a new method we recently developed to repeatedly image the activity of hundreds of identified neurons in the insular cortex of healthy and AN model mice combined with specific manipulations of PVT projections to BLA (PVT- > BLA), during operant behavior involving visual food cues, danger cues, or mixed-valence cues. First, we are testing whether specific neurons in BLA and in InsCtx encode food cues and danger cues to drive appetitive and defensive behaviors, respectively. We predict that hunger biases the competition between responses to mixed-valence cues in these two populations, resulting in food-seeking in fasted mice and threat avoidance in sated mice. Second, we are testing whether activation of PVT- > BLA neurons in hungry mice (directly or via removal of inhibition by hunger-related AgRP neurons) shifts behavioral and insular cortex cue responses towards aversive outcomes. Finally, we hope to test if a recent mouse model of AN exhibits a similar behavioral and neural hypersensitivity to danger cues, and whether this hypersensitivity is rescued by suppression of PVT- > BLA neurons, thereby providing a sensitive framework for development of pathway-specific therapies. We are testing these hypotheses in both male and female mice.

Results: Using chronic imaging of dozens of neurons in BLA using a GRIN lens and two-photon calcium imaging in $n = 24$ behaving mice (12 F/12 M), we have observed distinct populations of BLA neurons that respond to learned appetitive and to aversive cues. The shift from hunger to satiety blunts responses to food cues but enhances responses to aversive cues, consistent with the prediction that food restriction in anorexia may serve to decrease responses to aversive cues. We have also recorded from axons of dopamine neurons that project to BLA and find similar effects: a blunting of responses to food cues and an enhancement of responses to aversive cues as mice shift from the hungry to the sated state. This provides a potential mechanism underlying the findings in BLA. We are now beginning to extend these recordings to large populations of neurons in insular cortex.

Conclusions: Our findings suggest that satiety shifts in BLA neurons from a high sensitivity to food cues towards a high sensitivity to aversive cues. We predict that this is due to satiety-related increases in aversive-cue responses from PVT thalamus projections and VTA dopaminergic projections to BLA.

Disclosure: Nothing to disclose.

15.2 Cognitive Flexibility in Rodent Food Intake Behavior: A Translational Model for Eating Disorders

Rachel Ross

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Background: In eating disorders paradoxical decision-making is seen at both sides of the spectrum from underweight to morbid obesity. For humans, eating is influenced by cultural and emotional experiences, and animals also will also engage in different patterns of feeding in mixed valence settings. However, rodents will generally eat only that which they need to be metabolically sated and maintain a relatively consistent body weight unless they are placed in very extreme and stressful experimental settings, in which case they can model severe anorexia or diet-induced-obesity. These changes are not due to any perturbation in the normal metabolic pathways, but rather due to modulation of circuits that impinge on the feeding neurocircuitry, pointing to dysfunctional integration of these signals in higher order circuitry. Imaging studies in humans reveal dysfunction in the prefrontal cortex (PFC), in individuals with obesity shown food cues. Similarly, aberrant activity is found on functional MRI (fMRI) in this region in women with AN, which is thought to be related to cognitive rigidity. Rodent studies also demonstrate cortical responses to nutrient availability, and reward-based circuitry affecting food intake, but the role of the medial PFC in directing decisions toward feeding or energy expending behavior remains unknown.

Methods: To study the role of the mPFC in metabolic decision-making behavior, we focused on the melanocortin 4 receptor (MC4R) expressing cells in that region. MC4R is the most common gene linked to obesity, and its activity is influenced by hypothalamic neuropeptides that are responsive to nutrient status. First, we tested the effect that chemogenetic manipulation of this mPFC-MC4R population in male and female mice had on normal feeding behavior and on performance in a cognitive flexibility paradigm in different hunger states. Then we stereotactically infused the pharmacologic agonist and antagonist of the MC4R within the mPFC to determine the role that the receptor itself has on these pyramidal mPFC neurons and on mouse feeding and cognitive behavior.

Results: Using chemogenetic manipulation of mPFC-MC4R in male and female mice ($n = 6$ per group), we found that inhibition of these neurons in the hunger state leads to perseverative behavior in a decision-making task related to food, but no overt change in normal home cage feeding behavior, or in exploratory behavior in an open field. We saw a similar behavioral outcome in animals treated with the antagonist to MC4R, but no change in response to agonist treatment ($n = 5$ per group). Our next step is to investigate the pharmacologic effect of these peptides on MC4R expressing neurons in vitro to determine the molecular mechanism underlying this behavior change.

Conclusions: Our findings suggest this hypothalamic to prefrontal cortex melanocortinergic circuitry plays an important role in feeding behavior, specifically related to learned behavior

associated with feeding, and that state-based hunger information effects decision making. This implies that this circuit may be dysfunctional in settings of disordered eating.

Disclosure: Nothing to disclose.

15.3 Ghrelinergic Pathways Promoting Appetite and Food Intake in Response to Stress in Major Depressive Disorder

Laura Holsen

Harvard Medical School, Boston, Massachusetts, United States

Background: Divergent appetitive phenotypes in major depressive disorder (MDD) are largely understudied from a mechanistic perspective yet may yield insight into improved treatment strategies and related conditions such as anorexia nervosa and obesity. Compared to those with hypophagic depression (HypoMDD; decreased appetite/weight loss during an episode), individuals with hyperphagic depression (HyperMDD; increased appetite/weight gain) exhibit greater suicidality and medical comorbidities, with recent data suggesting that hyperphagic behaviors stem in part from disruption in mesolimbic regions governing reward. In rodents, chronic social defeat stress (a paradigm resulting in depressive-like behaviors) elevates serum levels of the orexigenic gut peptide, ghrelin, which leads to conditioned preference for high-fat diet. Further evidence from preclinical work supports the involvement of ghrelin in modulating DA transmission and reward signaling. We will present data from a study in humans examining effects of psychosocial stress on the relationship between ghrelin and brain activity to food reward.

Methods: Fifty-one participants [25 healthy control (HC; 12 F/13 M), 15 unmedicated Hypophagic MDD (HypoMDD; 9 F/6 M), 11 unmedicated Hyperphagic MDD (HyperMDD; 6 F/5 M)] each completed 2 study visits (~1 week apart) involving serial blood sampling (acyl ghrelin and cortisol), consumption of a meal, completion of the Maastricht Acute Stress Task (MAST; a potent psychosocial stressor) (Stress Visit) or a Non-Stress version of the MAST (Non-Stress Visit; order counterbalanced), and an fMRI scan (Food Incentive Delay task). Changes in behavioral ratings of hunger and stress, hormones, brain activity, and brain-hormone relationships were examined in the context of response to psychosocial stress.

Results: Across groups, psychosocial stress elicited significant increases ($p < 0.05$) in anxiety ratings and serum cortisol which were not observed during the Non-Stress Visit. However, unique to the HyperMDD group, during the Stress Visit (but not the Non-Stress Visit), we observed: (1) increases in ratings of hunger and ghrelin area under the curve (AUC); (2) greater BOLD response to food reward receipt in widespread limbic and paralimbic regions (amygdala, insula, OFC, mPFC; $p < 0.05$, FWE-corrected); and (3) significant associations between ghrelin AUC and BOLD activation in response to food reward receipt (OFC; mPFC; $p < 0.05$, FWE-corrected). These patterns of behavioral, hormonal, and brain reward responses to stress were not observed in HC or HypoMDD.

Conclusions: These data support the hypothesis that HyperMDD is promoted by ghrelinergic signaling in response to psychosocial stress, and that these ghrelin-specific effects are significantly associated with differential reward activity in mesolimbic and paralimbic regions. We posit that in the short-term, these effects may assist in regulating stress, but over time may lead to weight gain and obesity. Results will be discussed within the context of our previous findings on relationships between ghrelin and brain activity in women with chronic anorexia nervosa, weight-restored anorexia nervosa, remitted HypoMDD, and remitted HyperMDD.

Disclosure: Nothing to disclose.

15.4 Neuronal Mechanisms of Energy Balance and Obesity

Jason Tregellas

University of Colorado/Denver VA Medical Center, Aurora, Colorado, United States

Background: Given the high, increasing rate of global obesity, understanding the neuronal mechanisms involved in food intake, energy expenditure and propensity for obesity is an important goal. Towards this end, this session describes a series of studies in which the neuronal effects of diet, exercise and weight loss were examined in the context of obesity.

Methods: Multiple groups of participants who were either obesity-resistant or obesity-prone were examined with manipulations of short-term energy intake (e.g. over-feeding for two days prior to study day), acute energy intake (meal consumption) or long-term manipulation of energy expenditure (6 months of exercise). Neuronal measures, acquired with functional magnetic resonance imaging at 3 T, included response to visual food cues (compared to non-food objects) and activity in intrinsic networks (extracted via independent components analysis). In the most recent study, the effects of 10 kg of weight loss in participants with obesity were examined in 23 subjects (20 F, 3 M).

Results: Across multiple studies, visual food cues elicited response in a robust network of brain regions, including the insula, sensory cortex, parietal cortex and visual cortex. Short term overfeeding and acute feeding attenuated these responses in obesity-resistant, but not obesity-prone individuals (short term overfeeding interaction: insula, $p < 0.05$, corrected; hypothalamus, $p < 0.02$, corrected; acute feeding interaction: insula, $p < 0.05$, corrected). Greater neuronal activity observed in obesity, both in terms of task-independent intrinsic activity and response to visual food cues, was reduced by chronic exercise (task-independent: default network, $p < 0.003$, corrected; food task: insula, $p < 0.01$, corrected). In the most recent study, 10 kg of weight loss was associated with greater neuronal response ($p < 0.01$, corrected) to a meal (i.e., greater reduction from pre- to post-meal).

Conclusions: These studies suggest that neuronal responses to changes in energy balance (e.g., overfeeding, acute feeding) may be blunted in obesity. Results suggest that weight loss, achieved via diet or exercise, impacts these responses in a manner that may be consistent with improved sensitivity to energy balance.

Disclosure: Nothing to disclose.

Panel

16. Novel In Vivo Imaging and Virtual Reality Paradigms to Dissect Neural Circuits Underlying Brain Plasticity

16.1 Virtual Reality Conditioned Place Preference for In Vivo Investigation of Hippocampal Plasticity Underlying Drug-Paired Contextual Learning

Sidney Williams

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Background: Long term associations between a specific environment and drugs of abuse, such as opioids, can trigger craving and relapse in people with a previous drug misuse history. These

maladaptive memories are partly formed by structural and functional changes in the dorsal hippocampus. The timing and dynamics of these events and their potential relationship to the association between morphine reward and context are unknown. To observe neural networks in real time (as mor CPP and reinstatement take place), we have designed a virtual reality conditioned place preference (VR-CPP) paradigm paired with two-photon imaging. This innovative strategy will allow us to uncover the neuronal spatio-temporal dynamics of drug-induced contextual memories.

Methods: We used a three chamber VR-CPP apparatus composed of two conditioning chambers with distinct visual cues and connected by a third neutral chamber. Mice were head fixed in the VR environment and allowed to freely run on a Styrofoam ball suspended by air pressure. Movement of the ball is tracked by a computer mouse and converted to forward and yaw velocities by custom written software in LabView and then feds to a virtual reality engine written in Matlab, which updated the visual scene permitting the animal to navigate through the VR environment. To motivate animals to explore virtual reality animals underwent two training trials per day during which they received a H2O reward for spending 15 s in the H2O-paired compartment of the VR-CPP whereas no rewards were given in the other chamber. Within 8-12d the animals reach the learning criteria (> 70% of time spent in the H2O- paired compartment for 4 consecutive trials). Mice were then submitted to a biased mor CPP protocol in the VR environment. Briefly, a pre-conditioning preference test, used as a baseline measurement for time spent in each compartment of VR-CPP, was run and followed by 8days of saline/morphine (20 mg/kg) conditioning. A post-conditioning preference test confirmed the development of a preference for the morphine conditioned chamber.

Results: Mice demonstrate a switch in place preference for the mor-paired compartment following contextual MOR conditioning in the VR ($n = 13$; Paired, two-tailed t-test; $p < 0.002$). In an unpaired mor-conditioning paradigm in which mor is administered in both VR rooms, animals do not demonstrate a conditioned place preference for either Room A or Room B ($n = 7$; Paired, two-tailed t-test; $p > 0.05$). Consistent with the behavioral switch in preference, preliminary calcium imaging data analysis ($n = 1$) demonstrates a cell preference ratio ([μA- μB]/ [μA + μB]) switch following mor-conditioning in Room B. There is an increase in the percent of cells with higher mean activity in the mor- paired room post-conditioning.

Conclusions: We have established a novel virtual reality paradigm for mor CPP and operant conditioning behavioral testing. Current work is aimed at combining our VR-CPP paradigm with two-photon in vivo imaging. Preliminary calcium imaging analysis suggests that activity of hippocampal CA1 pyramidal cells is positively correlated to a switch in preference for a mor-conditioned contextual environment in VR.

Disclosure: Nothing to disclose.

16.2 Novelty-Associated Suppression of Hippocampal Inhibitory Circuits

Edward Han

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Background: The ability to modify behavior based on current environment is essential to survival. This ability to associate successful behavior with distinct environments relies on hippocampal-dependent learning, which itself is enhanced on exposure to novel environments. The mechanisms that support context-specific learning in novel environments remain unclear. One long-standing hypothesis is that suppression of inhibition in novel environments is necessary for downstream learning, perhaps by facilitating plasticity; however, the activity of identified interneuron types during behavior remains poorly understood.

Methods: Using in vivo, two-photon calcium imaging, we examined activity in genetically defined classes of CA1 hippocampal interneurons of male and female mice during spatial remapping and context-specific learning induced by novel visual environments during a virtual-reality (VR) track running task. We examined both soma targeting interneurons using parvalbumin (PV)-cre ($N = 3$ mice, $n = 114$ cells) and somatostatin (SOM)-cre mice ($N = 7$ mice, $n = 184$ cells). After mastering the task in a familiar virtual environment, animals were tested in remapping sessions, spending seven minutes in the familiar, then fourteen in a novel virtual environment (with identical task rules but changed textures on track walls, and distal landmarks altered and moved), then back to the familiar for seven minutes. During these remapping sessions we compared activity in inhibitory interneurons between familiar and novel environments using the Wilcoxon ranksum test. This familiar-novel-familiar sequence was repeated over five days, with the "novel" environment becoming more familiar with repeated exposures, as measured by increasing task performance.

Results: We found strong suppression of both PV and SOM-expressing interneurons upon exposure to novel environments. PV interneuron activity was suppressed 63% upon first exposure to a novel environment in comparison to familiar ($p = 1.3 \times 10\text{-}23$). SOM interneurons were similarly strongly suppressed, by 43% in comparison to familiar ($p = 2.4 \times 10\text{-}22$). Suppression of inhibition gradually decreased on repeated exposures to the "novel" environment. In PV interneurons, suppression of activity remained significant throughout all five days, decreasing to 23% suppression on day 5 ($p = 5.6 \times 10\text{-}8$). SOM interneuron activity was significantly suppressed until day 5, where suppression of 7% was not significant ($p > 0.05$).

Conclusions: Our findings reveal profound suppression of inhibition in novel environments in two genetically-identified populations of hippocampal interneuron. These two populations, SOM and PV-expressing interneurons, have distinct functional roles in the network with the former targeting the soma and the latter targeting dendrites for inhibition. Furthermore, the time course of inhibition suppression closely mimics that of learning, with strong suppression during initial exposures to a novel environment, that gradually decreases as the animal learns to perform the task in that new environment. This finding of strong modulation of inhibition, triggered by novel environments, encompassing two important interneuronal networks, and associated with behavior, suggests a critical role for inhibition suppression in learning. This suppression of inhibition may be a necessary first step for learning by increasing excitability in the hippocampal network, thereby facilitating the encoding of new learning through synaptic plasticity.

Disclosure: Nothing to disclose.

16.3 Neuromodulation and Function of De Novo Striatal Spinogenesis and Synaptogenesis

Yevgenia Kozorovitskiy

Northwestern University, Evanston, Illinois, United States

Background: The genesis and turnover of neuronal connections underlie development, learning, and disease. Decades of studies have yielded insights into the processes that regulate formation, pruning, function, and dysfunction of synapses. Still, making

causal inferences about the regulation of synapse birth remains difficult outside of cell culture systems due to technical challenges.

Methods: Here, we use multilaser 2-photon microscopy in order to probabilistically induce new dendritic spines and synapses to be produced de novo with high spatiotemporal precision on genetically targeted striatal spiny projection neurons. This is accomplished by 2-photon uncaging of photolabile glutamate, while imaging genetically targeted striatal spiny projection neuron (SPN) dendrites using a second laser. We use this ability to control dendritic spine formation and synapse genesis to gain insights into the mechanisms of modulation of dendritic spine birth and early stage synapse function.

Results: We find that the modulation of spine and synapse genesis operates at a subcellular level, is bidirectionally tuned by G protein-coupled receptor activation and differs across the sexes before puberty. We characterize the protein synthesis mechanisms important, selectively, for the genesis and the early stage stability of new dendritic spines.

Conclusions: This work is broadly relevant to the attendees of ACNP because the formation and function of neuronal connections is a fundamental property of neurons, perturbed in numerous metal health disorders. Here, we use 2-photon optical tools in order to both induce and study new neuronal connections within existing circuits. These focused experiments are highly complementary to the interrogation of subcellular, cellular and neural circuit activity patterns in animals behaving in VR paradigms, covered by other talks in this symposium.

Disclosure: Nothing to disclose.

16.4 Dynamic Encoding of Aversive Pain Perception Within Hierarchical Neural Ensembles

Gregory Corder

Stanford University, Palo Alto, California, United States

Background: Pain is a sensory and affective experience. An unpleasant percept dominates the affective dimension of pain, which provides a motivational drive to initiate protective behaviors that limit exposure to noxious stimuli. While detailed mechanisms underlying the sensory detection of noxious stimuli and spinal processing of nociceptive information have been uncovered, it remains unclear how brain circuits transform this emotionally inert information into an affective pain perception. Injury-induced plasticity within affective circuits, such as the basolateral amygdala (BLA), may lead to a miscoding of sensory information concomitant with the emergence of chronic pain.

Methods: To identify the principles of nociceptive information coding in the BLA, we used a head-mounted miniature microscope paired with viral expression of the $Ca2+$ indicator GCaMP6m to monitor the activity dynamics of individual BLA neurons in freely behaving mice presented with a diverse set of painful and innocuous stimuli. Concurrently, to monitor pain affect, we developed a method for objectively quantifying aversive behaviors evoked by noxious stimuli. This method categorizes and distinguishes reflexive withdrawal from the temporally delayed, non-stereotyped protective responses that indicate an aversive pain percept, such as attending to the painful tissue and adoption of an active escape. Furthermore, we tracked the longitudinal dynamics of BLA neural coding in mice before and after the development of neuropathic allodynia from a peripheral nerve injury (9,777 cells during 73 sessions over 3 months).

Results: We found that prior to nerve injury, multidimensional and population vector analysis of sensory-evoked $Ca2+$ transients revealed that a unique nociceptive neural ensemble in the basolateral amygdala, distinct from positive valence ensembles, encodes a diverse array of painful stimuli are encoded. Silencing of this ensemble alleviated pain affective-motivational behaviors without altering the detection of noxious stimuli, withdrawal reflexes, anxiety, or reward. After the establishment of neuropathic pain, the neural ensemble representations of prior innocuous and noxious stimuli became more similar.

Conclusions: Collectively, our results identify a neural representation of nociception in the amygdala that is necessary for the instantiation of the negative affective qualities of acute and chronic pain, possibly contributing to pathological psychological co-morbidities such as depression. Our approach

Disclosure: Nothing to disclose.

Panel

17. Modulation of Processes Underlying Mental Disorders via Neurofeedback Technologies

17.1 fMRI Informed EEG as a Reliable Neurofeedback Probe for Limbic Modulation in PTSD

Abstract not included.

17.2 Utility of Amygdala fMRI Neurofeedback Training With Simultaneous EEG in Depression and Combat-Related PTSD

Abstract not included.

17.3 Behavioral and Physiological Targets of Emotion Dysregulation for Amygdala Neurofeedback Training in Borderline Personality Disorder

Christian Paret

Central Institute of Mental Health, University of Heidelberg, Mannheim, Germany

Background: Pervasive emotion dysregulation as seen in Borderline Personality Disorder (BPD) is associated with hyperresponsiveness of the amygdala. Patients may benefit from neurofeedback to deliberately decrease the amygdala response to emotional cues. While feasibility of such training was shown previously, understanding of potential effects is limited.

Methods: Twenty-five female patients with BPD were administered several tests of emotion processing before and after a three-session functional magnetic resonance imaging (fMRI) neurofeedback protocol. In addition to pre and post assessments, we tested the stability of effects six weeks after. During the training patients viewed pictures with negative emotional content and down-regulated feedback from the right amygdala's blood oxygenation level dependent (BOLD) signal. Emotion processing was assessed with neural, peripheral physiological, behavioral and psychometric measures, including electromyography of the emotion-modulated startle response, ecological momentary assessment and diagnostic interview. Repeated measures analyses were used for significance testing.

Results: After the first session, patients significantly decreased their amygdala response to negative pictures compared to passive picture viewing ($P < 0.05$). Tested in the psychophysiology lab, patients improved emotion regulation skills after training, indicated by decreased startle response to negative pictures ($P < 0.05$). Repeated measures analysis of variance showed that this

effect did not persist until the follow-up test. After training patients indicated less feeling of inner tension and negative emotions, as well as lower hour-to-hour variability in these measures (Cohen's d = 0.76-0.78, P < 0.05, N = 21). BPD symptoms decreased in results from the Zanarini interview for BPD (d = 0.70, P < 0.05, N = 19). Mixed results were received from several psychometric scales and fMRI tasks assessing changes in emotion processing and regulation skills.

Conclusions: This one-arm clinical study revealed significant improvements in emotion regulation and reductions of emotional disturbance in daily life after fMRI neurofeedback in BPD. The treatment affected emotion processing on several systems levels, including psychophysiology, behavior and subjective experience. To retain stability, additional neurofeedback 'booster' sessions may be beneficial. Conclusions are limited due to the lack of a control group. A randomized controlled trial (RCT) is needed to confirm effectivity. The design of the RCT will be informed by present results.

Disclosure: Nothing to disclose.

17.4 Time Course of Clinical Change Induced by Neurofeedback

Abstract not included.

Mini Panel

18. Advances in Opioid Use Disorder From Clinical Translational Neuroscience

18.1 Quantifying Progression and Treatment in Opioid Use Disorder Using Computational Neuroeconomic Models of Decision Making

Anna Konova

New York University, New York, New York, United States

Background: Opioid use during opioid treatment increases risk for overdose and treatment failure. Understanding the cognitive mechanisms conferring vulnerability to use opioids in treatment-seekers could help improve treatment monitoring and intervention efforts by helping to identify when and possibly how best to intervene. We examined if computational measures of risky and impulsive decision making can provide quantitative estimates of opioid use disorder (OUD) prognosis. We hypothesized that decision processes will carry proximal, predictive information regarding an individual patient's heroin use vulnerability, and that this relationship would be mediated by the brain's canonical valuation system centered on the striatum and ventromedial prefrontal cortex (VMPFC).

Methods: 80 OUD individuals seeking medication-assisted treatment completed 1-15 sessions over 7 mos. (total = 750, mean = 6.5/patient). At each session we quantified individual decision-making behavior on validated tasks amenable to neuroeconomic modeling, assessed clinical status (symptom severity, adherence), and objectively monitored opioid use. This allowed us to, using time-lagged analyses, predict opioid use in the upcoming 1-4 weeks from behavior and clinical status on the current session. Drug-free controls (n = 55, 1-5 sessions/subject; total = 197, mean = 3) completed similar procedures, serving both as a comparison group and an independent sample in which to assess the test-retest reliability of our measures which was found to be excellent. 18 OUD and 18 controls additionally completed a single multi-band fMRI session. We modeled behavior by three parameters, risk and ambiguity tolerance (capturing response to known and unknown risk, respectively) and discount rate (capturing patience), and the fMRI data using model-based fMRI.

Results: Only an increase in ambiguity tolerance predicted upcoming opioid use at the timescale examined (P < 0.005, ~10% increased odds). Ambiguity tolerance remained a significant predictor (P < 0.05) in an extended model including known clinical predictors, suggesting its contribution cannot be explained by changes in symptom severity or recent use. Overall the diagnostic groups did not differ in ambiguity tolerance; by contrast, OUD were more risk tolerant and impatient (P < 0.01), suggesting that while all three decision parameters had clinical utility they serve different goals in detecting opioid use vulnerability: prognosis vs. diagnosis. Task-based fMRI revealed robust encoding of model-derived value in the striatum and VMPFC in all subjects (P < 0.05 corr); however, ambiguity level-dependent activity in these regions was higher in more ambiguity tolerant subjects (P < 0.04) and in more vulnerable OUD, suggesting changes in the brain's valuation system might underlie both the observed behavior change and heroin use vulnerability.

Conclusions: Treatment monitoring and intervention efforts aimed at specific decision processes (and thus the brain's valuation system) may help reduce incidence of opioid use in a population at high risk for overdose and treatment failure.

Disclosure: Nothing to disclose.

18.2 Cannabinoids: A Novel Strategy to Curb the Opioid Epidemic

Abstract not included.

18.3 Avoidance Learning: From Basic Circuits to Psychological Intervention

Abstract not included.

Panel

19. Is ECT Truly a Non-Focal Therapy? Circuit-Based Evidence for Anatomical and Dimensional Specificity

19.1 ECT Selective Modulation of the Anatomy and Connectivity of Reward Circuitry is Associated With the Clinical Improvement in Anhedonia and its Sub-Constructs

Joan Camprodon

Massachusetts General Hospital, Harvard Medical School, Boston, Massachusetts, United States

Background: ECT is the most effective treatment in psychiatry, and among the most effective in medicine. Despite its apparent non-focal effects leading to a generalized seizure, its therapeutic benefits are specific to a few clinical syndromes, including major depressive disorder (MDD) and bipolar depression (BD). These syndromes share core deficits in reward processing (i.e. anhedonia). ECT improves anhedonia across syndromes, implying selective effects on the functional and structural properties of reward networks.

Reward-related functions represent key behavioral dimensions of pathological relevance across clinical syndromes and have a central place as positive valence constructs in the RDoC matrix.

There has been a growing recognition that "anhedonia" does not represent a unitary dimension; two constructs emerge with clear relevance to behavior and disease: consummation (liking) and anticipation (wanting).

In this talk we will present results using multimodal MRI and dimensional measures of anhedonia to study patients receiving ECT, aiming to understand its effects on reward circuit biology and how these explain syndromal and dimensional clinical improvement.

Methods: We studied 17 patients with MDD before and after an acute course of ECT. Every visit consisted of an MRI scan (with structural, diffusion and resting functional sequences) and clinical assessments of syndromal depression severity (QUIDS) and anhedonia (SHAPS), in addition to specific reward subdomains of interest (TEPS anticipation and consummation subscales).

Voxel-based morphometry was used to assess volumetric changes induced by ECT and the relationship of these anatomical changes with improvement in depression severity and anhedonia.

The medial forebrain bundle (MFB), a primary white matter tract of the meso-cortico-limbic reward network, was defined using multi-tensor tractography. We compared fractional anisotropy (FA), radial diffusivity (RD), axial diffusivity (AD), and trace diffusivity indices before and after ECT and evaluated the associations between changes in diffusion and clinical response.

Results: ECT led to significant clinical improvement in depression severity, anhedonia and its consummatory and anticipatory components.

We observed a positive association between changes in reward anticipation (but not consummation) and regional GM volume increases in the right Nucleus Accumbens and the right anterior midbrain (Ventral Tegmental Area/Substantia Nigra). No significant correlations between GM volume changes and change in depression severity were observed.

ECT was associated with a significant decrease in AD in the right MFB. We observed a significant correlation between AD decrease and improvement in both depression severity and anhedonia.

Conclusions: Despite the diffuse biophysical properties of ECT, its mechanisms of action may be more specific than previously considered. Our results describe changes in the anatomy of key hubs of the reward system that selectively explain the improvement in anticipatory anhedonia, while changes in structural connectivity of the MFB reflect more general improvement in anhedonia and depression severity. A dimensional and circuit-based framework may prove useful to understand the mechanism of action of the most effective treatment in Psychiatry, offering new venues for target discovery and treatment development.

Disclosure: Apex Neuroscience, Advisory Board

19.2 Mouse Models of Electroconvulsive Seizure Induced Plasticity in Neural Circuits of Reward

Kerry Ressler

Harvard Medical School, Belmont, Massachusetts, United States

Background: Electroconvulsive Therapy (ECT) is the most effective treatment in psychiatry, with response rates of 70-90% in depression. Despite its great efficacy and rapid action, the mechanisms underlying ECT remain unknown. Our programs study the effects of ECT in rodents and human patients separately, with a shared focus on reward circuitry which is critically affected in mania and depression.

Methods: Lead by Stephanie Maddox, PhD, our group has developed a novel and clinically informed mouse model of ECT. This model more closely resembles the anesthesia and stimulation protocols used in current clinical practice than traditional pre-clinical rodent work which was largely conducted without anesthesia. While these previous studies observed changes in cortical and hippocampal plasticity and induction of neurotrophic factors and immediate early genes, anesthesia is known to alter synaptic plasticity and seizure threshold which occludes the translatability of these findings to the clinic.

The data presented will combine this mouse model of ECT with a combination of high-resolution 9.4 T DTI and viral-mediated dopaminergic axonal tract tracing to examine changes in connectivity between VTA and NAcc which accompany ECT. We will also utilize a combination of high-resolution 9.4 T voxel-based morphometry (VBM) imaging and cell-type specific profiling of dendritic and synaptic density alterations in the VTA and NAcc to examine the micro-scale anatomical alterations which contribute to structural changes observed with ECT.

Results: In validating our new mouse model we have replicated the induction of plasticity-related genes in the cortex and hippocampus and revealed for the first time a robust ECS-induction of brain-derived neurotrophic factor (BDNF) in the NAcc. This led us to postulate that alterations in the dopaminergic (DA) projections from VTA to NAcc, a pathway well-established in mediating reward-related behaviors, may account for the robust anhedonic response to ECT.

In addition to examining a number of molecular pathways, we will examine structural mechanisms underlying the mouse ECT response. We first report a voxel-based morphometry (VBM) study of mice following auditory fear conditioning complemented by confocal microscopy analysis of neuronal morphometric features. Following fear conditioning, significant VBM results included amygdala subregions and auditory cortex, with no significant VBM changes in a control brain area. Confocal analysis showed that fear conditioning led to a significantly increased density of shorter and wider dendritic spines. Of all the morphology metrics studied, the spine density was the only one to show significant correlation with the VBM signal. Ongoing analyses will perform similar studies as performed above on ECT-treated mice to examine macro- and micro-level structural change in NAcc.

Conclusions: These data demonstrate 1) a mouse model of ECT using similar anesthesia, number of seizures, and recovery may be possible as a translatable model to understand ECT mechanisms; 2) data suggest that this model is accompanied by molecular markers of neural plasticity within the NAcc; and 3) learning-induced structural changes detected by VBM may be partially explained by increases in dendritic spine density in a fear conditioning model. Ongoing analyses will describe observation with similar structural approaches in the mouse ECT model.

Disclosure: Resilience Therapeutics, Advisory Board; Biogen, Consultant

19.3 Differential Patterns of Structural Connectivity Associated With Latent Symptom Dimensions in Electroconvulsive Therapy

Benjamin Wade

University of California, Los Angeles, Los Angeles, California, United States

Background: Major depressive disorder (MDD) is a highly prevalent and symptomatically heterogeneous disorder. The Hamilton Depression Rating Scale (HDRS) is commonly used to evaluate clinical improvement, though different neural systems may account for changes in particular symptom profiles. Electroconvulsive therapy (ECT) shows rapid and robust clinical effects in patients with treatment resistant MDD, however, its underlying mechanisms of action in relation to symptom dimensions in MDD

remain poorly described. Here, we use data-driven methods and novel graph theory measures to identify and evaluate how symptom dimensions segregate, change, and relate to patterns of structural covariance and divergence over ECT index in a large multisite cohort of ECT patients with MDD.

Methods: One hundred eleven MDD patients (age = 52.16 ± 14.67; 68 female) from four independent sites participating in the Global ECT-MRI Research Collaboration were included and underwent structural MRI and 17-item HDRS assessment before and after ECT. Ninety-one patients received right-unilateral ECT. Exploratory factor analysis (EFA) was applied to the baseline HDRS item-level scores to identify more homogeneous latent symptom dimensions. Cortical thickness and subcortical volumetric measures were extracted for each subject with FreeSurfer. Novel graph theoretical measures of longitudinal structural connectivity and disconnectivity were modeled for each subject. Random forest (RF) regression models were then used to predict the degree of change along each latent symptom dimension using patients' regional node degree distribution, age, sex, site, and baseline severity.

Results: Three stable latent symptom dimensions were identified with EFA, largely segregating somatic (factor 1), core mood/anhedonic (factor 2), and insomnia symptoms (factor 3). Correlation between predicted and actual changes along each latent symptom dimension using the RF models was significant: factor 1 ($r = 0.46$; $p < 0.0001$), factor 2 ($r = 0.64$, $p < 0.0001$), and factor 3 ($r = 0.59$, $p < 0.0001$). Change along factors 2 and 3 were significantly better predicted than factor 1 ($p < 0.0001$). Change along factor 1 was primarily associated with networks of structural divergence from the left anterior cingulate to ventro-limbic, sensory and sensory association cortices. Divergence between right medial temporal and cingulate/striatal-pallidal networks, and from the right accumbens and medial temporal and fronto-temporal networks associated with change along factor 2. Covariance between the left putamen and bilateral medial temporal and frontal networks, along with divergence between occipital and medial temporal networks associated with change along factor 3.

Conclusions: We identified homogenous symptom dimensions and used machine learning to identify distributed patterns of structural connectivity associated with changes in each dimension over ECT index based on a novel graph theoretical approach. Symptom dimensions exhibited unique relationships between overlapping medial temporal structural networks, striatal/pallidal, and heteromodal frontal and occipital networks. Our results may inform more personalized treatment strategies and suggests that targeted neuromodulation of specific networks will affect specific symptom profiles.

Disclosure: Nothing to disclose.

19.4 ECT Pulse Amplitude and Medial Temporal Engagement

Christopher Abbott

University of New Mexico, Albuquerque, New Mexico, United States

Background: This investigation will examine the clinical and neurocognitive impact of targeted medial temporal lobe engagement as a function of pulse amplitude, one of several variables that influence the ECT charge. We hypothesize that the optimal pulse amplitude for an individual patient will induce medial temporal lobe neuroplasticity (clinical response) and minimize disruption of dominant hemisphere hippocampal cognitive circuitry (cognitive stability).

Methods: We will randomize subjects with major depressive disorder meeting the clinical indication for right unilateral ECT to 600, 700, and 800 milliamperes (mA). Subjects will receive clinical, neuropsychological, and imaging assessments before, during (after the sixth ECT treatment), and post-ECT. Whole brain electric field modeling will be completed with baseline (Pre-ECT) imaging data (T1, T2, DTI) and finite element modeling. We will assess the relationship between calculated hippocampal electric field, neuroplasticity, and clinical outcomes (efficacy and cognitive impairment) with regression models that control for demographics (age, gender).

Results: Our preliminary data included two data sets. The first data set included low amplitude groups as follows: 500 mA ($n = 3$, no responders), 700 mA ($n = 2$, responders), 800 mA ($n = 2$, responders), or 900 mA ($n = 7$, responders). The second data set included clinical and cognitive data from a larger RUL ECT dataset ($n = 30$). Right hippocampal electric field strength predicted change in the right hippocampal volume (Cohen's $f2 = 0.53$). This relationship was not evident in the left hemisphere ($p > 0.10$), which had a lower calculated electric field strength (< 150 volts/meter). The volume change of the right dentate gyrus was associated with Hamilton Depression Rating Scale (HDRS) change (Cohen's $f2 = 0.59$). Electric field strength was associated with disrupted functional connectivity between the left entorhinal cortex and the left precuneus (Cohen's $f2 = 0.89$). The connectivity between the entorhinal cortex and all of the regions within the default mode network predicted percent change in recall (Cohen's $f2 = 0.30$ to 0.98).

Conclusions: Our 500 mA ECT data is consistent with the view that the seizure is necessary but insufficient for clinical efficacy. The electric field must have sufficient strength to induce hippocampal volumetric change. Based on our preliminary data, the medial temporal lobe electric field strength around150 volts/meter may be consistent with the neuroplasticity threshold, the electric field strength necessary to induce neuroplasticity. The link between neuroplasticity and clinical efficacy was supported with the increased volume of the right dentate gyrus. The seizure activity and/or the electric field could be contributing to disrupted entorhinal connectivity and neurocognitive impairment for the subjects that completed ECT at the traditional 900 mA pulse amplitude. Evidence supporting the association with electric field strength includes the relationship between increased electric field strength and disrupted functional connectivity between the entorhinal cortex and precuneus. In addition, the relationship between disrupted functional connectivity and cognitive performance appears robust and specific for the left entorhinal cortex/default mode network.

Disclosure: Nothing to disclose.

Panel

20. Multiple Mechanisms of Ketamine Action—Beyond NMDA

20.1 Mechanisms Underlying Antidepressant Actions of Ketamine's (2 R,6 R)-Hydroxynorketamine Metabolite

Panos Zanos

University of Maryland School of Medicine, Baltimore, Maryland, United States

Background: Ketamine alleviates depressive symptoms in treatment-refractory patients suffering from major depression within 2 h following a single administration and this effect persists for many days. In both humans and rodents, ketamine is rapidly metabolized to norketamine, dehydronorketamine, hydroxyketamines and the hydroxynorketamines (HNKs).

Methods: Using mice, we investigated the antidepressant and anti-anhedonic effects of ketamine's metabolites. For understanding the mechanisms underlying ketamine metabolites' antidepressant actions, we performed in vitro field excitatory post-synaptic potential (fEPSP) recordings along with western blot measurement of synaptic proteins. Moreover, in vivo electroencephalogram (EEG) measurements of high frequency oscillations were performed as a measure of target engagement. To determine antidepressant-relevant concentrations of (2 R,6 R)-HNK, we measured (2 R,6 R)-HNK levels in the extracellular compartment of the hippocampus (using microdialysis), whole brain tissue, and plasma of mice following peripheral administration. We then systematically assessed the effects of (2 R,6 R)-HNK, compared to ketamine, on NMDAR activity in vitro on: (i) NMDAR-mediated field excitatory postsynaptic potentials in the mouse hippocampus, (ii) NMDAR-mediated miniature excitatory post-synaptic currents in rat CA1 pyramidal neurons, (iii) NMDA-evoked currents in CA1 pyramidal neurons, and (iv) function of recombinant GluN1/2 A, GluN1/2B, GluN1/2 C, and GluN1/2D NMDARs expressed in Xenopus oocytes. The effects of ketamine and (2 R,6 R)-HNK were also evaluated in vivo on NMDA-induced lethality in mice.

Results: We showed that metabolism of ketamine to its hydroxynorketamine metabolites is essential for the antidepressant actions of the drug. We also showed that (2 R,6 R)-HNK induces potent and sustained antidepressant, as well as anti-anhedonic actions in mice, both when it is administered peripherally, as well as following direct infusion into the brain. These actions require acute and sustained AMPAR activation. In vitro, (2 R,6 R)-HNK enhanced AMPAR-mediated synaptic potentiation and increased high (gamma) frequency EEG oscillations through a mechanism completely independent of any inhibition of the NMDAR. Both in vivo and in vitro, ketamine inhibited NMDAR-mediated responses with much greater potency than (2 R,6 R)-HNK, which showed moderate effects at doses irrelevant for the its antidepressant actions. (2 R,6 R)-HNK, unlike ketamine, lacks the sensory dissociation and abuse potential side effects in mice.

Conclusions: These findings indicate a novel mechanism of ketamine action as an antidepressant, which does not involve NMDAR blockade, but it requires the activity of a distinct metabolite. These findings have relevance for the development of next generation fast-acting antidepressant, especially considering the lack of side effects. NMDAR inhibition is a major determinant of ketamine's undesirable side-effects, and thus (2 R,6 R)-HNK, and next generation drugs sharing similar pharmacodynamics, may be better tolerated than ketamine.

Disclosure: Co-authors in patent applications related to the pharmacology and use of (2 S,6 S)- and (2 R,6 R)-hydroxynorketamine in the treatment of depression, anxiety, anhedonia, suicidal ideation and post-traumatic stress disorder, Patent

20.2 Ketamine Rescues Dysfunction of Glutamate Release and BDNF Dendritic Trafficking Induced by Both Acute and Chronic Stress: Implications for Pathophysiology and Treatment

Maurizio Popoli

University of Milano, Milano, Italy

Background: Stress represents a major risk factor for psychiatric disorders, in particular major depression, PTSD and anxiety disorders. Recently we dissected the destabilizing effects of both acute and chronic stress in prefrontal cortex (PFC) and hippocampus (HPC), respectively, with particular relevance to the glutamate (Glu) system.

Methods: The footshock stress protocol (FS, 40 min) was used as model of inescapable acute stress and the chronic mild stress protocol (CMS, 5 weeks) as model of chronic stress. Twenty-four hrs after FS and at the end of CMS rats were deemed resilient or vulnerable to the effect of stress on behavior (anhedonia) by using the sucrose preference test. Basal and depolarization-evoked release of Glu/GABA in PFC or HPC was measured after both stress protocols with the method of purified synaptosomes in superfusion. Dendritic morphology was assessed in Golgi-Cox stained sections. Expression and dendritic trafficking of BDNF transcripts were assessed by qPCR and in-situ hybridization, respectively. Sub-anhestetic racemic ketamine (10 mg/kg) was administered as a single i.p. injection at different times before or after FS, and 24 h before conclusion of CMS.

Results: Acute inescapable stress rapidly enhanced Glu release/transmission in PFC, an effect sustained for at least 24 h. Unexpectedly, after FS significant atrophy of apical dendrites was observed already at 24 h, and prolonged for at least 14 days. Ketamine blocked the acute stress-induced enhancement of Glu release when administered 24 or 72 h before, or 6 h after FS stress (2w-ANOVA; $p < 0,01$, $p < 0,05$).

With regard to chronic stress, Glu release was selectively reduced in HPC synaptosomes from CMS-vulnerable (CMS-V) but not CMS–resilient (CMS-R) rats. Significant reduction in expression of total BDNF and BDNF splice variants was found in all CMS rats. Reduced dendritic trafficking of BDNF mRNA and atrophy of apical dendrites was found in HPC of CMS-V only. In CMS-V rats, in just 24 h ketamine treatment completely restored anhedonic behavior and all the cellular/molecular changes observed, including Glu release, dendritic atrophy and BDNF dendritic trafficking (Multil. Covar. Analysis; $p < 0.001$), with the only exception of BDNF expression.

Conclusions: The present results show that a main action of ketamine, both in acutely and chronically stressed rats is to re-equilibrate dysfunctional Glu release. When Glu release is enhanced, after acute stress, ketamine restores normal level of release. When Glu release is impaired, after CMS, ketamine brings it back to normal level. In contrast with previous data, we showed that ketamine does not restore expression of total BDNF or BDNF splice variants in CMS-V rats, it rather restores the trafficking of BDNF to apical dendrites, in 24 h. Therefore, for the first time, this work suggests a relationship between vulnerability to chronic stress, Glu release and availability of BDNF mRNA at synapses. Overall, these results are consistent with a mechanism of ketamine involving re-establishment of synaptic homeostasis, through restoration of Glu release and dendritic BDNF for rapid synaptic translation.

Disclosure: Nothing to disclose.

20.3 Ketamine Evokes in Glia, a Rapid, cAMP-Dependent, NMDA-Receptor Independent Antidepressant "Biosignature" Wherein the G Protein, Gsalpha, is Translocated From Lipid Rafts Toward a Facile Association With Adenylyl Cyclase

Mark Rasenick

University of Illinois College of Medicine, Chicago, Illinois, United States

Background: All antidepressants examined thus far, translocate the G protein Gsalpha out of cholesterol-rich lipid rafts where it engages in a productive association with adenylyl cyclase and an increase in cellular cAMP. This can be measured directly or by fluorescence recovery after photobleaching (FRAP) in cultured glial cells expressing a fluorescent Gsalpha. For antidepressants other than ketamine, this requires 3 days incubation at plasma

concentrations of the drug. This mirrors the time required for antidepressants to sort, physically, into lipid raft fractions, as determined by mass spectrometry.

Methods: C6 glioma cells, primary astrocytes derived from P3 rats or astrocytes induced from iNSC from depressed human subjects were treated with ketamine (1 μM) for 15 minutes, following which the drug was removed from cultures. Effects of ketamine were assessed by cell fractionation on sucrose density gradients to determine raft-localization of Gsalpha. Gsalpha/adenylyl cyclase functional completes were determined by FRAP and cAMP production as determined with a fluorescent Epac sensor encoded in mammalianized baculovirus (Montana Molecular).

Results: Fifteen minutes of ketamine treatment promoted translocation of Gsalpha in the three cell types as determined by call fractionation (C6 cells) or FRAP and increased cAMP accumulation in response to forskolin or isoproterenol (all three cell types). The effect was transient and persisted 12, but not 24, hours after the withdrawal of ketamine. Ketamine exposure induced CREB phosphorylation and BDNF production, and both were blocked by the cAMP antagonist Rp-cAMPs. Elimination of NR1 (and functional NMDA receptor) with SiRNA had no effect on these actions of ketamine, and the ketamine metabolite 2 R,6 R hydroxynorketamine, which is reported to have no NMDA receptor antagonist properties, has similar effects to ketamine in these studies.

Conclusions: We have suggested that the translocation of Gs alpha from lipid rafts, is a cellular hallmark of antidepressant action and it may well provide a biosignature with which to identify compounds with antidepressant potential. Ketamine displays this signature along the rapid time course relevant for its antidepressant effects, and shows reversion to the initial state, following a lag time, after drug withdrawal. Thus, it is possible that these results not only reveal a mechanism for ketamine action but are also consistent with a cAMP-dependent mechanism of action of many classes of antidepressants.

Disclosure: Pax Neuroscience, Board Member

20.4 Dopamine System Dysregulation in Negative Affective States: Reversal by Ketamine

Abstract not included.

Study Group

21. No Longer Tarred With the Same Brush? Evidence for the Therapeutic Potential of Cannabidiol: Implications for Regulatory Policy

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New York University School of Medicine, United States

Study Group Summary: Cannabidiol (CBD) is a non-psychoactive cannabis component with preliminary evidence of therapeutic effects across a remarkable range of domains: neuroprotective, antiepileptic, anxiolytic, antipsychotic, analgesic, anti-inflammatory, anti-asthmatic, and antitumor properties have all been ascribed to CBD. However, CBD, as a cannabis derivative, is controlled in Schedule I by the U.S. Drug Enforcement Agency, which hinders research to determine which of these many potential indications will bear out in large-scale clinical trials.

Despite the obstacles, compelling data on the efficacy of CBD for treating refractory seizures recently led to a unanimous recommendation by an FDA advisory panel endorsing approval for Epidiolex, purified CBD proposed for marketing approval by GW Pharma for treatment of Dravet and Lennox-Gastaut syndromes. The panel also noted that CBD's safety profile appears promising with relatively minimal adverse effects. Pediatric and adult participants have tolerated CBD well at doses up to 1500 mg/day. The World Health Organization (WHO) recently found that there is no evidence to support dependence or abuse potential or associated public health related concerns for CBD. The WHO report concluded, "As CBD is not currently a scheduled substance in its own right (only as a component of cannabis extracts), current information does not justify a change in this scheduling status nor does it justify scheduling of the substance."

Accordingly, this study group, composed of CBD investigators, along with scientists from NIH and FDA, will consider the following questions in anticipation of the next phase of CBD clinical translational research.

Questions for the CBD Study Group:

1. Assuming CBD is approved for treatment of refractory seizures, should it be removed altogether from the DEA schedule? What evidence would be required to justify its declassification?
2. What are the specific ethical considerations regarding using CBD in pediatric populations? In children with autism spectrum disorder (ASD)? ASD can be reliably diagnosed as young as age 2 years. Assuming earlier treatment is better, what safeguards need to be in place to consider trials of CBD in 2-year olds with ASD?
3. Although CBD toxicity appears to be minimal, elevated liver function tests have resulted from treatment combinations with some antiepileptic compounds. What other concerns about drug interactions might there be?
4. What do we know about the cellular and synaptic effects of CBD?
5. Does CBD have a potential role in the treatment of drug use disorders?
6. How should we dose CBD? Varying doses have been found to be most effective for targeting seizures vs. anxiety-like symptoms or social behaviors. How might this relate to underlying mechanisms?

Disclosure: Nothing to disclose.

Study Group

22. Challenges and Strategies for Strengthening the Pipeline of Physician Scientists in Psychiatry and Neuroscience

Maria Oquendo, M. Mercedes Perez-Rodriguez, Eric Nestler, Rene Kahn, David Ross, Ruth O'Hara, David Kupfer, Alan Schatzberg, Jeffrey Borenstein, Neal Swerdlow, Mark Chavez, Erika Forbes, Eduardo Leonardo, Joshua Gordon, Antonia New*

Perelman School of Medicine University of Pennsylvania, United States

Study Group Summary: Recent advances in neuroscience and genetics set an ideal stage for groundbreaking translational research in psychiatry. The National Institutes of Health, The US Department of Veterans Affairs, and other federal agencies and foundations have recently invested billions of dollars to understand the mechanisms of psychiatric disorders and develop new

treatments based on discoveries in genomics, neuroscience, and behavioral science.

However, while funding and discoveries in neuroscience have grown exponentially over the past few decades, the physician-scientist workforce that is required to translate these research findings into clinically meaningful interventions and diagnostic tests has remained stagnant. In fact, throughout this "neuroscience revolution," the percentage of MD/PhD graduates entering residency training in psychiatry has remained unchanged at only 5%. Not surprisingly, this has contributed to an enormous practice gap (i.e., it can take many years before critical findings from neuroscience research are implemented in psychiatric practice and can have an impact on individual patients).

The overall goal of this study group is to foster a discussion around challenges and novel strategies for enhancing recruitment, training and retention of a diverse workforce of physician scientists within the field of psychiatry. Specifically, we will discuss strategies that have been recommended and/or already implemented to achieve the goal of increasing the physician-scientist workforce in the field of psychiatry, such as: 1) Flexibility in clinical training to allow more time for research and avoid gaps between research training periods (e.g., innovative NIH-funded residency research track programs); 2) Bridging the transition to faculty and research independence through coordinated research training linked to post-residency research fellowships and other strategies; 3) Financial incentives through debt repayment and stipend supplements, as well as closing the pay gap between research and clinical positions; 4) Fostering the recruitment of a diverse physician scientist workforce, including women and underrepresented minorities; 5) Novel approaches to provide and support research training to non-PhD psychiatry residents (e.g., the NIMH-funded combined residency plus PhD program); 6) Early outreach during medical school to address inaccurate perceptions of psychiatry held by MD and MD/PhD students.

Disclosure: Bristol Myers Squibb, Stock / Equity (Spouse) Research Foundation for Mental Hygiene, Royalties

Panel

23. Neurobiology of Opioid Addiction: Hyperkatifeia and Negative Reinforcement as a Driving Force

23.1 Neurobiological Correlates of Pain Avoidance Behavior in Opioid Vs. Alcohol Dependence

Abstract not included.

23.2 Pain Recruits the Dynorphin-Kappa Opioid System in the Nucleus Accumbens Affecting the Pattern of Fentanyl Self-Administration

Nicolas Massaly

Washington University in St. Louis, St. Louis, Missouri, United States

Background: Pain is frequently accompanied by emotional disorders such as negative affective states. Pain is also a risk factor for drug abuse and accidental overdose, particularly for pain-relieving opioids. Indeed, 25% of patients experiencing pain misuse drugs of abuse, a maladaptive behavior that can lead to involuntary overdose. Prior work has revealed that the dynorphin-kappa opioid receptor (KOR) system, in discrete brain regions, decreases the reinforcing properties of rewards and induces dysphoria and aversive behaviors. The abundance of dynorphin neurons and KORs in the nucleus accumbens shell (NAcSh), a brain structure highly involved in reward processing, led us to hypothesize a role for this system in pain-induced negative affect and alterations in opioid consumption.

Methods: We used an inflammatory pain model together with a wide range of complementary techniques including pharmacology, optogenetics, chemogenetics, physiology, biochemistry and rodent PET imaging to investigate on pain-induced negative affect in both rats and mice. Furthermore, to evaluate the effect of pain on opioid misuse, we conducted dose-response analyses for fentanyl self-administration using a fixed ratio (FR) schedule of reinforcement in rats. When appropriate ANOVAs followed by post-hoc tests were used to assess statistical differences in between treatments/groups.

Results: We reveal that inflammatory pain recruits dynorphin neurons through a disinhibition mechanism ($p = 0.014$) and increases kappa opioid receptor function ($p < 0.001$) in the NAcSh. These results are further corroborated by our PET imaging data demonstrating an increase in receptor binding pocket occupancy during inflammatory pain ($p = 0.0022$). Using a combination of pharmacological approaches during a motivation task for sucrose, we confirm that the KORs in the NAcSh are both necessary ($p = 0.022$) and sufficient ($p = 0.0064$) to drive in vivo pain-induced negative affect. In addition, using chemogenetics, we demonstrate that dynorphin containing neurons in the NAcSh are necessary for pain-induced negative affect ($p < 0.0001$). Lastly, using intravenous opioid self-administration in rats, we reveal that inflammatory pain impacts patterns of consumption of fentanyl. Indeed, rats in pain display bursts of consumption interrupted by periods of "rest" while control rats consume fentanyl at a highly conserved frequency ($p = 0.013$).

Conclusions: Our current work demonstrates that inflammatory pain recruits the dynorphin-KOR system in the NAcSh to drive pain-induced negative affect. These negative affective states strongly impact motivation to perform goal-directed behavior and opioid consumption patterns in animals experiencing pain. Altogether, the current work thoroughly demonstrates that the dynorphin-KOR system in the NAcSh represents an important target for therapeutical approaches in the treatment of pain-induced negative affect. More extensive studies on this topic might open avenues for innovative and necessary pharmaceutical treatments to alleviate the emotional component of pain and ultimately tackle pain-induced opioid misuse.

Disclosure: Nothing to disclose.

23.3 Negative Emotional Learning Brain Circuits in Compulsive-Like Heroin Seeking

Stephanie Carmack

National Institute on Drug Abuse/National Institutes of Health, Baltimore, Maryland, United States

Background: Negative emotional states associated with opioid abstinence (e.g., pain and anxiety) may promote compulsive-like opioid use via negative reinforcement. Our previous work has shown that environmental cues can be classically conditioned to withdrawal-induced negative emotional states to drive heroin taking. Here, we used functional magnetic resonance imaging (fMRI) to test the hypothesis that the extended amygdala stress circuit (central amygdala, CeA; nucleus accumbens shell, NAc Shell; and bed nucleus of the stria terminalis, BNST) is sensitized in opioid dependence and has a key role in learning that contributes to the maintenance of compulsive opioid seeking.

Methods: Male Long Evans rats were trained to self-administer heroin in limited (1 h per day, $n = 11$) or extended (12 h per day, n

= 10) access sessions. Odor cues were paired with heroin intoxication or naloxone-precipitated heroin withdrawal in a within-subjects design. Rats underwent scanning 24 h into acute heroin withdrawal. Data were acquired during presentation of the odor cues in the absence of heroin. Differences in BOLD response were calculated for the whole brain. Resting BOLD data were acquired and differences in functional connectivity (rsFC) were calculated using seeds in the CeA, NA shell, and BNST.

Results: Odor cues previously paired with withdrawal-induced aversive states increased operant responding for heroin, promote reinstatement of lever pressing behavior following extinction, and increase sensitivity to painful stimuli in rats given extended, but not limited access to heroin ($p < 0.05$). Whole-brain analysis of the BOLD response following cue presentation in the absence of heroin showed an interaction between heroin access and cue type in several brain regions, including the extended amygdala and hypothalamus ($p < 0.01$). Generally, the odor cue previously paired with withdrawal-induced aversive states increased activation in rats given extended, but not limited, access to heroin in these regions. Rats given extended access to heroin had significant rsFC increases relative to rats given limited access to heroin in the: (1) CeA and BNST; (2) NA shell and CeA; and (3) BNST and NA ($p < 0.02$).

Conclusions: Repeated, intense cycles of heroin intoxication and withdrawal sensitized extended amygdala stress circuits in acute heroin withdrawal. Cues previously paired with heroin withdrawal also activated hypothalamic and extrahypothalamic stress circuits in the absence of heroin. These allostatic changes may contribute to negative emotional states that promote compulsive heroin taking and seeking.

Disclosure: Nothing to disclose.

23.4 Glucocorticoid Receptors Play a Critical Role in Compulsive-Like Opioid Intake

Leandro Vendruscolo

National Institute on Drug Abuse, Baltimore, Maryland, United States

Background: Negative emotional states (e.g., anxiety, dysphoria, and pain) are hypothesized to drive compulsive-like drug taking and seeking. These behavioral changes are mediated by maladaptive allostatic changes in the brain reward and stress systems. Our hypothesis is that potent stimulation of the hypothalamic-pituitary-adrenal axis by opioid intoxication and withdrawal leads to glucocorticoid-mediated reward hypofunction and the sensitization of extrahypothalamic brain stress systems.

Methods: Male Wistar rats were allowed short access (1 h sessions; ShA) or long access (12 h sessions; LgA) to intravenous heroin self-administration. Mifepristone (RU-486) and vehicle administration were conducted using pellets for chronic release or intraperitoneal for acute effect.

Results: Our results indicate that the chronic blockade of glucocorticoid receptors (GRs) with mifepristone reduced the escalation of heroin self-administration, attenuated the greater motivation for heroin, decreased naloxone-induced heroin intake, and lowered mechanical hypersensitivity during withdrawal in LgA rats. The same treatment had no effect in ShA rats. Once heroin self-administration escalated, acute mifepristone treatment significantly decreased heroin intake in LgA rats.

Conclusions: Drugs that normalize a hypofunctional reward system and desensitize brain stress systems have the potential to effectively block compulsive opioid taking and seeking.

Disclosure: Nothing to disclose.

Panel

24. Big-Data Approaches to Identifying the Neurodevelopmental Trajectories of Psychiatric Illness: Novel Emerging Results From the Adolescent Brain and Cognitive Development Study

24.1 Neural Correlates of Psychotic-Like Experiences in School-Age Children: Findings From the Adolescent and Brain Cognitive Development Study

Deanna Barch

Washington University, Saint Louis, Missouri, United States

Background: A number of researchers have argued that psychosis occurs along a continuum of severity. For example, 5-8% of adults without a clinical diagnosis experience subclinical psychotic symptoms, or "psychotic-like experiences." This percentage is even higher among children and adolescents, with rates between 13 and 15%. Further, childhood psychotic-like experiences are associated with greater risk of developing a psychotic disorder later in life, especially if the psychotic-like experiences persist over time. However, little is known about the behavior and neural correlates of psychotic-like experiences in young children. A number of studies have found abnormal functional connectivity to be present across many different stages of the psychosis spectrum, particularly in relationship to cognitive function– the cingulo-opercular network, fronto-parietal network, and default mode network. Also, a number of studies have found reduced volume of brain regions such as the thalamus, hippocampus, insula, dorsal anterior cingulate and the dorsolateral prefrontal cortex. The goal of the current presentation is to examine the relationship between childhood psychotic-like experiences and both functional connectivity and brain volume to understand potential continuities with clinical psychosis.

Methods: We used data from 4524 children in the first wave of the Adolescent Brain and Cognitive Development Study, a prospective longitudinal study aimed at assessing risk factors associated with adverse physical and mental health outcomes from ages 9-10-years-old into late adolescence/early adulthood. Children were recruited from twenty-one research sites across the United States. We assessed psychotic-like experiences using the Prodromal Questionnaire-Brief Child version, a modified version of the validated adult/adolescent Prodromal Questionnaire-Brief and assessed cognitive function using the NIH Toolbox as well as additional cognitive measures. We assessed brain structure using high resolution T1 images segmented through FreeSurfer and functional brain connectivity using up to 20 minutes of resting state functional magnetic resonance imaging data.

Results: Cognitive deficits (e.g., working memory), motor coordination impairments, increased internalizing symptoms, and family history of psychotic disorders were associated with increased psychotic-like experiences. In addition, decreased cingulo-opercular ($t = -4.83$, $p < .001$ $B = -076$), and default mode ($t = -4.00$, $p < .001$, $B = -064$), and functional connectivity were associated with increased psychotic-like experiences, even after accounting for cognitive test performance, internalizing symptoms, and family history of psychotic disorder. Further, decreased whole brain gray matter volume was also associated with increased psychotic-like experiences ($t = -2.40$, $p = .017$, $B = -036$), though individual regions did not survive after controlling for whole brain.

Conclusions: These data advance our understanding of the spectrum of psychotic experiences, indicating that psychotic-like experiences in childhood relate to variation in neural and

behavioral indices that have also been associated with clinically diagnosed psychotic symptoms in adolescence and adulthood. Thus, in combination with previous findings, the results provide evidence that correlates of childhood psychotic-like experiences may be useful in predicting risk for psychosis and helpful in understanding its developmental trajectory.

Disclosure: Nothing to disclose.

24.2 Callous-Unemotional Traits and its Correlates in the ABCD Study

Samuel Hawes

Florida International University, Miami, Florida, United States

Background: Childhood antisocial behavior (AB), including aggression, violence, and theft, puts youth at risk for chronic AB, comorbid mental health problems, and criminality across the lifespan (Moffitt, Caspi, Harrington, & Milne, 2002). Moreover, the greater use of health and education services by children with AB, as well as the harmful effects of crime and AB on communities and families more broadly, confers a significant monetary cost to society (Rivenbark et al., 2017). A growing body of literature suggests that Callous-Unemotional (CU) traits, which refer to a lack of empathic concern, shallow affect, and low moral regulation (Frick, Ray, Thornton, & Kahn, 2014a), can be used to delineate a subgroup of youth with a particularly recalcitrant and severe form of AB thought to develop, in part, via a unique neurobiological pathway. Early identification of youth exhibiting elevated levels of CU traits is critical for developmental, diagnostic, and clinical models of antisocial behavior (AB).

Methods: This study used data from the initial data release of the Adolescent Brain and Cognitive Development (ABCD) Study ($n = 4{,}524$) to derive a brief measure of CU traits in order to examine unique associations with theoretically-relevant mental health and neurocognitive outcomes, among youth exhibiting elevated levels of CU. The ABCD study is a multi-site longitudinal investigation focused on assessing risk factors of mental and physical health, as well as neurodevelopmental trajectories beginning in childhood (ages 9-10) and extending into late adolescence/early adulthood. Moderated non-linear factor analysis (MNLFA) was used in the development of the CU measure and a latent variable model framework was used to assess convergent and divergent associations with study outcomes.

Results: The CU traits measure demonstrated strong psychometric properties (e.g., measurement invariance, lack of differential item functioning) and exhibited evidence of convergent validity as CU traits were moderately related to (but distinguishable from) other early indicators of AB (e.g., ADHD, ODD, conduct problems; $r's = .31–.43$, $p < .001$). Notably, a cross-over suppression effect for the association between CU traits and anxiety provided evidence of discriminant validity, as a significant and positive bivariate association ($r = .15$, $p < .001$) became significant in the negative direction ($r = -.08$, $p < .001$), after controlling for overlap with conduct problem behaviors—providing support for theoretical links between models of CU and low anxiety. Although several small significant effects were found between CU traits and relevant cortical (e.g., OFC) and subcortical brain volumes (e.g., amygdala), there was little evidence of specificity among these associations after controlling for overlap with other externalizing constructs.

Conclusions: In a large, multi-site study, a brief measure of CU traits was developed to help identify youth at early risk for chronic AB. The derived measure provides the scientific community with a method to assess CU traits in the ABCD sample, which can be combined with other neurocognitive, genetic, and environmental outcomes.

Disclosure: Nothing to disclose.

24.3 Using a Dimensional Measure of Gender Identity to Examine the Influence of Gender Identity on Mental Health in a Community Sample of Youth

Alexandra Potter

University of Vermont College of Medicine, Burlington, Vermont, United States

Background: Transgender and gender nonconforming youth experience staggering rates of mental health problems, including depression, anxiety, self-harm, and suicidality compared to cisgender peers. However most large studies to date have used binary (male/female) or categorical measures of gender limiting understanding of individual differences in gender identity and of relations between gender identity and mental health? We hypothesized that gender identity could be measured dimensionally in a community sample, and that emotional and behavioral problems would increase as gender identity becomes less cisgender.

Methods: Data for this study is from the Adolescent Brain and Cognitive Development Study (ABCD); which is a large, longitudinal study of health and behavior. Children and a parent/guardian are enrolled at ages 9 or 10 and then participate in yearly comprehensive assessments including the Child Behavior Checklist which measures emotional and behavioral problems. At the 1 year follow up assessment, a dimensional measure of gender identity is administered in which youth rate how much they feel like their sex assigned at birth; and how much they feel like the other sex (not assigned at birth). Data from the 1-year assessment ($n = 1{,}652$ youth) were used to examine the influences of gender identity and sex assigned at birth on children's emotional and behavior problems. Hypotheses were tested using general linear modeling and controlled for the influence of site.

Results: Scores on the gender identity measure indicated that youth used the whole range of response options with 81% of youth identified "totally" with their sex assigned at birth and "not at all" with the sex they were not assigned at birth. 0.5% of the youth identified "not at all" with their sex assigned at birth and "totally" with the sex they were not assigned at birth.

Gender identity and sex assigned at birth interacted in their influence on the Child Behavior Checklist Total Problems Score [$F(20,1632) = 4.77$, $p < .0001$; $R\text{-}sq = .055$]; with greater emotional/behavioral problems associated with being less cisgender identified. For male assigned at birth subjects, gender identity was significantly correlated with internalizing, externalizing, and total problems; while for female assigned at birth subjects, gender identity was significantly correlated with externalizing and total problems, but not with internalizing problems. Gender identity for female assigned subjects was associated with rule-breaking problems while for males it was not, and male-assigned subjects had a relationship between gender identity and withdrawn/depressed problems while females did not.

Conclusions: This study demonstrates the feasibility of a dimensional measure of gender identity for youth and demonstrates a relationship between gender identity and emotional/behavioral problems. Thus, a dimensional measure of gender identity may inform mental health across the spectrum of gender identities and not just for transgender youth. Further, there was a different pattern of mental health issues associated with the strength of cisgender identity in male assigned at birth subjects compared to female assigned at birth subjects.

Disclosure: Nothing to disclose.

24.4 Screen Media Activity and Brain Structure in Youth: Evidence for Diverse Structural Correlation Networks From the ABCD Study

Martin Paulus

Laureate Institute for Brain Research, Tulsa, Oklahoma, United States

Background: The adolescent brain undergoes profound structural changes and is influenced by many factors. Screen media activity (SMA; e.g. watching television or videos, playing video games, or using social media) is a common and important recreational activity in children and adolescents; however, its effect on brain structure is not well understood.

Methods: This investigation used a multivariate approach with the first data release from the Adolescent Brain Cognitive Development (ABCD) Study, a multi-site, longitudinal study of 9-10-year-olds, to understand the relationship between screen media activity and structural brain characteristics. Data from participants who had usable structural imaging and SMA data ($N = 4277$ of 4524) were subjected to group factor analysis (GFA) to identify latent variables that relate SMA to cortical thickness, sulcal depth, and gray matter volume. These latent variables were used in generalized mixed models to investigate the effect on internalizing and externalizing psychopathology, as well as fluid and crystalized intelligence.

Results: There were four SMA-related GFAs that explained 30% of the variance. SMA-related GFAs correlated with brain areas that support homologous functions. Some but not all of the factors related to SMA were related to higher externalizing (effect size (ES) 0.06-0.1) but not internalizing psychopathology and lower crystalized (ES: 0.08-0.1) and fluid intelligence (ES: 0.04-0.09).

Conclusions: Taken together, these findings support the notion of maturational coupling or structural correlation networks and shows that SMA is significantly related to brain structure with mixed consequences for psychopathology and cognitive performance.

Disclosure: Nothing to disclose.

Panel

25. Recent Advances in MRI Studies of Schizophrenia

25.1 State- and Trait-Like Functional Imaging Biomarkers for Onset of Psychosis

Hengyi Cao

Yale University, New Haven, Connecticut, United States

Background: Pinpointing neural markers predictive of psychosis in individuals at clinical high risk (CHR) remains a challenge in psychiatry. Using data from the second phase of the North American Prodrome Longitudinal Study (NAPLS) consortium, our lab has previously reported progressively reduced prefrontal cortical thickness as a potential structure-based predictor. This change likely reflects excessive synaptic pruning during neurodevelopment and may further lead to abnormalities in brain functioning. Here, we report recent findings from functional imaging using this dataset and demonstrate that the observed biomarkers can be both state-dependent (i.e., present during a particular task) and state-independent (i.e. present across paradigms).

Methods: We report a total of three studies. The first study investigated baseline brain functional alterations during memory processing in 155 individuals at CHR (including 18 subjects who later converted to psychosis) and 108 healthy controls (HC) using brain activation and brain network analyses. The second study used graph theoretical analysis to examine longitudinal changes in resting-state brain networks in a sample of 72 subjects at CHR (8 converters) and 48 HC. The third study further investigated whether any state-independent biomarkers can potentially predict the onset of psychosis, regardless of paradigm. To this end, principal component analysis and network-based statistic were performed in a sample of 182 subjects at CHR (19 converters) and 120 HC scanned with five different paradigms. Both sexes were included in these studies.

Results: The results showed that 1) Subjects at CHR had significantly higher activation during memory retrieval in the prefrontal, parietal, and temporal cortices ($PFWE < 0.035$), in particular converters. The hyperactivation was correlated with retrieval reaction time during scan ($P = 0.009$), prodromal symptom severity ($P < 0.003$) and memory ability scores ($P < 0.01$), suggesting that memory retrieval related hyperactivation may mark processes linked to conversion to psychosis; 2) There were progressive reduction in global efficiency ($P = 0.006$) and increase in network diversity ($P = 0.001$) in converters compared with non-converters and HC, both with large effect size (Hedge's $g > 1.05$). These alterations were primarily driven by progressively diminished local efficiency in the default-mode network ($P = 0.004$) and enhanced node diversity across all networks ($P < 0.05$). Change rates of both measures were significantly correlated with change rate of cortical thinning in the prefrontal cortex ($P < 0.03$) and visuospatial memory scores ($P < 0.04$). These results suggest that longitudinal reconfiguration of brain networks during resting state may implicate the progression to full psychosis; 3) Individuals at CHR displayed an intrinsic abnormality in functional brain architecture characterized as increased connectivity in the cerebello-thalamo-cortical circuitry, a pattern that was significantly more pronounced among converters ($PFWE = 0.005$). This alteration was independent of fMRI paradigm ($P < 0.05$), significantly correlated with disorganization symptoms ($P = 0.02$) and predictive of time to conversion to psychosis ($P = 0.04$). These findings suggest a state-independent neural signature for psychosis prediction.

Conclusions: The presented data provide the first evidence for several state- and trait-like functional imaging alterations that are prior to and potentially mark the onset of psychosis. Such alterations show potential as biomarkers for psychosis prediction.

Disclosure: Nothing to disclose.

25.2 Heterogeneity of Brain Structure Alterations in Patients With Never-Treated First Episode Schizophrenia

Su Lui

West China Hospital of Sichuan University, Chengdu, China

Background: Schizophrenia is a heterogeneous clinical syndrome. Several recent studies have used cluster analysis to delineate discrete homogenous subgroups of patients within psychiatric disorders. The diverse data obtained from modern MRI neuroanatomic studies may provide the basis for resolving neurobiological heterogeneity within schizophrenia. We studied a large sample of antipsychotic-naïve first-episode schizophrenia (FES) patients to identify patient subgroups based on gray matter

features and compared this clustering with that in a sample of chronic treated patients.

Methods: High resolution 3D T1 structural MRI data were acquired from 163 FES patients and 163 controls, and chronic treated patients from the B-SNIP study (133 patients and 133 controls). Three major anatomical features (cortical thickness, surface area and cortical volume) were extracted. Concatenating 68 regions*3 neuroanatomical features for each subject, a 204 x N matrix was generated. We employed principal component analysis to obtain each principal component and the corresponding eigenvalues. Using Matlab function "pdist" with Mahalanobis distance, we obtained a NxN dissimilarity matrix for a cluster analysis. A density peak-based clustering algorithm was employed to intuitively classify schizophrenia patients into subtypes with distinct neuroanatomical patterns. The topological properties of gray matter connectivity were also analyzed among the different subtypes. All imaging features were compared between subtypes and controls, and the clinical outcome at one year follow up in the FES group was also analyzed.

Results: We found three subtypes of neuroanatomic alterations in the FES sample. Subtype one showed increased cortical surface area and volume especially in lateral orbitofrontal cortex, fusiform and precentral gyri. Subtype two showed increased cortical thickness and volume including orbitofrontal and lateral occipital cortex. Subtype three showed decreased cortical area and volume including cuneus and precentral cortex. The topological properties of gray matter connectivity were also different among the 3 subgroups. Although subgroups did not differ in baseline symptoms, subtype 2 patients showed poorer clinical outcome relative to the other two groups ($p < .05$). Statistically significant similarities and differences of clustering features between FES and BSNIP data were also found and will be discussed.

Conclusions: We show three novel and clinically relevant subtypes of schizophrenia patients with distinct patterns of regional structural alterations and topological properties at illness onset prior to treatment. The subtype with increased cortical thickness and volume at a drug-naïve state showed poorer clinical outcome.

Disclosure: Nothing to disclose.

25.3 Reconsidering the Diagnostic Boundaries of Schizophrenia: Novel Biomarker-Based Approaches to Classification of Psychosis

Elena Ivleva

University of Texas Southwestern Medical Center, Dallas, Texas, United States

Background: One enduring question in schizophrenia (SZ) research is how the neurobiology of nonaffective and affective psychotic disorders differ. The B-SNIP consortium was designed to address this question and to determine whether a profile of biological phenotypes could be used to develop novel classifications which we refer to as Biotypes (Clementz et al., 2015). Presenting our consortium's recent advances in MRI studies will be the focus of this presentation.

Methods: Whole brain structural characteristics were examined in probands, their relatives, and healthy subjects, grouped by DSM-IV diagnosis [SZ, schizoaffective disorder (SAD), psychotic bipolar disorder (BD)] and then by Biotype ($n = 1{,}409$). 3 Tesla T1-weighted images were merged across 6 sites, using rigorous data compatibility protocols, and analyzed via optimized Voxel-Based Morphometry (VBM8/SPM8/DARTEL). Stepwise regression and machine learning analyses examined predictive features of conventional diagnoses and Biotypes.

Results: SZ and SAD showed broadly distributed and strikingly similar gray matter density (GMD) reductions; BD had modest, primarily frontal reductions, compared to controls. Structural changes in relatives followed the regional patterns in probands. Analysis of B-SNIP Biotypes showed step-wise GMD changes: Biotype1 (B1), extensive and diffusely distributed cortical/subcortical reductions; B2, intermediate in magnitude, more localized reductions (the largest effect sizes in insula, frontotemporal cortex); B3, small GMD reductions in anterior limbic cortex. Relatives grouped by Biotype showed regionally distinct GMD changes: B1, primarily anterior/frontotemporal reductions; B2, temporoparietal/cerebellar reductions; B3, normal GMD. Biotype was a strong predictor for total GMD [$F(1,551) = 24.7$, $p < .0001$, $R2 = .043$], and remained significant after diagnosis was added to the stepwise regression model. The diagnosis, when fit first, was a significant predictor for GMD [$F = 6.47$; $p = .011$; $R2 = .012$]. However, when Biotype was added, diagnosis was no longer significant ($p = .18$), while Biotype was ($p < .0001$). The highest classification accuracy (71%) was observed for B1, the group with the most severe cognitive and neurophysiologic deficits.

Conclusions: Conventional diagnoses showed considerable overlap in GMD characteristics, especially in SZ and SAD, suggesting their limited specificity across psychotic disorders. In Biotypes, GMD biomarkers were consistent with their cognitive and neurophysiologic profiles. The Biotypes showed stronger between-group discrimination, predictive function and classification accuracies, based on GMD. Our findings reveal informative differences in the neurobiology of SZ and BD but indicate that the Biotype approach may provide an improved strategy for classifying biologically discrete subtypes of psychotic disorders.

Disclosure: Nothing to disclose.

25.4 White Matter Abnormalities in Never-Treated Patients With Long-Term Schizophrenia

Yuan Xiao

West China Hospital of Sichuan University, Chengdu, China

Background: Several studies have reported progressive cerebral alterations in schizophrenia. However, the degree to which these changes are progressive over the longer-term course of illness and the degree to which they are secondary to antipsychotic treatment remain unclear. Separating influences of these two mechanisms remains challenging because nearly all patients are appropriately treated following diagnosis, and antipsychotic drugs have robust effects on brain anatomy. Comparing never-treated and antipsychotic-treated long-term schizophrenia patients could shed light on neuroanatomic changes over the longer-term course of illness. We aimed to test for differences of white matter integrity between illness duration-matched never-treated and treated long-term schizophrenia patients, and for differential changes in relation to age in these two groups relative to healthy controls.

Methods: In this cross-sectional diffusion tensor imaging (DTI) study, 31 never-treated and 45 matched antipsychotic-treated patients with long-term schizophrenia and 58 healthy controls were included. Routine DTI preprocessing was performed using FSL software. Then, we used Automatic Fiber Quantification software to identify 20 white matter tracts (JHU altas) in individual subjects. Fractional anisotropy (FA) of white matter tracts were extracted and compared among groups using two-way (3 groups $\times$ 20 regions) ANOVAs. Significant effects were followed by post-hoc one-way ANOVAs comparing groups on each tract separately, and then pairwise post hoc tests to determine which of the 3 groups differed significantly. Linear regression analysis was

used to explore the association between age and FA among the three groups.

Results: FA significantly differed among the three groups in 14 of 20 white matter tracts defined in the JHU white-matter template. Never-treated patients displayed greater reduction of FA than antipsychotic-treated patients in left anterior thalamic radiation, left cingulum-hippocampus pathway, splenium and genu of corpus callosum and left superior longitudinal fasciculus, and greater FA in right uncinate fasciculus. Both patient groups showed multiple reductions relative to controls. Never-treated patients showed an accelerated and clinically relevant age-related reduction of FA in the genu of the corpus callosum relative to both treated patients ($F = 16.26$, $P < 0.001$) and controls ($F = 4.60$, $P = 0.035$).

Conclusions: These findings provide insight into the regional distribution of white matter deficits in the years after illness onset in long-term schizophrenia patients without potential confounds of antipsychotic medication. Findings of greater impairments in never-treated than treated patients, and a greater age-related reduction in the genu of the corpus callosum in never-treated patients, suggest that long-term antipsychotic treatment does not adversely affect white matter tracts over the longer-term course of illness, and may via direct and indirect mechanisms confer benefits.

Disclosure: Nothing to disclose.

Panel

26. Optimism in Targeting Microglia and Astrocytes to Treat Psychiatric Disorders

26.1 Astrocyte-Microglial Communication via Innate Immune Signals in the Developing Brain

Anna Victoria Molofsky

University of California, San Francisco, San Francisco, California, United States

Background: Synapses are a key regulated variable in the developing brain and are dysfunctional in neurodevelopmental diseases including autism and schizophrenia. Synaptic pruning is critical to the regulation of synapse numbers and excessive pruning has been implicated in the pathogenesis of schizophrenia. Coincident with this, the immune system has been linked to the pathogenesis of schizophrenia through genetic and epidemiologic studies. Glial cells are the silent majority of the brain and play an increasingly appreciated role in both the formation and pruning of synapses. We have recently shown that two types of specialized glial cells -- astrocytes and microglia -- communicate via the cytokine Interleukin-33 (IL-33) to promote synapse pruning during brain development. Astrocytes adjacent to synapses produce IL-33 to drive synapse engulfment by microglia, which are the only cells that express the IL-33 receptor. We found that this circuit is developmentally required to limit synapse numbers and promote normal neural circuit function and behavior, and that IL-33 deficient animals have abnormal sensorimotor startle behaviors. Therefore, IL-33 signaling represents a mechanism by which immune signals regulate the development of brain synapses, with important implications for neuropsychiatric diseases.

Methods: We use genetically modified mice to detect IL-33 expression and to conditionally delete IL-33 and its receptor from the relevant cell types. We then study the effects of these manipulations on synapse numbers, dendritic spines, and circuit function, and behavior using immunohistochemistry, electrophysiology, among other techniques. Both sexes are studied for all assays and analyzed separately. Most analyses involved comparisons between two groups with normal distributions, these were performed with student's t-test. In some cases, three or more groups were compared (e.g. wild type, heterozygous, and knockout). In these cases, one-way ANOVA with tukey's post-hoc comparison was used for statistical analysis.

Results: In the present study, we investigated the relevance of the IL-33 signaling pathway in the remodeling of synapses in the hippocampus. We found that neurons in the dentate gyrus and CA1 regions of the hippocampus express IL-33, and this neuronal expression can be increased by raising animals in an enriched environment ($p < 0.01$). Conditional deletion of IL-33 from neurons or its receptor from microglia led to a decreased number of dendritic spines on both CA1 ($p < 0.01$) and dentate gyrus neurons, and this effect was more pronounced after environmental enrichment ($p < 0.001$). Ongoing studies investigate the requirement of this signaling pathway for neuronal function and hippocampal dependent anxiety behaviors.

Conclusions: Our data demonstrate that innate immune signaling to microglia is required for normal dendritic spine formation in the adult hippocampus, and that this pathway is required for the synapse-promoting effects of environmental enrichment. These data have important implications for understanding how the immune system regulates synapse turnover in the hippocampus for optimal brain function and learning and could have relevance to understanding psychiatric diseases including schizophrenia, autism, and others.

Disclosure: Nothing to disclose.

26.2 What Makes a Microglia? Measuring the Effects of Origin and Environment on Microglial Identity

Abstract not included.

26.3 P2X7 Receptor Signaling in Psychiatric Disorders: From Synaptic Physiology to Animal Models

Beata Sperlagh

Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest, Hungary

Background: The purinergic P2X7 receptor (P2X7R) is a ligand-gated non-selective cation channel sensitive to high extracellular ATP levels. P2X7Rs are expressed in the central nervous system by microglia, astrocytes and nerve terminals. Whilst under physiological conditions P2X7Rs are thought to be silent, elevation of extracellular ATP during increased neuronal activity, cellular damage or inflammation could activate P2X7Rs and trigger a diversity of actions that might contribute to disease pathophysiology in the CNS. The hallmark of P2X7R activation is that it serves as a maturation signal for post-translational processing and subsequent release of pro-inflammatory cytokines such as IL-1beta, IL-6 or IL-18, through the formation of NLRP3 inflammasome. In addition, P2X7R activation promote the release of neurotransmitters from nerve terminals and astrocytes and stimulate microglial cell proliferation. Previous studies unequivocally demonstrated that genetic deletion or pharmacological inhibition of P2X7R alleviates depression-like behavior, amphetamine-induced hyperactivity and certain aspects of schizophrenia-like behavior in rodents.

Methods: Taken into account the role of P2X7Rs in the modulation of inflammatory response, we examined whether

P2X7R receptors participate in shaping of the autistic-like phenotype in the maternal immune activation (MIA) model of autism spectrum disorder (ASD). Wild-type and P2rx7 gene deficient (P2rx7-/-) pregnant mouse dams were injected with 3 and 1.5 mg/kg of Poly(I:C) i.p. on E12.5 and E17.5 days respectively. Then a battery of behavior tests (three-chamber social interaction test, rotarod, self-grooming, marble burying and open field tests) were performed as well as biochemical and morphological alterations (cerebellar Purkinje cell dropout, synapse ultrastructure) were examined on young adult male offspring.

Results: MIA induced by poly(I:C) injections to wild-type mouse dams elicited an autism-like phenotype in their offspring. These treatment related alterations, i.e. decrease in social preference (Two-way ANOVA, F interaction genotype*treatment(1,39) = 12.044; $p = 0.00128$); impairment of motor coordination (Finteraction genotype*treatment(1, 28) = 5.433; $p = 0.0272$), and increased repetitive behaviors such as self-grooming (F interaction genotype*treatment (1, 38) = 5.1135; $p = 0.0296$) and marble burying (F interaction genotype*treatment (1, 39) = 0.37383; $p = 0.0049$ vs. wild-type, Fischer LSD post hoc test), were not observed in P2rx7 deficient mice. Further, poly(I:C) induced decrease of Purkinje neurons in cerebellar lobe VII, and increased proportion of malformed synaptosomes were not observed either in P2rx7-/- offspring. The effect of P2rx7 gene deficiency were replicated by maternal treatment with the specific P2rx7 antagonist, JNJ7965567 on MIA induced offspring phenotype. Genetic deletion and pharmacological inhibition of maternal P2X7Rs also counteracted the induction of IL-6 in the maternal plasma and fetal brain, and disruption of brain development by poly(I:C). Interestingly, postnatal P2X7R inhibition also alleviated behavioral and morphological alterations in the offspring.

Conclusions: In conclusion, activation of maternal P2X7 receptors is necessary and sufficient to transduce MIA to an autistic phenotype in offspring. Our findings point to the P2X7R-signaling pathway as potential therapeutic target for psychiatric disorders.

Disclosure: Nothing to disclose.

26.4 The P2X7 Ion Channel as Therapeutic Target for Mood Disorders: Drug Discovery to Early Development

Abstract not included.

Panel

27. Glutamate Receptor Plasticity in Cognition and Neuropsychiatric Disorders

27.1 Study of Rare Variants of NMDA Receptor Subunits Associated With Neurological Disorders

Katherine Roche

National Institute of Neurological Disorders and Stroke/National Institutes of Health, Bethesda, Maryland, United States

Background: Synaptic dysfunction is implicated in a variety of neurological disorders. De novo mutations in certain synaptic proteins are pathogenic. In particular, rare variants of two NMDA receptor subunits, GluN2A and GluN2B, underlie some cases of epilepsy and neurodevelopmental disorders. In fact, Grin2A and Grin2B are genes identified as being associated with autism in the Simons Variation in Individuals Project (Simons VIP).

Methods: Disease-associated rare variants of GluN2A and GluN2B were studied using biochemical assays and immunofluorescence microscopy. First, we generated the relevant missense mutations located within the C-terminal domain of GluN2A or GluN2B. These mutant subunits were expressed in dispersed cultured hippocampal neurons and compared to WT subunits in assays of surface expression, endocytosis, and effects on spine density. In addition, we evaluated the binding of these mutants to other synaptic proteins such as PSD-95 family members. Furthermore, for one previously published variant, GluN2B S1415L (Liu, et al., 2017), we generated a knock-in mouse expressing the rare variant and have conducted biochemical experiments to examine expression of synaptic proteins in this mouse.

Results: We find that expression of a rare variant associated with autism (GluN2B S1415L) in hippocampal neurons displays decreased surface expression, impaired NMDA receptor synaptic currents, and decreased spine density (Liu et al., 2017). We have now extended our studies to evaluate this variant in an in vivo model system by generating a knock in mouse. We find that there are significant deficits in synaptic proteins in the PSD fraction from the hippocampus of these mice, but not in cortex indicating region specific-effects on synaptic function. In similar studies on a de novo mutation in GluN2A associated with epilepsy, we find the mutation affects NMDA receptor trafficking and endocytosis. Furthermore, we observe impaired GluN2A association with specific interactors and reduced spine density.

Conclusions: Our studies validate a 'bedside to bench' approach of studying rare variants associated with disease to better understand the function and signaling properties of NMDA receptors. We anticipate our results will allow us to better understand the structure, function and signaling regulated by the C-termini. Furthermore, by characterizing these variants in vitro, in situ, and in vivo, we hope to elucidate common pathways of synaptic dysfunction underlying these neurological disorders that will open up new avenues for discovering therapeutics.

Disclosure: Nothing to disclose.

27.2 Role of Synaptic Cell Adhesion Proteins in Glutamatergic Synapse Plasticity

Robert Malenka

Stanford University School of Medicine, Stanford, California, United States

Background: Synaptic cell adhesion proteins are among the most ubiquitous of the large number of synaptic proteins that are genetically associated with a range of psychiatric disorders. Elucidating their detailed roles in circuit development and mature synaptic function will therefore be of critical importance for understanding the pathophysiology of mental illnesses. Here we use genetic deletion strategies to examine the roles of neuroligins and leucine-rich repeat transmembrane proteins (LRRTMs) at excitatory synapses on hippocampal CA1 pyramidal neurons, arguably the most extensively studied glutamatergic synapses in the mammalian brain.

Methods: Quadruple conditional knockout mice of all four neuroligins and double conditional knockout mice of LRRTM1, 2, the two main LRRTMs found in CA1 pyramidal neurons, were used in these studies. In vivo genetic deletion of neuroligins or LRRTM1, 2 from CA1 pyramidal neurons in young adult mice was accomplished by stereotactic injection of lentiviruses expressing Cre recombinase and EGFP. Rescue experiments were performed using lentiviruses expressing Cre recombinase along with wildtype

or mutant forms of neuroligin 1 or LRRTM2. Two to three weeks later, acute hippocampal slices were prepared and whole cell voltage clamp recordings were made from CA1 pyramidal neurons while stimulating Schaffer collateral/commissural axons in stratum radiatum.

Results: Genetic deletion of either neuroligins or LRRTM1, 2 dramatically impaired NMDA receptor (NMDAR)-dependent LTP while having no detectable effect on NMDAR-dependent LTD. Neuroligin deletion significantly reduced NMDAR-mediated excitatory postsynaptic currents (NMDAR EPSCs) while having no detectable effect on AMPA receptor-mediated EPSCs (AMPAR EPSCs). Deletion of LRRTM1,2 had the opposite effects; resulting in a decrease in AMPAR EPSCs but no detectable effect on NMDAR EPSCs. Surprisingly, the rescue of LTP by neuroligin 1 in the neuroligin knockout cells and by LRRTM2 in the LRRTM1,2 knockout cells did not require the intracellular domains of these proteins but did require their extracellular domains and the binding of these domains to presynaptic neurexins. The spine growth which accompanies LTP was also dramatically impaired by genetic deletion of neuroligins and exhibited the same molecular requirements for neuroligin 1 binding to neurexins as functional LTP. Surprisingly, the rescue of NMDAR EPSCs by neuroligin 1 had a different molecular requirement; it required the intracellular domain of neuroligin 1 but not the extracellular domain binding to neurexins.

Conclusions: At mature glutamatergic synapse on hippocampal CA1 pyramidal neurons, LTP requires both neuroligin 1 and LRRTM2, both of which appear to require binding to presynaptic neurexins to fulfill their roles in LTP. Spine growth during LTP also requires neuroligin 1 binding to neurexins while the maintenance of basal NMDAR-mediated synaptic transmission does not require this molecular interaction but does require the intracellular domain of neuroligin 1. These findings provide a foundation and an approach, which should be useful for understanding how the mutations in synaptic proteins associated with psychiatric disorders influence the synaptic and circuit dysfunction that underlie mental illness symptoms.

Disclosure: Circuit Therapeutics, Inc., Advisory Board; Cerevance, Advisory Board

27.3 Imaging AMPA Receptor Gamma-8 Tarp Expression in Healthy Volunteers: [18F]JNJ-64511070 Pet

David Bredt

Janssen, San Diego, California, United States

Background: The transmembrane receptor regulatory protein TARP gamma-8, which is preferentially expressed in the hippocampus, controls hippocampal AMPA receptor expression, pharmacology and synaptic plasticity. We previously reported preclinical results obtained with the novel AMPA TARP gamma-8 PET tracer, [18 F]JNJ-64511070, and will now disclose first in human imaging results.

Methods: The novel AMPA TARP gamma-8 imaging agent, [18 F]JNJ-64511070 was developed using a series of in vitro assays and its specific binding was evaluated in in vitro autoradiography (ARG) experiments on rat brain tissue sections and through in vivo imaging in rats and non-human primates in the absence and presence of JNJ-284, a selective AMPA receptor gamma-8 negative modulator. A Phase 0 exploratory study of [18 F]JNJ-64511070 was initiated with the goal of evaluating the safety of a single injection of the tracer and to visually and quantitatively assess brain uptake and pharmacokinetics of [18 F]JNJ-64511070 in healthy subjects.

Results: JNJ-64511070 is a highly potent (IC50 for human AMPA receptor gamma-8 TARP, FLIPR™ is 0.05 nM) and selective PET ligand. Rat and non-human primate PET imaging demonstrated the expected uptake pattern in the hippocampus and frontal cortex, which was completely blocked by JNJ-284. We report results from human [18 F]JNJ-64511070 PET scans in five healthy subjects. Dynamic PET scans were acquired for 120 minutes with an arterial input function. The tracer was generally well tolerated, with excellent initial brain uptake (average cortical SUVpeak 2.5). Regional volume of distribution (VT) was estimated for 1 and 2 tissue compartment models, the latter provided the best fit. Hippocampus and amygdala had the highest VT; thalamus and brain stem the lowest. Metabolism for the tracer was slow and showed low variability between

Conclusions: The initial clinical evaluation of [18 F]JNJ-64511070 suggests that this tracer is a suitable candidate PET ligand for AMPA receptor gamma-8 TARP and supporting its use for clinical target engagement studies and as a biomarker of synaptic density and plasticity.

Disclosure: Johnson and Johnson, Employee

27.4 The NMDAR/CaMKII Complex is the Master Synaptic Signaling Hub

Abstract not included.

Study Group

28. Medical Cannabis From a Neuroscience Perspective: How Did We Get Here and Where Should We Go?

Bryon Adinoff, Ziva Cooper, Matthew Hill, Rosalie Liccardo Pacula, Marcel Bonn-Miller*

University of Colorado Medical School, United States

Study Group Summary: The medical cannabis (medicinal marijuana) debate arises not so much from cannabis' therapeutic effectiveness as it does from our values, politics and culture. This controversy is particularly relevant to the ACNP. The ACNP and its members have long-standing partnerships with government and pharmaceutical organizations to develop medications showing promise for the treatment of psychiatric disorders. These pharmaceuticals undergo a rigorous examination of safety and efficacy prior to FDA approval. Legalization of medical cannabis by (to-date) 29 states and Washington DC (plus many others providing protections for the use of cannabinol) has bypassed this federal mandate. The conflict between state and federal regulations, coupled with variable between-state approaches, has resulted in an unstable legal and medical environment.

Whether approving medical cannabis through a popular vote or legislation is beneficial or harmful remains uncertain. Regardless, there are ethical and scientific issues that merit discussion. Furthermore, the shifting federal political environment will likely heighten the controversy surrounding issues related to medicinal use of cannabis.

This Study Group will explore the following topics:

- Has the Schedule I status of cannabis and its individual constituents hampered research into its clinical utility? If so, should the status be changed? How might recent proposed legislation to remove certain cannabinoids from DEA scheduling affect cannabis research?
- What are the implications of medical cannabis legalization for our system of pharmaceutical evaluation/regulation in general?

- How should the opioid crisis inform our view of medical cannabis?
- How can translational neuroscience guide cannabis policy and our understanding of the potential therapeutic utility and adverse effects associated with cannabinoids?
- What is the most responsible path forward, given the increasing public support favoring both medical and recreational cannabis and the rapidly changing political landscape, while also keeping in mind the public health concerns related to legalization?

Our panel consists of leading scientists in cannabis research, with expertise in translational research, health policy and clinical trials of pharmaceutical cannabis. Ziva Cooper's human translation laboratory utilizes double-blind, placebo-controlled studies to understand variables that impact both the therapeutic and adverse effects of cannabis and cannabinoids. Marcel Bonn-Miller has extensively studied the therapeutic effects and negative consequences associated with cannabis use in psychiatric populations, including the specific effects of individual cannabinoid constituents therein. Matt Hill's preclinical laboratory examines the neurobiology of endocannabinoid signaling in the brain and how it regulates stress and anxiety. His work demonstrates how basic science can help to create a framework to understand the potential mechanisms by which cannabis can influence neural function and behavior. Rosalie Liccardo Pacula, a health economist, assesses the supply and demand for intoxicating substances, including cannabis and opioids, and the responsiveness of these medical and non-medical markets to supply and demand shocks, prices, and the relative effectiveness of various government policies. Bryon Adinoff will moderate the study group.

Disclosure: Journal Editor, Board Member; EnLiSense LLC, Consultant; DemeRx, Board Member

Panel

29. Understanding Cognition in Schizophrenia - Perspectives on Causes, Circuitry Dysfunction, Course and Cures: Building Upon Dr. Larry Seidman's Work

29.1 Cognitive Pathways to the Development of Psychosis and the Course of Schizophrenia

William Stone

Harvard Medical School/Beth Israel Deaconess Medical Center, Boston, Massachusetts, United States

Background: Larry Seidman was among a handful of researchers in the 1980's who established the core relationships between cognitive dysfunction and schizophrenia illness. His 40 years of work in the field helped to establish not only the significance of cognition in producing disability but the developmental and diagnostic roles they played in the development of psychosis. This presentation demonstrates the continuation of those efforts and the exciting new findings they are continuing to yield by focusing on recent and as yet unpublished NIMH-supported findings from the North American Prodrome Longitudinal Study 3 (NAPLS 3) and from a study of cognition in untreated psychosis underway in Ningxia, China.

Methods: Adolescents and young adults of both genders in NAPLS 3 met diagnostic criteria for clinical high risk based on the Structured Interview for Prodromal Syndromes (SIPS) and other measures. Between 200 and 400 subjects were available for the current analyses. Potential subjects in the Ningxia study were identified through the Provincial Health Registry and assessed for schizophrenia with the SCID. Data from 100 to 150 subjects were available for these analyses in each of the untreated, treated (with antipsychotic medication) and the control (non-schizophrenic) groups. Subjects in both studies received portions of the MATRICS Cognitive Consensus Battery (MCCB). Data were analyzed with ANOVAs, ANCOVAS and repeated measures designs.

Results: All findings reported were significant at $p < 0.05$. The NAPLS 3 results showed both stable cognitive deficits compared to controls in the clinical high risk (CHR) subjects and also additional cognitive deficits that differentiated CHR subjects who developed psychosis (i.e. 'converted') from those who did not. Tests that emphasized processing speed and learning were particularly sensitive in distinguishing converters from non-converters. Results from the Ningxia study showed that untreated subjects with schizophrenia performed worse than treated and control subjects in most cognitive measures. Subjects with greater durations of untreated psychosis showed declines in the same MCCB measures that best distinguished converters from non-converters in the NAPLS 3 study.

Conclusions: These findings show that certain cognitive measures or domains predict both the development of psychosis in CHR and continued decline in untreated psychosis. The findings in these novel data sets show that some cognitive trajectories are more sensitive than others in predicting the natural course of schizophrenia and may provide particularly important treatment targets for therapeutic interventions.

Disclosure: Nothing to disclose.

29.2 Aberrant Modular Organization of the Functional Connectome Predicts Conversion to Psychosis in Clinical High-Risk Youth From the Sharp Program

Guusje Collin

Harvard Medical School, Beth Israel Deaconess Medical Center, Boston, Massachusetts, United States

Background: The prodromal or Clinical High-Risk (CHR) phase of schizophrenia is the period of imminent risk for psychosis when subthreshold psychotic symptoms first emerge and cognitive function declines. The CHR phase typically occurs in adolescence or young adulthood, a period of late brain development when major changes take place in the neural circuits supporting higher integrative functions. During this critical window of cognitive system development, functional changes in brain connectivity and organization may have a particularly profound and lasting impact on cognitive and clinical outcome in at-risk youth. In this study, we examine whether abnormal functional brain organization precedes psychosis onset.

Methods: A total of 251 subjects, including 158 CHRs and 93 age- and sex-matched healthy controls (HCs), participated in this study. During a mean (sd) follow-up of 392 (77) days, 23 CHRs developed psychosis (converters), while 135 did not (non-converters). Prodromal symptoms and cognition at baseline were assessed using validated procedures. Structural and functional MRI scans were acquired and processed using Freesurfer v6.0 and CONN v17.d software. The resulting functional connectome maps were examined using graph theoretical analysis, focusing on modular brain network organization.

Results: The modular organization of the functional brain network was abnormal in CHR converters, but not in CHRs non-converters (overall group effect $F(2, 245) = 4.08$, $p = .018$). Using Kaplan Meier analysis, CHRs with abnormal modular connectome organization at baseline were found to show worse psychosis-free survival ($z = 2.41$, $p = 0.016$), with a Hazard ratio of 3.1 indicating a three-fold conversion rate. Cox regression showed that abnormal brain network organization ($z = -2.37$, $p = .018$), lower IQ ($z = -2.48$, $p = .013$), and being male ($z = 1.92$, $p = .036$) predicted shorter time to conversion.

Conclusions: Abnormal functional organization of the brain network precedes the onset of psychosis and is associated with increased conversion rates in CHR subjects. Our results provide new insights into functional mechanisms on the connectome level that may underlie psychosis development. Moreover, finding that brain network organization is predictive of conversion is critically important to efforts to improve early detection and prevention of psychosis and the quest to identify biomarkers for transition to psychosis.

Disclosure: Nothing to disclose.

29.3 Cerebello-Thalamo-Cortical Hyperconnectivity: A State-Independent Functional Biomarker for Psychosis Prediction

Tyrone Cannon

Yale University, New Haven, Connecticut, United States

Background: Understanding the fundamental alterations in brain function that lead to schizophrenia remains a major challenge in clinical neuroscience. Recent work suggests that the brain possesses an intrinsic and state-independent functional architecture, and functional networks during different paradigms are shaped primarily by this "standard" architecture and secondarily by paradigm-specific features. Here, using multi-paradigm fMRI data from two independent cohorts, we investigated whether common functional network abnormalities shared across different paradigms predict the onset of psychosis among those in a prodromal state.

Methods: A total of 182 male and female subjects at clinical high risk (CHR), including 19 who later converted to psychosis, and 120 demographically similar controls completed a battery of five fMRI paradigms at baseline evaluation: resting state, verbal working memory, episodic memory encoding, episodic memory retrieval, and emotional face matching. We constructed whole brain connectivity matrices representing the pairwise connectivity between 270 nodes for each individual during each paradigm. Individual-level principal component analysis was performed on these connectivity matrices, followed by connectome-wide network-based statistics to discern any differences by outcome group.

Results: The first principal component scores explained ~70% of the total variance in the connectivity matrices across paradigms, to an equivalent degree across outcome groups. Controlling for age, sex, IQ, site, mean frame-wise displacement, and antipsychotic dosage, individuals at CHR displayed an intrinsic "trait-like" abnormality in functional brain architecture characterized as increased connectivity in the cerebello-thalamo-cortical circuitry, a pattern that was significantly more pronounced among converters compared with non-converters ($PFWE = 0.005$). This alteration was correlated with disorganization symptoms and predictive of time to conversion to psychosis in CHR cases. As a test of robustness, we examined the presence of this connectome-based biomarker in an independent cohort including 50 patients with schizophrenia, 49 patients with bipolar disorder, 40 patients with attention-deficit hyperactivity disorder and 123 healthy controls. The same hyperconnectivity pattern predictive of psychosis in the CHR sample was reliably detected and specifically present in patients with schizophrenia.

Conclusions: These findings implicate cerebello-thalamo-cortical hyperconnectivity as a robust state-independent neural signature for psychosis that can be ascertained in those with schizophrenia and those at risk prior to onset of psychosis. These results appear broadly consistent with the "cognitive dysmetria" theory of schizophrenia, which holds that dysfunction of the cerebello-thalamo-cortical circuitry results in impaired synchrony or coordination of mental processes. Given the cerebellum's role as a general "error detection and correction" center, which receives, integrates and computes error information regarding both movement and thought from the cerebral cortex and provides adaptive feedback via the cerebello-thalamo-cortical circuitry, the hyperconnectivity in this circuitry among those with and at risk for psychosis may reflect excessive error input from the upstream cerebral cortex.

Disclosure: Boehringer Ingelheim Pharmaceuticals, Consultant; Lundbeck A/S, Consultant

29.4 Cognitive Enhancement Approaches in Early Course Psychotic Disorders: Efficacy and Mechanisms

Matcheri Keshavan

Harvard University, Boston, Massachusetts, United States

Background: Cognitive deficits are a core aspect of schizophrenia and underlie the functional disability in this illness. As shown by seminal studies led by late Prof. Larry Seidman, cognitive deficits in schizophrenia are persistent, being present in the prodromal and early phases of this illness. These deficits do not respond optimally to pharmacological interventions.

Methods: I review here systematically the existing literature on efficacy and therapeutic mechanisms underlying cognitive enhancement therapy (CET) in the early phase of psychotic disorders and in individuals at high risk for psychosis. We also adapted CET to clinical high risk (CHR) populations. This manualized approach (Cognition and Learning for Use in Everyday Situations, CLUES) was shortened (to 6 from 18 months), made more developmentally relevant, and included principles of acceptance and commitment therapy (ACT). We have completed an open label study ($n = 17$) in CHR.

Results: Our prior work and the extant literature as well as preliminary results from an ongoing Independent randomized clinical trial showed that CET is effective, compared to supportive therapy in early course psychotic disorders in social cognition, neurocognition and social adjustment ($n = 104$; effect sizes .5-.8; $p < .05$- .01). CLUES showed promising results on feasibility and efficacy on global social function ($t(16) = -4.20$, $p = .001$, $d = 1.02$).; and psychomotor speed ($t(15) = 2.09$, $p = .054$, $d = .52$). Of the 17 participants, 15 continued to meet CHR criteria at follow-up, and two experienced a remission of CHR symptoms. None of the participants experienced a transition to full psychosis.

Conclusions: Cognitive enhancement interventions are effective, and have an impact on functioning in the early course of psychotic disorders and in CHR. Their effects are based on the principles of experience dependent neuroplasticity. Further studies are needed to confirm these observations and identify potential predictors of treatment response.

Disclosure: Nothing to disclose.

Study Group

30. I Can't Wait: Challenges and Successes in Translational Research on Impulsivity

Harriet de Wit, Kate Nautiyal, Catharine Winstanley, Valerie Voon, James Jentsch, Anna Konova, Martin Paulus, Carlos Blanco*

University of Chicago, United States

Study Group Summary: This study group will address key challenges in translational research on impulsivity. Understanding the behavioral and neurobiological basis of impulsive behavior is essential for understanding psychiatric disorders such as substance use disorder, gambling disorder, ADHD, and suicide. High impulsivity is a known risk factor for drug abuse and is likely to contribute to other disorders. However, the relationship between impulsivity and drug abuse is complex, and depends on the subtype of impulsivity (choice versus action), its time course (state versus trait), the phase of drug use (escalation versus abstinence), age (adolescent vs adult), and substance (stimulants versus depressants). Recent developments in both the methodology used to assess impulsive behavior, and the findings from preclinical and clinical studies, have advanced our understanding of how impulsive tendencies both lead to, and result from, drug use. In this study group basic (animal) and clinical (human) researchers will come together to discuss recent advances in this field, to identify gaps and sources of inconsistences, and to identify avenues for improving the translatability of preclinical and clinical research.

The goals of this study group are to: (1) Understand the commonalities and inconsistencies across measures of impulsivity, including self-report and behavioral tests in humans, animal paradigms, and computational models. (2) Identify areas for improvement to generate meaningful comparable results from bench to bedside. (3) Brainstorm about how we can move translational impulsivity research forward by developing meaningful parallel paradigms and tools.

Harriet de Wit (University of Chicago) is a leading expert in the measurement of impulsive behavior in humans and will chair the Study group. Catharine Winstanley (University of British Columbia) has expertise in developing and using rodent models of impulsive behavior and will act as the moderator. Katherine Nautiyal (Dartmouth College) will discuss some ways in which current animal models fail to represent human behavior and make back-translating difficult. Valerie Voon (University of Cambridge) will discuss her successful experience translating her impulsivity work from rodents to humans. J. David Jentsch (Binghamton University) will share how he has utilized a cross-species approach, integrating analyses of impulsivity in laboratory rodents and non-human primates. Anna Konova (New York University) will contribute her work incorporating measures and models of impulsivity in the clinical setting to aid in the treatment monitoring and prognosis of abstinence. Martin Paulus (Laureate Institute for Brain Research) will review computational components of impulsivity using the stop signal task. Finally, Carlos Blanco (National Institute on Drug Abuse) will discuss NIDA's translational efforts.

Disclosure: Insys Therapeutics, Grant

Panel

31. Novel Pharmacological Targets for Neuropathic Pain: Beyond Opioids

31.1 Nociceptor Translational Profiling Reveals MNK1-EIF4E Signaling as a Novel Target for Treatment of Neuropathic Pain

Theodore Price, University of Texas at Dallas, Richardson, Texas, United States

Background: Neuropathic pain is a devastating disease with poor treatment outcomes. Existing treatments produce only minor pain relief in patients. Chemotherapy-induced peripheral neuropathy (CIPN) is the major dose-limiting side effect of cancer treatment. No drugs are approved to treat this form of pain. Nociceptors, are the cellular origin of pain caused by injury to nerves. The dynamics of nociceptor gene expression in painful neuropathy at the genome wide scale is not known. We used translating ribosome affinity purification (TRAP) to comprehensively characterize up- and down-regulated mRNA translation in Scn10a (Nav1.8)-positive nociceptors in CIPN.
Methods: We employed the TRAP technology, using Nav1.8Cre mice to create sensory neuron-specific ribosome tagging with enrichment in the nociceptor population. We used these Nav1.8-TRAP mice to identify transcripts associated with ribosomes specifically expressed in nociceptors isolated from animals with or without CIPN. Four biological replicates per condition were used for profiling. We used behavioral methods in transgenic mice and with pharmacological treatments to determine the efficacy of MNK1-eIF4E signaling in CIPN.
Results: We find ~250 mRNAs with increased translational efficiency and about 100 with decreased translational efficiency in nociceptors in CIPN. An underlying mechanism driving these changes in mRNA translation is a sustained mTORC1 activation driven by MNK1-eIF4E signaling. RagA, a GTPase controlling mTORC1 activity, is a novel target of MNK1-eIF4E signaling, demonstrating a new link between these distinct signaling pathways that is strongly induced by CIPN. RagA translation is strongly attenuated (~75%) by genetic ablation of eIF4E phosphorylation, MNK1 elimination or treatment with the MNK inhibitor eFT508. All of these genetic manipulations also reduce CIPN pain behaviors and treatment with eFT508 almost completely eliminates behavioral signs of CIPN.
Conclusions: Our work demonstrates dysregulation of the mTORC1 signaling network in CIPN that reveals a complex interplay between eIF4E-mediated translation control of RagA and mTORC1 function. Our work shows that in the context of CIPN, increased eIF4E phosphorylation leads to enhanced RagA protein levels driving mTORC1 activation and neuropathic pain. Our findings demonstrate that MNK and eIF4E phosphorylation can be targeted genetically or with eFT508, a drug in late phase clinical trials, to prevent or reverse CIPN pain. These discoveries suggest that MNK inhibitors can be used for the treatment of neuropathic pain.
Disclosure: CerSci Therapeutics, Stock / Equity; Ted's Brain Science, Board Member; Merck, Grant

31.2 Novel Melatonin MT2 Receptor Ligands in Neuropathic Pain

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McGill University, Montreal, Canada

Background: Melatonin (MLT), is a neurohormone acting on two G-protein coupled receptors called MT1 and MT2 receptors. Recent studies have attempted to better characterize the functions of these receptors and exploit their therapeutic effects, by synthetizing selective ligands. Considerable evidence has suggested the MT2 receptor may be implicated in the analgesic functions of the melatonin system and are localized in brain areas implicated in the control of pain. Here, we determined the effects of the novel selective MLT MT2 receptor partial agonist N-{2-([3-bromophenyl]-4-fluorophenylamino)ethyl} acetamide (UCM924) in neuropathic pain models in rats and examined its mechanism of action.

Methods: Rat L5–L6 spinal nerve ligation (SNL) and spared nerve injury (SNI) models were used to evaluate neuropathic pain and in vivo electrophysiology recording of ON and OFF cells of the periaqueductal grey (PAG) projecting the in rostroventralmedulla (RVM) were recorded to determine the mechanism of action. Moreover, opioid agonists and antagonists were used to understand how the melatoniergic system interacts with the opioid system.

Results: In SNL and SNI models, UCM924 (20-40 mg/kg, subcutaneously, s.c) produced a prolonged antinociceptive effect that is: (1) dose-dependent and blocked by the selective MT2 receptor antagonist 4-phenyl-2-propionamidotetralin, (2) superior to a high dose of MLT (150 mg/kg, sc) and comparable with gabapentin (100 mg/kg, sc), but (3) without noticeable motor coordination impairments in the rotarod test ($n = 6$-7 for each experimental group). Using in vivo electrophysiology, combined with tail flick, we observed that microinjection of UCM924 into the ventrolateral PAG decreased tail flick responses, depressed the firing activity of ON cells, and activated the firing of OFF cells ($n =$ 6-7 for each group, $p < 0.001$); all electrophysiological effects were MT2 receptor–dependent since blocked by the MT2 selective antagonist.

Further experiments suggest that the MT2 receptor and μ opioid receptor (μOR) are both localized in the PAG and MT2 receptor interacts with μOR: indeed, in vivo analgesic effects of UCM924 are blocked by the μOR antagonist CTOP (D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH2, 10 μg, intra-PAG, $p < 0.001$) and naloxone (1 mg/kg, sc or 10 μg intraPAG, $p < 0.001$), but not by the delta opioid receptor ∂OR antagonist naltrindole (10 μg intra-PAG), in the SNI model. Moreover, using in vivo electrophysiology, CTOP prevented UCM924 from inhibiting the ON cells and activating the OFF cells neurons of the descending antinociceptive pathways. By using in vivo electrophysiology of VTA dopaminegic neurons we also found that UCM924 (1-20 mg/iv) does not produce activation of the dopaminergic rewarding system, unlike other substances of abuse.

Conclusions: Altogether, these data demonstrate that selective MT2 receptor partial agonists have analgesic properties through modulation of brainstem descending antinociceptive pathways and indirectly interact with the opioid system. MT2 receptors may represent a novel target in the treatment of neuropathic pain.

Disclosure: McGill University, Patent; Cosmas Therapeutics, Stock / Equity

31.3 Role of N-Palmitoylethanolamide (PEA) and N-Acylethanolamines in the Molecular and Neuronal Mechanisms of Neuropathic Pain

Livio Luongo

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Background: The endocannabinoid system, originally described as a signaling system encompassing the cannabinoid CB1 and CB2 receptors, their endogenous agonists (the endocannabinoids), and metabolic enzymes regulating the levels of such agonists, is now viewed as being more complex. The function and dysfunction of this signaling system in the molecular and cellular mechanisms of pain transduction has been widely studied over the last two decades. In this study we have evaluated the capability of the N-palmitoylethanolamide (PEA), an endogenous lipid mediator, to modulate the neuropathic pain-associated sensorial and neuropsychiatric dysfunctions.

Methods: Spared nerve injury (SNI) mouse model of neuropathic pain was used to assess pain responses (Dynamic Plantar Aesthesiometer test, Plantar test), learning and memory tasks (Y-maze test, Novel Object Recognition Test, Morris Water Maze test) and neuropsychiatric behavior (Tail suspension test and Marble Burying test). In addition, long-term potentiation (LTP) in vivo experiments were performed by stimulating the lateral enthorinal cortex (LEC) and recording the EPSPs of granule cells in dentate gyrus (DG) of the hippocampus. Moreover, glutamatergic synapses were analyzed by measuring extracellular Glutamate and GABA levels in DG (Microdialysis in vivo), AMPA and metabotropic glutamate receptor (mGlu) receptors expression (Western Blot analysis) and synaptogenesis in the DG (Electron microscopy and Immunofluorescence assays) were also explored. Finally, endocannabinoid levels were quantified (LC-APCI-MS) and CB1R/β-arrestin2 co-localization in the entorinal cortex was analyzed. All the experiments were carried out in Sham (false operated) or SNI mice, treated with vehicle or with PEA (10 mg/Kg, i.p. 15 days), starting 15 days after SNI induction. All the data were repeated onto the PPARαnull mice, to investigate a possible mechanism by which PEA exerts its effect.

Results: We found that PEA reduced the tactile allodynia, restored depressive-like behaviour without affecting anxiety-related behaviour in SNI mice. Moreover, we found deep impairment in both spatial and discriminative memory tasks accordingly with the compromised LTP in the LEC-DG pathway 7-14 and 30 days after injury. PEA chronic treatment significantly improved behavioural and synaptic flexibility. The SNI also decreased the postsynaptic density, the volume and dendrite arborization of DG whereas it increased mGluR1, mGluR7 and AMPA receptor GluR1 subunit and the levels of glutamate in the DG. The quantity of the endocannabinoid 2 arachidonoylglycerol (2-AG) was increased in the LEC after SNI. In this case, PEA restored the level of glutamate and the expression of phosphorylated GluR1 subunits, postsynaptic density and neurogenesis in DG in SNI animals. Finally, PEA mostly failed to show its neuroprotective effect in PPARα null mice, adding further support to the key role of PPARα signaling pathway in pain responses and the related cognitive impairment.

Conclusions: All together these data highlight a possible role of the PPARα receptors in the brain plasticity associated with neuropathic pain and that the PEA could be a great candidate in pain treatment at least as an adjuvant therapy for reducing opioid or other analgesic drugs dosage.

Disclosure: Nothing to disclose.

31.4 A3 Adenosine Receptor Agonists (A3AR) as Non-Narcotic Analgesics for the Treatment of Neuropathic Pain

Abstract not included.

Panel

32. Harnessing the Cannabinoid System for Psychosis Treatment

32.1 Endocannabinoid Metabolism in Early Psychosis

Romina Mizrahi

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Background: The endocannabinoid (eCB) system modulates brain responses to factors affecting psychosis risk and relapse including cannabis and stress. To date, in vivo imaging of the eCB system in psychosis is limited to the CB1 receptor, which is altered in psychosis. No other component of the eCB system has been investigated in the brain in vivo in psychosis. We aim to investigate the eCB system in schizophrenia patients using positron emission tomography (PET) imaging with [11 C]CURB, a ligand for Fatty Acid amide Hydrolase (FAAH), the enzyme responsible for setting brain levels of anandamide. An exploratory aim is to investigate whether FAAH is related to symptom severity.

Methods: In this pilot study, we recruited 13 schizophrenia patients (age: 26.3 ± 6.1 years) and 24 healthy control (age: 23.9 ± 4.5 years) participants. Healthy controls had no current or past DSM IV axis I diagnosis and no family history of psychotic disorders. All participants tested negative for drugs of abuse including cannabis.

Each participant underwent a 60-minute [11 C]CURB PET scan on a high-resolution PET scanner and a structural MRI scan. [11 C]CURB binding (λk3) was calculated using an irreversible 2-tissue compartment model with a metabolite-corrected arterial plasma input function. We determined FAAH rs324420 genotype for all participants given that carriers of the rs324420 minor A-allele exhibit lower levels of FAAH. Data were analyzed using a linear mixed model with group (2 levels) and region (10 levels) as predictors, controlling for rs324420 genotype. Seven schizophrenia patients were taking antipsychotic drugs at any dose (mean: 387.8 ± 459.3 chlorpromazine equivalents) all others were antipsychotic-free at the time of the [11 C]CURB PET scan.

Results: Results of linear mixed model analysis revealed that [11 C]CURB was lower in schizophrenia patients than in healthy controls ($F(1,33) = 6.27$, $p = .017$). Results of planned contrasts revealed reductions of FAAH in schizophrenia in the striatum ($p = .012$; Cohen's $d = 0.96$), amygdala ($p = .018$; Cohen's $d = 0.91$), and DLPFC ($p = .036$, Cohen's $d = 0.70$). Post hoc tests (adjusted for 7 regions) revealed that FAAH was also lower in occipital lobe ($p = .042$). After controlling for sex and genotype, reductions in FAAH in schizophrenia remained significant overall ($p = .050$) and in amygdala ($p = .036$), but not in striatum ($p = .065$) and DLPFC ($p = .204$). Lower [11 C]CURB binding in the striatum was associated with greater severity of positive psychotic symptoms ($r = -.573$, $p = .041$) on the positive and negative syndrome scale, controlling for genotype. In patients, lower [11 C]CURB binding in the medial prefrontal cortex was associated with greater self-rated anxiety ($r = -.618$, $p = .032$), controlling for genotype.

Conclusions: Data from this pilot sample provide the first in vivo evidence that FAAH is altered in schizophrenia. FAAH was lower in schizophrenia than in healthy controls. Within schizophrenia, lower FAAH was associated with greater pathology, supporting a link between reduced FAAH levels and disease severity. An expanded sample is required to better understand the involvement of eCB-metabolizing enzymes in psychosis.

Disclosure: Nothing to disclose.

32.2 Cannabidiol, Endocannabinoids and Dopamine

Abstract not included.

32.3 Cannabidiol in the Treatment of Early Psychosis

Abstract not included.

32.4 Safety and Effectiveness of Cannabidiol in Psychosis

Abstract not included.

Panel

33. Psychiatric Neurogenetics: Genetics-First Deep Phenotyping as a Lens into the Biology of Common Psychiatric Disorders

33.1 Probing Genomic Variation in 22q11.2 Affecting Brain-Behavior Phenotypes of Social Processing in Human and Mouse Model

Raquel Gur

University of Pennsylvania, Philadelphia, Pennsylvania, United States

Background: Rare CNVs are implicated in neuropsychiatric disorders. 22q11DS confers ~25% risk for schizophrenia (SZ). There is need to: 1. Examine youth with 22q11DS for psychotic symptoms (PS), neurobehavioral and neuroimaging measures compared to idiopathic PS and typically developing (TD). 2. Link human brain circuitry of social processing to a mouse model of 22q.

Methods: We examined PS, neurobehavioral and neuroimaging parameters in 22q11DS ($n = 331$), idiopathic PS ($n = 2,083$) and TD ($n = 2,906$). Multimodal neuroimaging included measures of brain structure and function were available on subsamples. In 22q mice ($n = 12$) and WT ($n = 16$), social function was measured on multiple tasks and virally transfected, neuron-specific DREADDs were used to induce or salvage deficits detected.

Results: PS in 22q11DS was similar to idiopathic PS, with negative symptoms preceding the positive symptoms. Social processing provided the best predictors for PS in both samples. MRI showed lower gray matter volume in cortical and subcortical regions, including hippocampus (p-value = 0.002); reductions in hippocampal gray matter were associated with psychotic symptoms. DTI showed aberrant WM microstructure across patient groups with specific deficits associated with psychosis features. A GWAS on mean hippocampal volumes in 22q11DS identified two globally significant loci (p-values < 9-10), SEMA5B and ASTN2, both with known roles in neuronal migration. Mice with 22q deletion showed specific deficits in social discrimination, associated with a hyperactive ventral CA1. Excitatory DREADDs in ventral CA1 recreated social discrimination deficits in WT mice while inhibitory DREADDs in this brain area rescued social discrimination performance in deleted mice.

Conclusions: Psychotic features are common in 22q11DS with onset and time course resembling idiopathic PS. Neurocognitive impairment is likewise similar, with social processing deficits being markers of psychosis features. The neuroimaging measures indicate aberrant brain parameters with temporolimbic abnormalities associated with social deficits. The mouse model strengthens the link between aberrant limbic functioning and psychosis.

Disclosure: Nothing to disclose.

33.2 Estimating and Predicting the Effect of Copy Number Variants on Cognition and Behaviour

Sébastien Jacquemont

University of Montreal, Sainte Justine Research Center, Montreal, Canada

Background: With the routine implementation of whole genome chromosomal microarrays and exomes in the clinic, "pathogenic" Copy Number Variants (CNVs) and single nucleotide variants (SNVs), are currently identified in 15 to 20 % of children with NDs. It is well known that rare deleterious mutations affect general intelligence but data quantifying the effect-size of rare variants on IQ are only available for a handful of genes and genomic loci including deletions and duplications of the 16p11.2, 22q11,2 15q11.2 loci.

In addition, most "pathogenic" CNVs and SNVs are "non-recurrent." Because they are observed only once or a few times in patients, it is impossible to reach the statistical power required for individual association studies.

Methods: To address this issue of undocumented CNVs and SNVs, we propose a novel strategy. Instead of conducting individual association studies for each mutation, we propose to model effects sizes of rare CNVs on cognitive, behavioral and neuroimaging traits relying on genetic and functional annotations.

We investigate several general population ($n = 20000$) and autism cohorts ($n = 5000$) to train linear and non-linear models. CNV calling was performed for all cohorts using data from genotyping arrays. CNVs were called using a combination of 2 softwares: PennCNV and QuantiSNP.

CNVs are scored using intolerance to haploinsufficiency metrics, brain expression measures (including the Differential Stability score and a developmental brain trajectory transcription score), gene lists (e.g. postsynaptic density of the human cortex). Non-coding regions are annotated using eQTLs regulating genes expressed in the brain.

General intelligence was measured using different IQ tests. Autism diagnosis and abnormal behavior were measured using standardized assessments and questionnaires.

Results: The effect-size of rare deletions on IQ can be modeled and estimated using several haploinsufficiency scores. Results are consistent across all cohorts, regardless of clinical manifestations and unaffected by sensitivity analyses. There is a 0.75 concordance between the effect size on IQ estimated by our model and IQ loss calculated in previous studies of 15 recurrent CNVs. There is a close association between effect size on IQ and the frequency at which deletions occur de novo. We also successfully modeled the genome-wide effect of duplications on IQ using the same strategy.

The same strategy was extended to the effect size of non-recurrent CNVs on autism risk and Autism-related behaviors.

Conclusions: Models trained on non-pathogenic CNVs in the general population reliably estimate the effect size of pathogenic CNVs and suggest omnigenic association of CNVs with IQ. It represents a new framework to study variants too rare to perform individual association studies and can help estimate the effect size on cognition and behavior of undocumented CNVs identified in patients referred to the neurodevelopmental clinic. Algorithms developed in this project are available as online tools to help clinicians quantify the contribution of rare variants to the neurodevelopmental symptoms in their patients.

Disclosure: Nothing to disclose.

33.3 Contrasting Behavioral and Brain Phenotypes in the 7q11.23 Hemizygous Deletion of Williams Syndrome and the 7q11.23 Duplication Syndrome: From Genes to Neural Circuits to Behavior and Back Again

Karen Berman

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Background: Williams syndrome (WS), an extremely rare condition, caused by hemideletion of some 1.6 megabases at chromosomal band 7q11.23 has a characteristic profile of hypersociability combined with differential impact on cognitive functions—some, such as language and face processing, preserved or only mildly affected, while others, particularly visuospatial construction, are severely impaired. Individuals with duplication of these same 7q11.23 genes (Dup7) have contrasting behavior: rather than the hypersociability that typifies WS, these individuals often have problematic social function, including risk for autism; Dup7 is also characterized by delayed speech, and some individuals have relative strengths in visuospatial processing. Because the genes involved are known, because the copy number variation (CNV) is stereotyped and well-studied, and because the clinical phenotypes have contrasting features, studying persons with WS, together with those having Dup7, affords a privileged setting for investigating how genes are translated in the brain to produce complex cognitive and behavioral features.

Methods: Identification of brain phenotypes in WS via multimodal imaging (structural MRI, fMRI, DTI, myelin mapping) has motivated experiments aimed at linking specific 7q11.23 genes to neural and behavioral features of the syndrome. Identified neural phenotypes are examined 1) in persons with CNVs involving just a few 7q11.23 genes, 2) in the general public in replicated samples via association analyses with SNPs in 7q11.23 genes, and 3) searching for brain/gene-dosage relationships in participants with WS vs. those with Dup7.

Results: First, extremely rare individuals with small deletions that include only a subset of the genes deleted in classic WS—LIMK1 and genes extending telomerically, but not GTF2I—show the visuospatial deficits and altered structure and function in the dorsal stream, but not hypersociability. Second, and further implicating LIMK1 in the visuospatial processing deficits, allelic variation in this gene was associated with reduced dorsal stream gray matter volume in two cohorts of healthy individuals ($Ns > 244$, $Ps < .001$). In contrast, the WS hypersocial personality and its underlying neural substrate appear to be linked to the GTF2I hemideletion. Third, we have also found associations of brain structure and function with 7q11.23 gene-dosage (1 copy in WS vs. 2 copies in the general population vs. 3 copies in Dup7) at FDR-corrected $Ps < 0.01$.

Conclusions: The incisive approach afforded by these well characterized CNVs and their contrasting cognitive/behavioral profiles provides a unique opportunity for the study of neurogenetic mechanisms of complex human variability. Further work longitudinally documenting the developmental trajectory of these brain phenotypes in children with 7q11.23 CNVs will clarify how brains develop over time in the face of these particular genetic landscapes.

Disclosure: Nothing to disclose.

33.4 Studying Sex Chromosome Aneuploidies as Models of Biological Risk for Psychopathology

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Background: Sex chromosome aneuploidies (SCAs) are a collectively common (prevalence ~ 2.3/1000) family of gene dosage disorders defined by presence of X- and/or Y-chromosome counts other than the typical XX for females and XY for males. SCAs increase risk for cognitive impairment and psychopathology, but the biological bases for these associations remain unclear. Addressing this gap in knowledge is not only important for affected individuals and their families, but also carries important consequences for our understanding of gene dosage effects on psychiatric risk more generally. Furthermore, by modeling X- and Y-dosage effects, SCAs offer a unique window into potential genetic contributions to the broader phenomenon of sex-biases in risk for psychopathology.

Methods: Our laboratory conducts comparative genetics-first studies in a globally-unique sample of > 200 individuals with one of several different SCAs (e.g. XXY, XYY, XXX, and XXYY syndrome), and a large (n > 600) sample of karyotypically normal controls. Phenotypic comparisons across SCA groups allows us to triangulate X- and Y-chromosome dosage effects, and integration across phenotypes within each SCA group allows us trace pathways of biological risk through several levels spanning gene expression, neuroimaging, cognition and psychopathology. Shared pathways across SCAs represent data-driven candidates for biological stratification of groups with idiopathic mental disorders. Finally, we conduct parallel neuroimaging-genomic studies in mice with X- and Y-chromosome aneuploidies which aid mechanistic inference regarding GDV effects on brain development.

Results: Eep-phenotypic analysis indicates that X- and Y-chromosome dosage variations induce a patterned shift in cognition and risk for diverse common behavioral syndromes including autism spectrum, attention deficit hyperactivity, tic and mood disorder. Neuroimaging reveals robust (effect size > 1) and reproducible effects of X- and Y-chromosome dosage variation in regional brain anatomy, which target cortical, subcortical and cerebellar nodes within distributed systems for language, social cognition, affect regulation and reward processing. We dissect potential genomic bases for these focal changes in macroscopic brain organization through bioinformatic alignment of imaging and gene-expression brain maps in humans and mice with SCA, as well as genome-wide studies of gene expression in SCA.

Conclusions: Taken together, these studies emphasize the importance of studying SCAs as neurogenetic disorders in their own right and underline the as yet untapped potential for SCAs as powerful models of genetic risk for psychopathology. Our findings pinpointing specific biological pathways through which X- and Y-chromosome variation impart clinical risk in psychiatry, and also reveal novel organizing principles for effects of gene dosage variation on brain and behavioral development more generally.

Disclosure: Nothing to disclose.

Panel

34. Novel Cellular, Circuit, and Brain Region Specific Mechanisms of Rapid Acting Antidepressants

34.1 Ketamine and Other Rapidly Acting Antidepressants Cause Disinhibition of CA1 Pyramidal Cells

Abstract not included.

34.2 Initial Cellular Trigger in the PFC and Projections Required for the Rapid Antidepressant Actions of Ketamine

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Background: Recent studies demonstrate novel, rapid, and efficacious treatments for depression, including the NMDA receptor antagonist ketamine, the metabolite (2 R,6 R)-hydroxynorketamine ((2 R,6 R)-HNK), and the NMDA partial agonist Rapastinel. Interestingly, the antidepressant effects of these diverse agents have in common a requirement for increased glutamate-AMPA receptor activity in the medial prefrontal cortex (mPFC). However, the exact cellular trigger underlying the convergent effects of these agents, whether indirect on GABA interneurons or direct on glutamate neurons, the subtypes of glutamate neurons, and the projection targets of these neurons have not been determined.

Methods: We used viral GluN2B shRNA for cell specific knockdown of GluN2B on GABA vs. glutamate neurons in Camk2a-, Gad1-, Sst-, or Pval-Cre recombinase lines. For principle neuron subtypes we used Drd1- and Drd2-Cre lines to express channelrhodoposin or DREADDs to stimulate or inhibit neurons in the mPFC and terminal field regions to identify projections for ketamine responses. Mice were tested for responses in the forced swim test (FST) novelty suppressed feeding (NSF), and female urine-sniffing test (FUST).

Results: Viral-mediated knockdown of GluN2B on GABA interneurons in the mPFC of Gad1-Cre mice blocked the antidepressant actions of ketamine as well as (2 R,6 R)-HNK in the FST and NSFT. In addition, knockdown of GluN2B in Sst- and Pvalb-Cre mice blocked the effects of ketamine. In contrast, knockdown of GluN2B on pyramidal neurons in Camk2a-Cre mice did not alter the response to these agents. Surprisingly, differential effects were observed with Rapastinel: GluN2B knockdown in Camk2a- but not Gad1-Cre mice blocked the effects of this agent. In studies of principle neuron subtypes, optogenetic stimulation of Drd1 + but not Drd2 + cells in the mPFC produced rapid and sustained antidepressant responses, while inhibition of Drd1 + cells blocked the antidepressant actions of ketamine. We also found that stimulation of mPFC Drd1 + terminals in the basolateral nucleus of amygdala (BLA) was sufficient to reproduce the antidepressant actions of mPFC Drd1 + cell body stimulation.

Conclusions: The results indicate that blockade of NMDA receptors on tonic firing GABA interneurons in the mPFC acts as a "cellular trigger" for ketamine and (2 R,6 R)-HNK, while partial agonist effects at NMDA receptors on glutamate principle neurons mediate the actions of Rapastinel. These different initial effects converge to increase AMPA, synapse number/function, and increase network connectivity that block or reverse the effects of stress. The results also show that stimulation of mPFC Drd1 + principle neurons and their projections to the BLA are necessary and sufficient for the actions of ketamine. Together these finding demonstrate novel cellular mechanisms and circuits underlying the effects of different classes of rapid acting agents.

Disclosure: Naurex, Consultant; Taisho, Consultant, Grant; Eli Lilly, Consultant; Aptinyx, Consultant, Grant; Navitor, Consultant, Grant; Allergan, Grant; Relmada, Grant

34.3 Ketamine Blocks Burst Firing in LHb to Cause Rapid Anti Depression

Abstract not included.

34.4 Behavioral and Electrophysiological Evidence of Homeostatic Balance in Ketamine's Mechanism of Action

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Background: Recent evidence suggests that disruptions in synaptic homeostasis may underlie depression. In the ketamine mechanism of action (Ket-MOA) study, multi-modal biomarkers obtained before, during, and after ketamine in patients with treatment-resistant depression (TRD) allowed us to contrast biological factors in the same individuals when depressed vs during response or remission. This permitted us to identify biologically-defined subgroups of TRD and to provide mechanistic insights into treatment response across units of analysis.

Methods: Thirty-five drug-free with TRD and a MADRS ≥ 20 and 26 healthy controls completed a crossover trial with ketamine (0.5 mg/kg) or placebo two weeks apart. Subjects underwent comprehensive phenotyping using DTI, MRS, fMRI and MEG, including resting state acquisition as well as acquisition during somatosensory stimulation, the emotional face dot-probe task, and an emotional evaluation task. Imaging modalities were performed at five time points: baseline (before randomization), 0-2 days post-infusion 1, 10-13 days post-infusion 1, 0-2 days post-infusion 2, and 10-13 days post-infusion 2.

Results: Robust increases in gamma power were observed in both controls and TRD subjects. Regions include those involved in the central executive network (CEN), salience network (SN), and default mode network (DMN). TRD subjects exhibited increases post-ketamine to levels commensurate with those seen in controls post-placebo. Gamma power at baseline moderated the relationship between change in gamma power and antidepressant response. Post-ketamine, large increases in gamma power were associated with a more favorable antidepressant response in subjects with low baseline gamma power at baseline but with a worse antidepressant response in subjects with greater gamma power at baseline. The interaction between baseline gamma power and MADRS response was most notable in the thalamus ($p < 0.001$) and the right insula ($p < 0.001$). Next, we replicated a previous finding describing cortical excitability as a plasticity measure of antidepressant response to ketamine. In post-hoc tests, the difference in evoked gamma power between ketamine and placebo sessions was significant only in the ketamine responders ($p = 0.008$). Using fMRI, we further found that ketamine had opposite effects on brain activation with cognitive and emotional processing in TRD participants versus controls. Finally, a significant interaction between response to ketamine, fMRI connectivity, and gamma power in the tracts connecting left and right hippocampus was found.

Conclusions: First, our results suggest that ketamine may normalize aberrant brain activity, both at a cellular level (as measured via gamma power) and at a systems level (as measured via both task-based and resting state fMRI). Second, the resting state connectivity changes in the anterior insula in TRD suggest that ketamine may normalize the interaction between the DMN and SN, supporting the triple network dysfunction model of depression. Third, our gamma power results suggest that where subjects lie on a continuum of homeostatic inhibition/excitation balance influences how TRD patients will respond behaviorally to gamma increases induced by ketamine, suggesting the existence of functional subgroups. Finally, our somatosensory task results suggest that only patients who responded to ketamine showed synaptic potentiation.

Disclosure: Dr. Zarate is a full-time U.S government employee and is an inventor on several patent and patent applications related to ketamine and ketamine metabolites that have all been assigned to the U.S. Government. He will share a percentage of any royalties that may be received by the Government in accordance to NIH policy., Patent

Panel

35. Cellular and Circuit Mechanisms of Anhedonia: Experiment-Based Targets for Intervention?

35.1 Aberrant Structural and Functional Circuit Maturation Underlies Anhedonia: Novel Findings Using Viral-Genetic Approaches and High-Resolution Neuro-Imaging in Experimental Systems

Tallie Z. Baram

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Background: Anhedonia is considered to denote aberrant function of pleasure and reward circuits, yet its neurobiological basis is not fully understood. Anhedonia often presages depression, schizophrenia and other emotional disorders, providing strong impetus to uncover its basis and test mechanistic preventative or mitigating interventions. We have identified several measures of anhedonia in both rats and mice that had experienced aberrant sensory signals (fragmented unpredictable sensory signals from maternal care; FRAG). Here we reasoned that disruption of cognate sensory input is known to distort the development of visual, auditory and motor circuits, and probed if disrupted maternal-derived sensory signals distort the maturation of the pleasure-reward circuit, promoting anhedonia

Methods: In mice, we employed viral-genetic tracing methods as well as immunohistochemistry to examine molecularly-defined pathways bridging nucleus accumbens (NAc) and amygdala in control and FRAG mice. We employed DREADD technology to probe the functions of pathways that differed in these groups. We used sucrose preference, estrous-female-scent and 3- chamber tests of anhedonia. In rats, we examined structural brain circuits using high-resolution diffusion tensor imaging (DTI) in FRAG and control cohorts, and cellular/regional activation was probed using cFos. We employed viral-genetic approaches to manipulate the expression of candidate genes. Finally, we tested for anhedonia using sucrose preference and social play.

Results: Several measures of FRAG-induced anhedonia were apparent in both mice and rats. Augmentation of CRH + -BLA-NAc pathways was found in FRAG mice compared to controls. In rats, DTI-tractography revealed increased structural connectivity of amygdala to medial prefrontal cortex in FRAG cohorts, as well as dispersion of DTI-apparent tracts between amygdala and NAc, indicating altered connectivity across fear/anxiety networks with pleasure/reward networks. Viral manipulation of these pathways reversed FRAG-induced anhedonia, suggesting that the long-term effects of early-life FRAG are modifiable in adulthood.

Conclusions: In experimental systems 'anhedonia' that follows disturbed patterns of sensory signals to the developing brain involves aberrant maturation of pleasure/reward circuits. Collaborative prospective studies in humans are examining the contribution of unpredictable and fragmented early-life

environmental and maternal signals (in the context of other risk factors) to the emergence of anhedonia symptoms and employ MRI to assess the underlying circuit changes. Thus, the mechanistic insights provided by viral-genetic tracing and gene manipulations, feasible in experimental systems, should be immensely valuable to our understanding of human anhedonia a dimensional entity which is a core feature of several serious mental illnesses

Disclosure: Nothing to disclose.

35.2 Is Anhedonia a Risk Factor for Trauma-Related Disorders?

Victoria Risbrough

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Background: Anhedonia, the diminished ability to experience pleasure, indicating dysfunction of the pleasure/reward circuitry, is an important dimensional entity linked to depression, schizophrenia and other emotional disorders. The neurobiological underpinnings of anhedonia may confer risk for development of mood, anxiety disorders and substance use disorders. Indeed, Anhedonia in adolescence/early adulthood is recognized as a risk for subsequent psychopathology. Here we examined the contribution of anhedonia symptoms in otherwise healthy subjects to the prediction of later development of mood, anxiety and alcohol abuse. Our study was a prospective longitudinal study of service members before and after a combat deployment (N = 2600).

Methods: The Marine Resiliency Studies (MRS) is a prospective longitudinal study of risk factors for combat-related trauma disorders in Marines deployed to Afghanistan and Iraq. Participants underwent psychiatric symptom assessments (clinician-administered PTSD scale, CAPS) and completed self-report scales for anxiety, mood and alcohol use symptoms prior to deployment and again at 3 and 6 months after returning from deployment. A principal components analysis (PCA) with varimax rotation was conducted on CAPS & BDI items to define separate factors for anhedonia, depression and PTSD symptoms. An anhedonia factor was identified and items making up this factor were used to define a latent variable of anhedonia in subsequent structural equation models. Comparisons of anhedonia with anxiety and depression latent variables were also conducted to confirm independent contribution of anhedonia to subsequent psychopathology.

Results: Pre-deployment anhedonia significantly increased the likelihood of PTSD symptoms after deployment (standardized beta = 0.16, $p < 0.01$; cross lag latent variable analyses). The associations of pre-deployment anhedonia with post-deployment PTSD symptoms persisted when depression or anxiety were added to the model, indicating independent contribution of anhedonia to PTSD risk. In addition, pre-deployment anhedonia increased risk for post-deployment PTSD diagnosis (assessed by DSM-IV symptom via CAPS [Main effect of Pre-deployment Anhedonia beta = 0.21, z value = 4.61, $p < 1e\text{-}05$; Main effect of Deployment trauma beta = 0.37, z value = 12.784, $p < 1e\text{-}05$; trauma X anhedonia interaction was not significant]. Anhedonia also significantly predicted increases in CAPS scores from pre- to post-deployment. Finally, pre-deployment anhedonia predicted increased post-deployment alcohol use. The longevity of the predictive value of anhedonia is now being determined in a sub- group assessed 5 years after deployment.

Conclusions: Anhedonia in early adulthood is a strong, independent risk factor for deployment-related psychopathology. Specifically, anhedonia indicates a vulnerability for development of future pathology after deployment-trauma exposure. Future studies will focus on understanding the circuit and cellular changes that promote anhedonia, and how they are further influenced by the 'second hit' of deployment trauma to provoke trauma-related psychopathology.

Disclosure: Nothing to disclose.

35.3 Inflammation-Related Disruptions in Corticostriatal and Amygdala-Prefrontal Circuits in Depression: Associations With Anhedonia, Anxiety and Response to Dopaminergic Therapy

Jennifer Felger

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Background: Neuroimaging studies have shown that cytokine therapies or the experimental administration of inflammatory stimuli cause changes in the neural activity and functional connectivity in reward and threat-related circuits that are involved in psychiatric disorders like depression. Translational findings in humans and laboratory animals indicate that these changes in circuity may be due to the effects of inflammation on neurotransmitters such as mesolimbic dopamine. For example, our work in non-human primates has demonstrated that inflammation causes decreased dopamine availability and release, which was correlated with effort-based sucrose consumption and reversed by the dopamine precursor levodopa. Inflammation is reliably increased in a subset of patients with major depressive disorder (MDD) and thought to contribute to symptom severity, yet little work has investigated the impact of inflammation on neurotransmitters and neurocircuits that may contribute to symptoms of MDD like anhedonia.

Methods: We studied whether increased inflammation (plasma inflammatory cytokines and C-reactive protein [CRP]) in depression affects reward and threat-related circuits in association with symptoms of anhedonia and anxiety using resting-state fMRI. Whole-brain, voxel-wise functional connectivity was examined as a function of increasing inflammation using seeds for ventral striatum and amygdala in 48 medically-stable, unmedicated adult outpatients with a primary diagnosis of MDD. Global network analysis was also performed. Additionally, we examined whether acute administration of levodopa reverses inflammation-related changes in functional connectivity in a pilot study in MDD.

Results: Increased CRP and inflammatory cytokines were associated with decreased functional connectivity between ventral striatum and ventromedial prefrontal cortex (vmPFC), which in turn correlated with symptoms of anhedonia ($r = -0.47$, $p < 0.05$). Plasma CRP additionally predicted decreased connectivity in a distinct circuit involving amygdala and vmPFC, which correlated with symptoms of anxiety ($r = -0.33$, $p < 0.05$). Global connectivity analysis further identified vmPFC as a primary hub for the whole-brain, network-level effects of inflammation on functional connectivity ($p < 0.002$/voxel + 640 mm3; $p < 0.05$). Mediation analyses revealed that variability in functional connectivity within these circuits mediated relationships between CRP and symptoms of anhedonia and anxiety ($z = \sim 2.2$, $p < 0.05$). Preliminary data also suggested that inflammation-related changes in functional connectivity can be improved by the administration of levodopa ($p < 0.05$), but only in patients with high CRP (> 3 mg/L).

Conclusions: Results indicated that inflammation compromises reward and threat-related subcortical to vmPFC circuits in association with symptoms of anhedonia and anxiety in patients with depression. Preliminary findings from depressed patients administered levodopa, combined with our translational work in non-human primates, suggest a role for dopamine in these circuit changes. Furthermore, inflammation-related deficits in functional connectivity may serve as imaging markers for the efficacy of anti-inflammatory or pro-dopamine therapies targeting improved symptom severity in MDD.

Disclosure: Nothing to disclose.

35.4 The Role of Dopamine in Depression: In Vivo PET, Postmortem, and Pharmaco-Imaging Approaches

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Background: Dopamine (DA) has been strongly implicated in reinforcement learning and motivation–functional domains that are impaired in Major Depressive Disorder (MDD), indicating that MDD may be characterized by reductions in DA signaling. The dopamine transporter (DAT) regulates clearance of extracellular striatal DA and modulates DA neurotransmission. Notably, preclinical studies (especially those involving inescapable stressors) have shown that DAT density decreases when DA signaling is reduced, suggesting that DAT density is sensitive to DA levels. In spite of compelling preclinical data, evidence of reduced DAT in MDD is inconclusive. In Study 1, we used positron emission tomography (PET) to evaluate DAT binding in MDD. In Study 2, we evaluated whether a pharmacological challenge hypothesized to increase dopaminergic signaling would normalize striatal responses to reward in MDD.

Methods: In Study 1, we used the highly selective DAT tracer [11 C]altropane to investigate DAT binding in 25 unmedicated individuals with MDD and 23 healthy controls. Levels of DAT expression were also evaluated in post-mortem tissues from donors with MDD who died by suicide ($n = 15$) and healthy controls ($n = 15$). In Study 2, using a double-blind placebo-controlled design, 46 unmedicated depressed participants and 43 healthy controls were randomized to receive either placebo or a single low dose (50 mg) of the D2/D3 antagonist amisulpride 1.5 h before performing a monetary incentive delay task during fMRI scans.

Results: In Study 1, relative to controls, individuals with MDD showed significantly lower in vivo DAT binding potential in the bilateral putamen ($p < 0.029$; Cohen's $d = -0.66$) and VTA ($p < 0.018$; Cohen's $d = -0.71$), and both effects were exacerbated with increasing numbers of major depressive episodes (putamen: $r = -0.36$, $p < 0.014$; VTA: $r = 0.36$, $p < 0.013$). Unlike healthy controls, the MDD group failed to show age-related reduction in striatal DAT binding, with young MDD individuals being indistinguishable from older healthy controls. Moreover, in MDD, DAT binding potential in the ventral tegmental area was lowest in individuals reporting feeling trapped in stressful circumstances from which one cannot escape ($r = -0.43$, $p < 0.032$). Reduced DAT in the putamen was replicated in post-mortem analyses ($p = 0.0091$, $d = -1.15$). In Study 2, relative to depressed participants receiving placebo, those receiving amisulpride had significantly increased striatal activation (e.g., $F(1,85) = 6.73$, $p = 0.011$) and potentiated corticostriatal functional connectivity between the nucleus accumbens and midcingulate cortex ($F(1,67) = 5.76$, $p = 0.019$) in response to monetary rewards.

Conclusions: MDD, particularly with recurring episodes and perception of being trapped by stressful situations, is associated with decreased striatal DAT expression, which might reflect a compensatory down-regulation due to low DA signaling within mesolimbic pathways. Findings from Study 2 indicate that a pharmacological challenge hypothesized to momentarily increase DA signaling can "rescue" blunted reward-related striatal activation and corticostriatal functional connectivity in MDD.

Disclosure: Akili Interactive Labs, Consultant; Boehringer Ingelheim, Consultant; BlackThorn Therapeutics, Consultant; Takeda Pharmaceuticals, Consultant

Study Group

36. Event-Related and Spontaneous Oscillations: Siblings, Cousins, or Unrelated?

Daniel Javitt, Peter Uhlhaas, Daniel Mathalon, Elliot Hong, Jason Tregellas, Antigona, Martinez, Cindy Ehlers, Robert Greene, Atheir Abbas, Tobias Teichert, Peter Lakatos, Thilo Womelsdorf*

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Study Group Summary: Neurophysiological recording and analysis approaches are among the most effective techniques for translational investigation into neural mechanisms underlying brain dysfunction across neuropsychiatric disorders. In clinical situations, several neurophysiological biomarkers including P50, mismatch negativity (MMN), steady-state synchronization and P300 are well established biomarkers of attentional and cognitive impairments. Recent neurophysiological studies have mapped neural activity associated with these measures into specific beta (10-24 Hz), theta (4-7 Hz), and delta (1-4 Hz) frequency bands.

In addition, alterations in the generation of both spontaneous and task-related gamma (> 24 Hz) frequency activity in various brain systems have been tied to impaired cognitive function. In intracranial studies, periods of enhanced oscillatory activity are recorded during behavioral testing and linked to specific cognitive operations. The neural circuitry underlying these processes, moreover, has been increasingly clarified, with higher frequency oscillations involving primarily parvalbumin (PV) interneurons in excitatory / inhibitory circuits in the cortex, as well as in the purely inhibitory circuits in the striatum, while lower frequency oscillations involve circuits that may additionally involve somatostatin (SST) interneurons or alternative circuit motifs.

At present, the relationship between stimulus-related oscillations, as typically obtained in human clinical studies, and the diverse sustained and transient oscillatory activity signatures obtained in animal in vivo recordings or ongoing human EEG remains controversial, with limited cross-talk between the two fields of research. The goal of this study group is to establish a dialog between clinical and basic investigators using oscillatory measures as their primary experimental "read-outs" and explore the similarities and differences between information obtained in different recording contexts.

In general, oscillatory activity can be considered spontaneous, "evoked" or "induced" based upon the need for, and level of phase-locking to, eliciting stimuli. Stimuli may also induce both increases ("event-related synchronization") and decreases ("event-related desynchronization") of ongoing activity. The study group will discuss the utility with which these approaches are applicable to both event-related and more continuously emerging oscillations and develop strategies to bridge across levels of analysis.

In addition, the study group will evaluate the degree to which both event-related and spontaneous oscillatory activity can be used to evaluate excitatory/inhibitory balance within clinical populations involving both PV- and SST-neuron components, as well as the degree to which circuit-level oscillatory findings in intracranial recording studies may be used to inform mechanisms underlying clinical abnormalities.

The study group incorporates researchers with expertise across complementary "units of analysis" including molecules, cells, circuits, physiology and behavior, and represents a unique opportunity for dialog between research groups using similar neurophysiological constructs within disparate research contexts.

Disclosure: NeuroRx, Board Member, Stock / Equity, Patent; Glytech, Patent, Stock / Equity; AASI, Patent; Promentis, Advisory Board; Phytecs, Advisory Board; Lundbeck, Consultant; Concert,

Consultant; Autifony, Consultant; Forum, Consultant; Takeda, Consultant

Mini Panel

37. Neuropsychopharmacology of Social Behaviors: Important Lessons From Non-Traditional Animal Models

37.1 A Non-Traditional Model of Early Life Stress: Neonatal Paternal Deprivation Alters Neuroendocrine, Neuroinflammatory, and Behavioral Responsiveness in a Sex-Dependent Manner in Adult California Mice (Peromyscus Californicus)

Erica Glasper

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Background: Peromyscus are emerging as a nontraditional model system in studies of behavior and behavior. The California mouse (P. californicus) is genetically monogamous and biparental. Males forego opportunities to sire new litters outside the nest and devote resources to caring for their offspring. This life strategy is rare, occurring in less than 6% of mammalian species, including most Peromyscus species that typically exhibit polygynous or promiscuous mating systems that results in males providing no parental care. Early-life experience with caregivers can significantly alter developmental trajectory and while most of our knowledge of parent-offspring relationships stem from mother-offspring interactions, increasing evidence suggests father-offspring interactions can prevent social, behavioral, and neurological impairments. The California mouse is a rodent model uniquely suited to investigate neural and behavioral consequences following a species appropriate early-life stressor, namely paternal deprivation [(PD); i.e., removing the father on postnatal day 1]. We have recently demonstrated that PD leads to decreased neonatal survival as well as decreased exploratory behavior and increased passive-stress coping behavior in adulthood. Interestingly, a sex-dependent effect of PD on short-term survival of newborn cells in the dentate gyrus (DG) of the hippocampus was also observed. Specifically, PD reduced cell survival in female, but not male, young adult California mice.

Methods: Given that numerous factors are implicated in reduced neuronal survival and disrupted behavioral functioning, we have performed new experiments with the goal of elucidating potential mechanisms contributing to sex differences in brain and behavior as a result of PD in this unique model system. In adulthood, male and female mice ($n = 3$-10, for each endpoint) were behaviorally assessed for novel object recognition, generalized anxiety, social recognition, and social novelty preference. Basal and stress-induced serum corticosterone (CORT) was measured via radioimmunoassay. Total number of corticotrophin releasing factor (CRF) immunolabeled cells was quantified in the paraventricular nucleus of the hypothalamus (PVN) and DG. Hippocampal, frontal cortex, and hypothalamic proinflammatory cytokine concentrations were quantified via Luminex technology. For all analyses, a 2-way ANOVA (sex X rearing) was performed.

Results: PD prevented the resolution of an acute stressor-induced CORT elevation in males, but not females ($p = 0.04$). Chronic variable stress resulted in a hyporesponsive stress axis (e.g., CRF labeling in PVN) in PD males, but not females ($p = 0.03$). A similar sex-dependent effect of PD was observed following behavioral testing, such that generalized anxiety was increased in PD males, but not females; PD increased social recognition memory and preference for social novelty in males, but not females. Interestingly, PD increased IL-1β hippocampal concentration in males, but not females ($p = 0.03$).

Conclusions: Together, these findings suggest that PD results in sexually dimorphic disruptions to behavioral, neuroendocrine, and neuroimmune responsiveness, with the greatest disruptions observed in adult male offspring. On the whole, these data also highlight the importance of taking the model system into consideration when asking questions related to brain and behavior disorders commonly observed in humans.

Disclosure: Nothing to disclose.

37.2 Titi Monkeys: A Non-Traditional Model for the Neurobiology of Social Bonding

Karen Bales

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Background: Many studies of social behavior in neuropsychopharmacology are conducted in mice or rats, species in which males do not form selective social bonds as adults. Socially monogamous species, in which adult animals form pair bonds, are ideal for the purpose of assessing pharmacological effects on adult social bonds in both sexes. Most previous neuroscience research in socially monogamous animals has been focused on prairie voles, a rodent model. In order to increase translatability to humans, a non-human primate model is critical. For this purpose, we have been developing titi monkeys (Plecturocebus cupreus), a socially monogamous non-human primate, as a model for the neurobiology of pair bonding and parenting. As part of this process, we have carried out identical pharmacological studies of chronic intranasal oxytocin (OT) in prairie voles, C57BL/6 J and BTBR T + ltpr3tf/J mice, and titi monkeys. The goal of these studies was to assess the chronic behavioral, neurobiological, and reproductive effects of chronic intranasal OT exposure, which has been proposed as a treatment for autism spectrum disorders. In prairie voles, we found that prior chronic exposure to OT caused deficits in male pair bonding behavior. In mice, we found that the exposure to OT had no effects at all. In this talk, I primarily present the data from titi monkeys and discuss the overall implications of this comparative experiment.

Methods: Intranasal OT (0.8 IU/kg), or saline was administered once daily to male and female titi monkeys ($n = 29$, 7-8 per group) in the peri-adolescent period, from 12 months of age till 18 months of age. During the period of administration, we measured behavior within the family; preference for parents over strangers; anxiety-like behavior; glucose uptake in the brain; and pubertal maturation. At 2.5 years of age, one year after administration had ended, we paired the subjects with a reproductively proven mate. We then measured the development of preference for the partner; distress upon short separation; mating behavior; and fertility.

Results: We found that OT-treated monkeys of both sexes spent more time grooming their family members ($F1 = 8.97$, $p = 0.006$). In a preference test, OT-treated animals of both sexes spent more time in total social behavior ($F1 = 8.35$, $p = 0.005$) than saline-treated animals, including more time in proximity to their parents ($F1 = 4.54$, $p = 0.003$), as well as more time in proximity to a strange pair ($F1 = 4.86$, $p = 0.03$). OT-treated animals also showed longer latencies to approach novelty ($F1 = 10.99$, $p = 0.009$), indicating higher anxiety-like behavior. Pubertal maturation was not affected by OT treatment. Preliminary analysis of adult pair bonding shows that males treated with OT formed a preference for their partner faster. While treatment did not affect female partner preference, females treated with OT vocalized less when separated from their pair mate. There was also a trend for OT-treated animals to more successfully raise their first infant.

Conclusions: We obtained very different results from testing in three different species; even though the tests were done by the same laboratory and using the same methods. It remains to be seen which species better reflects the results found in humans, although current studies appear to be finding modest positive effects, as we found in the titi monkeys. This study reflects the need for careful choice of study species depending on the dynamics of the system being studied and suggests that typical laboratory species may not always be the best choice.

Disclosure: Nothing to disclose.

37.3 Microtine Rodent Models for Studying Selective Social Attachment

Zoe Donaldson

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Background: Microtine voles provide a powerful model for studying the neural substrates underlying complex social behaviors. Closely-related species exhibit strikingly different social behaviors. For instance, monogamous prairie voles (Microtus ochragaster) form life-long pair bonds; if they lose a partner in in the wild, they rarely take a new partner. In contrast, promiscuous meadow voles (M. montanus) do not form sociosexual bonds. Comparative neuroendocrine research in these animals has led to translationally-relevant insights into the modulatory systems that underlie adult bond formation. Now, with the expansion of novel molecular-genetic tools to monitor and manipulate neural activity, my lab has begun exploring the circuit and cellular dynamics underlying social bonds. Specifically, we are testing the hypothesis that differences in activation of neuronal ensembles selectively in reward-associated brain regions contribute to differences in attachment between these two vole species.

Methods: We are using in vivo $Ca2+$ imaging and molecular genetic tools to identify and manipulate mate-active neuronal populations in prairie and meadow voles. By directly comparing ensemble dynamics across the two species, we have a powerful means to uncouple the encoding of familiarity and sexual experience, from those that uniquely represent the partner reward association in pair bonded animals.

Results: Consistent with our hypothesis, we have found that in monogamous prairie voles, overall $Ca2+$ spiking activity in the nucleus accumbens does not differ when the test animal interacts with its monogamous partner or a novel individual. Rather, we have identified subsets of neurons whose $Ca2+$ spiking activity is selective for the partner, providing a putative substrate underlying a partner-specific neuronal ensemble in prairie voles. We are currently assessing the stability of tuning within these partner-active neurons across time. In addition, we have demonstrated that we can use immediate early gene-mediated labeling of this putative neuronal ensemble via the Rapid Activity Monitor (RAM) system, which will enable us to subsequently manipulate activity within these cells

Conclusions: This work uses complementary approaches to identify the neural framework that encodes social bonds. Such work is not feasible in commonly-used rat and mouse models, which do not form selective sociosexual bonds. Such an understanding of the basic neurobiology of adult attachment is a key first step towards elucidating the processes that may be disrupted in disorders associated with under- and over-attachment, including separation anxiety disorders and complicated grief, and for identifying novel and relevant pharmacological approaches for treating dysfunctional attachment.

Disclosure: Nothing to disclose.

Study Group

38. The Debate Regarding Maintenance Treatment With Antipsychotics In Schizophrenia

Michael Davidson, Donald Goff, Jeffrey Lieberman, John Krystal, Robin Murray, Rene Kahn, Wolfgang Fleischhacker, Mark Weiser, Stephen Marder, David Daniel, Christoph Correll, Jari Tiihonen, Dolores Malaspina, Iris Sommer, Robert Buchanan, John Kane, Jonathan Rabinowitz*

Tel Aviv University and Minerva Neurosciences, Israel

Study Group Summary: Several large meta-analyses of maintenance trials confirmed that patients suffering from chronic schizophrenia randomized to placebo are likely to experience earlier symptomatic worsening then patients randomized to a DA blocking drug. These findings have let expert groups to issue treatment guidelines which recommend treatment with DA blocking drugs for many years to indefinitely. These recommendations were accepted by the majority but not all the experts. The later propose a targeted therapy approach by which DA blocking drugs are discontinued upon symptomatic remission and, renewed only in case of symptoms re-emergence.

The case for continuous maintenance treatment is straightforward since the risk for symptoms re-emergence persists long after resolutions of the acute symptoms. Since there exists no diagnostic markers to distinguish between those individuals who can maintain very long period of symptoms' remission in absence of DA blocking drugs and, these who would experience impending exacerbation, all individuals affected by chronic schizophrenia should be encouraged to accept continuous and long-term maintenance treatment. Critics of this approach have questioned the benefit/risk ratio and pointed out that the trials are too short to be informative, that some of the outcome measures have poor ecological validity.

The case for the targeted approach treatment claims that like almost all medical interventions, maintenance treatment has immediate and accumulating adverse effects, therefore its use should be restricted to the minimum in terms of dose and time. Supporting this approach are several follow-up studies indicated that schizophrenic patient on targeted treatment have better long-term outcome in term of social and vocational performance than those on continuous treatment. Critics of this approach have pointed out that the studies are biased by reversed causality and other methodological limitations.

The debate between continues and targeted treatment approaches arises from disagreements regarding scientific and ethical questions. Scientifically, the debate focuses on the quality and interpretation of the supporting and detracting evidence regarding each treatment option. For example, what is the percentage of individuals who can maintain stability off drugs, what is the percentage on individuals who exacerbate despite maintenance treatment, what is the percentage of individuals who experience drug related adverse effect, how to interpret results of open label, non-randomized trials targeted trials. On the ethical question the debating sides disagree on how to weight the impact of the decrease risk for exacerbation versus the certainty of adverse effects on the patient quality of life and, how to reach a patient-therapist shared decision within the constraints of mental illness. Participant in the study group will address elements of this debate and will present data on the characteristics of patients who may benefit from each of the two treatment approaches.

Disclosure: Minerva Neurosciences, Employee

Panel

39. Large-Scale Developmental Neuroimaging of Dimensional Psychopathology

39.1 The Influence of Maternal Prenatal Stress Trajectories During Pregnancy on Offspring Brain and Behaviors From 0-24 Months of Age

Damien Fair

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Background: The period from conception to birth is confers rapid brain development that occurs in an environment exclusively defined by maternal physiological and psychological health. Perturbations to this environment can alter fetal brain development potentially impacting long-term infant health outcomes.

The majority of studies that examine maternal distress during pregnancy primarily consider the magnitude of distress as the 'risk factor;' however, psychosocial stress, particularly in pregnancy, is dynamic and variable. As a result, it remains unclear which aspects of stress most strongly impact offspring development – is it simply the scale of stress during pregnancy, or does the trajectory matter as well?

Under this context the present study aims to examine heterogeneity in maternal prenatal/perinatal distress by defining individual longitudinal trajectories, and their impact on offspring brain and behavior.

Methods: Data from a previously collected sample of healthy mother-infant dyads ($n = 114$) were used for the preliminary studies. Mothers were recruited during the first trimester of pregnancy and complete measures on depression, anxiety, and stress from the first trimester through the first two years of infant life (i.e., 9-timepoints). Structural and functional brain MRI was acquired in neonates (male and female) shortly after birth, along with the IBQ (negative affect sub-scale) completed at 3, 6, 9, 12, and 24 months of age. The findings reported here were based on a new approach we've termed the Functional Random Forest (FRF), which allows for capturing distinct longitudinal (or trajectory) heterogeneity in psychosocial measures of stress across pregnancy.

Results: We found that the trajectory of maternal prenatal distress predicts infant negative affect development over the first two years of life, even after controlling for maternal postnatal distress covariates. Trajectories that emphasized increased stress between the 2nd and 3rd trimesters had the greatest effects on infant outcomes. These maternal stress trajectories relate to neonatal limbic system functional connectivity, which also predicts characteristics of infant negative affect, as well. Overall, the data highlight that the trajectory, above and beyond magnitude, of maternal prenatal distress may contribute to offspring brain and affective development.

Conclusions: These findings provide new evidence in humans linking trajectories of maternal stress during pregnancy with newborn brain and emerging behavioral phenotypes relevant for psychiatric disorders. A better understanding of intrauterine conditions that influence offspring disease susceptibility is warranted to inform targeted early intervention and prevention efforts.

Disclosure: Nothing to disclose.

39.2 The Temporal Cascade in the Underlying Neurobiology of Emerging Psychopathology in Children and Adolescents: A Population-Based Study

Tonya White

Erasmus University Medical Centre, Rotterdam, Netherlands

Background: Major psychiatric disorders, such as schizophrenia and affective disorders often have their origins in adolescence, a time when a number of brain regions are continuing to develop. However, most studies evaluating the neurobiology of major psychopathology study individuals who either are in the prodromal phase, or already have the illness. Thus, these studies face the 'chicken versus the egg' dilemma, where the question is whether the findings are a primary result of the illness, or the result of a downstream process secondary to earlier changes in other brain regions or networks. To address this question, it is important to recruit children or adolescents prior to the emergence of prodromal or early symptoms.

Methods: The Generation R Study is a large population-based birth cohort based in Rotterdam, the Netherlands in which over 5,000 children have participated in brain MRI acquisitions and we are currently collecting our third neuroimaging wave. In addition, fetal head ultrasounds were obtained during prenatal life at three time points in over 5,000 pregnant mothers. Diagnostic measures of psychopathology along a continuum have been obtained for a number of different disorders, including autism spectrum disorders (ASD), ADHD, and the dysregulation disorder phenotype. Mixed model longitudinal designs and structural equation modeling using cross-lagged models will be used to determine the temporal relationship between changes in brain and behavior.

Results: Interestingly, we find few differences in the brains of younger children with dysregulation disorder phenotype. However, those children who continue to have behavioral problems over time show subsequent decrease in gray matter volume over time, especially in subcortical regions. We find little evidence of early differences in prenatal and postnatal head growth in children who later develop ASD. However, we do demonstrate a number of early environmental factors that contribute to the later development of ASD.

Conclusions: Our findings raise the question as to whether continued behavioral problems results in brain changes either because these regions become 'unmasked,' or alternatively, in a sort 'kindling' model of psychopathology. If so, this could have important ramifications for prevention and treatment.

Disclosure: Nothing to disclose.

39.3 Using Machine Learning to Discover Networks Associated With Dimensions of Psychopathology in Youth

Theodore Satterthwaite

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Background: During adolescence and young adulthood, the human brain undergoes protracted structural maturation. Abnormal development of specific networks may confer vulnerability to differing types of psychopathology. Here, I will review a set of recent unpublished cross-sectional and longitudinal studies that seek to delineate abnormalities of brain development associated with dimensions of psychopathology across clinical diagnostic categories using recently-developed machine learning techniques.

Methods: Participants included 1,394 youth as part of the community-based Philadelphia Neurodevelopmental Cohort

(PNC), as well as a subset of 144 participants who completed longitudinal follow-up imaging (median interval ~4 years). In both cross-sectional and longitudinal data, cortical thickness was estimated from the T1 image using the top-performing tools included in ANTs. Structural covariance networks were defined using non-negative matrix factorization (NMF), an advanced unsupervised analysis technique. A dimensional measure of psychopathology was constructed using a bifactor model of item-level data from a psychiatric screening interview, which delineated four factors (fear, anxious-misery, psychosis, and behavioral symptoms) plus a general factor, which represented overall psychopathology present across disorders. Longitudinal follow-up focused on irritability, which was quantified with the Affective Reactivity Index. Group level analyses utilized generalized additive mixed models with penalized splines to capture both linear and nonlinear developmental effects. Multiple comparisons were accounted for using the False Discovery Rate ($Q < 0.05$). All analyses carefully controlled for potentially confounding variables such as data quality.

Results: At baseline, the dimensional index of fear symptoms was associated with thinner cortex within multiple networks relevant for salience detection. Specific regions impacted included the anterior and posterior cingulate cortex, the anterior insula, and the temporal-parietal junction (all FDR-corrected p values < 0.05). Notably, this association with fear was independent of associations with overall psychopathology, which was most strongly linked to total gray matter volume. In contrast, irritability at follow-up was associated with thinner cortex within networks implicated in emotion regulation, including the orbitofrontal and medial temporal cortex (corrected $p < 0.05$). Furthermore, evaluation of longitudinal change revealed that higher levels of irritability at follow up was associated with accelerated longitudinal thinning within these same networks over time.

Conclusions: These results delineate specific abnormalities of structural brain network development associated with clinically-relevant dimensions of psychopathology that are present across clinical diagnostic categories. Notably, each dimension of psychopathology demonstrated a dissociable linkage to deficits in specific cortical networks implicated in the pathogenesis of each symptom domain.

Disclosure: Nothing to disclose.

39.4 Dimensions of Psychopathology: Neurocognitive Correlates and Treatment Outcomes

Patricia Conrod

Université de Montréal, Montreal, Canada

Background: Using large pediatric prospective studies and new computational methods, Dr. Conrod's team has shown that the structure of psychopathology in adolescents comprises hierarchically-organized general and specific latent dimensions of risk for psychopathology. Using these datasets, this team has also identified neural, cognitive and personality correlates of these dimensions, which have helped to inform new targeted and early interventions strategies. For the first time, this team will present analyses from a large randomized trial of brief psychosocial interventions for at risk youth to evaluate the impact of early intervention on these latent dimensions of psychopathology.

Methods: Using data from the Co-Venture Trial and its parallel imaging study, NeuroVenture, we investigate the cognitive and neural correlates of dimensions of psychopathology identified from a general-specific hierarchical model. These correlates are investigated over the course of adolescence, from 12 until 17 years of age. Using data from the Adventure Trial will investigate the impact of early and brief cognitive behavioural interventions for youth at risk of mental health and substance use disorders within this same dimensional framework.

Results: Results indicate that the structure of psychopathology across adolescence includes a general psychopathology factor, and three specific factors capturing thought disorder symptoms, internalizing symptoms and externalizing symptoms. These latent factors of psychopathology were dissociated on personality, cognitive and neural factors. Treatment effects of personality-targeted interventions were revealed on some specific factors and only marginally on the general factor, and there was some evidence of targeted effects, at least with respect to impulsivity-targeted interventions.

Conclusions: Understanding how targeted interventions impact on common and specific vulnerability to psychopathology will lead to more refined targeted intervention strategies and a better assessment of the true benefits of early intervention approaches.

Disclosure: Nothing to disclose.

Panel

40. Full Frontal: Translational Studies Providing New Insight Into Pathological and Therapeutic Mechanisms in the Prefrontal Cortex

40.1 The Role of Prefrontal Interneuron Subtypes in Spatial Encoding and Long-Range Synchrony

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Background: Interneurons play crucial roles in cortical function and have been hypothesized to underly oscillatory synchrony. Spatial working memory in rodents relies on oscillatory synchrony between the hippocampus (HPC) and medial prefrontal cortex (mPFC) to support encoding of spatial information in mPFC neurons. We therefore hypothesized that parvalbumin- (PV), somatostatin- (SOM) and/or vasoactive-intestinal peptide- (VIP) positive interneurons might be required for synchrony and spatial representations during a working memory task.

Methods: To test this hypothesis, we used the light-activated proton pump Arch3.0 to selectively silence prefrontal PV, SOM or VIP interneurons in the medial prefrontal cortex of mice performing the delayed non-match to sample T-maze test of spatial working memory. We simultaneously recorded neural activity in the medial prefrontal cortex (mPFC) and other brain areas known to be involved in working memory, including the dorsal and ventral hippocampus (dHPC and vHPC). We used measures of LFP-LFP and spike-LFP synchrony to characterize the functional connectivity between these structures and a maximum margin linear classifier to examine spatial coding. $n = 7$-17 male mice per group of various genotypes were used for the experiments. Non-parametric statistics were used throughout, with correction for multiple comparisons where appropriate.

Results: Silencing SOM ($p = 0.027$, rmANOVA) but not PV ($p = 0.51$) interneurons during the sample or delay phases of the task significantly impaired working memory performance. SOM silencing during the sample phase of the working memory task was also associated with a decrease in phase locking ($p < 0.01$), as measured by PPC, between mPFC neurons and vHPC theta oscillations and dHPC theta oscillations. SOM silencing during the sample phase was also associated with impaired spatial

representations of goal location by mPFC neurons ($p < 0.01$). Work continues on experiments involving inhibition of VIP neurons and we hope to have data to present on this by the annual meeting.

Conclusions: Our evidence is consistent with SOM interneurons supporting spatial encoding during working memory and facilitating hippocampal-prefrontal synchrony.

Disclosure: Nothing to disclose.

40.2 Using In Vivo Calcium Imaging to Study Prefrontal Cortex Contributions to Behavioral Dysfunction Relevant to OCD

Elizabeth Manning

University of Pittsburgh Medical Center, Western Psychiatric Institute & Clinic, Pittsburgh, Pennsylvania, United States

Background: Functional imaging studies have strongly implicated prefrontal cortex (PFC) dysfunction in the pathophysiology of obsessive compulsive disorder (OCD). However, the mechanisms by which abnormal activity gives rise to OCD symptoms are unclear. While hyperactivity is typically observed at baseline and during symptom provocation, impaired recruitment of PFC is observed across a variety of neurocognitive paradigms, including reversal learning. This raises the question of whether overlapping or distinct populations of neurons contribute to these different neural activity patterns and associated symptoms. To address this question, we are using miniature microscopes to perform in vivo $Ca2+$ imaging in the Sapap3 knockout mouse (KO) model, to directly compare activity of individual neurons during paradigms relevant to the cognitive impairments (i.e. reversal learning) and compulsive behaviors (i.e. compulsive grooming) observed in OCD.

Methods: Expt.1: Sapap3KOs and wildtype (WT) littermate controls were tested in compulsive grooming and instrumental reversal learning paradigms (males & females; $n = 36$ KO/31 WT). Expt.2: Reversal learning-associated immediate early gene (cFos) expression was examined across 10 cortical and striatal regions of interest (12 KO/11 WT males). Expt.3: Male and female Sapap3KOs ($n = 15$) and WT controls ($n = 8$) were injected with AAV-GCaMP6f and implanted with prism lenses in mPFC to visualize $Ca2+$ activity during grooming and reversal learning. $Ca2+$ signals were extracted using CNMFe, converted to $\Delta F/F$, and aligned to specific behavioral events during reversal learning (correct/incorrect responses, reward retrieval) and compulsive grooming (bout initiation/ termination). To determine whether cells were responsive to behavior, fluorescent signal was shuffled (1000 iterations) to create a null-distribution, and behavior-associated fluorescence > 1.5 SD from null distribution was considered significant.

Results: As in previous studies, KOs displayed compulsive grooming ($p < 0.0001$). KOs also showed reversal learning impairments (RMANOVA, $p < 0.001$), in which ~half (17/36) were unable to acquire a reversed contingency during 5 days of training, while the others performed similarly to WT. This heterogeneity in reversal learning was unrelated to severity of compulsive grooming. General linear mixed-effects models revealed unique associations between reversal learning in Sapap3KOs and mPFC cFos on day 1 of training, with elevated activity associated with impaired performance ($p < 0.001$). In vivo calcium imaging in mPFC indicated that the proportion of grooming responsive neurons is increased in KOs (25% vs 11%; $p = 0.01$).

Conclusions: Our studies are among the first to describe cognitive impairments in a transgenic mouse model with relevance to OCD. Our cFos results suggest that abnormal reversal learning in Sapap3KOs is associated with elevated mPFC activity, and our in vivo imaging data during compulsive grooming also demonstrate a larger percentage of grooming-responsive neurons in KOs. Ongoing analysis in this dataset is 1) examining mPFC activity during reversal learning in KO vs WT; and 2) using longitudinal tracking to compare activity of individual neurons during compulsive grooming and reversal learning to determine whether distinct or overlapping neural populations are associated with these behaviors.

Disclosure: Nothing to disclose.

40.3 High Density Continuous Theta Burst Stimulation (HD-CTBS) to the Medial Prefrontal Cortex– a New Neuromodulation Approach to Dampening Appetitive Drive and Increasing Executive Control Circuity

Colleen Hanlon

Medical University of South Carolina, Charleston, South Carolina, United States

Background: The competing forces of appetitive drive and executive control have shaped scientific and philosophical discussions of human behavior for centuries. Now, through modern molecular and optical imaging techniques is it possible to directly manipulate the neural circuits that govern cue-reactivity through stimulation of the prefrontal cortex. Building upon the preclinical studies in the preceding presentations, this talk will introduce, for the first time, an innovative neuromodulation technique which has shown utility as a tool that can simultaneously increase executive control circuitry and decrease appetitive drive in several clinical populations.

Methods: Specifically, we will first introduce this technique – high density continuous theta burst stimulation (HD-cTBS) and its translational relevance. We will then present functional neuroimaging data (cue-evoked BOLD signal) from three clinical trials which all demonstrate that this technique causes a dampening of cue-reactivity in limbic control areas and an increase in cue-evoked activity in executive control areas. The basic experimental design in all three studies was congruent: functional MRI of cue-evoked BOLD signal before and after a course of real or sham HD-cTBS wherein the primary outcome was a change in cue-evoked BOLD signal in the brain in the real versus sham group. Statistical analyses: FMRI data was processed with standard techniques and general linear modeling with a full factorial design was used to evaluate the interaction between time and TMS condition (minimum inclusion for significance: $p < 0.05$ family wise error corrected).

Results: In the first trial 30 treatment-seeking alcohol users (30% women) received 10 days of real or sham HD-cTBS to the medial prefrontal cortex (EEG: FP1 target). Real HD-cTBS led to a significant increase in BOLD signal in executive control regions when individuals were shown alcohol cues 2months after treatment. There was also a significant increase in gray matter volume in the dorsolateral prefrontal cortex. In the second trial, 16 compulsive eaters with obesity (60% women) received 5 days of real or sham HD-cTBS. Real HD-cTBS led to a significant increase in BOLD signal in executive control regions. Finally, in the third trial, 20 individuals with chronic pain and opiate use disorder (65% women) received HD-cTBS and had a significantly attenuation of brain response to pain. As above, all statistical differences reported were $p < 0.05$ corrected at the cluster level and controlled for scalp-to-cortex distance.

Conclusions: Considered together these neuroimaging and brain stimulation studies demonstrate that this new neuromodulation technique may have transdiagnostic efficacy as a tool to rebalance the neural circuits involved in executive control and limbic drive. These data will serve as a foundation for future studies involving diseases with pathology involving fear, reward,

or motivation which seek to use the VMPFC as a biological target for treatment development.

Disclosure: Brainsway, Inc, Consultant

40.4 Sustained Rescue of Prefrontal Circuit Dysfunction by Antidepressant-Induced Postsynaptic Spine Formation

Conor Liston

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Background: Depression is a fundamentally episodic form of mental illness, yet the neurobiological mechanisms underlying the induction and remission of depressive episodes over time are not well understood. Converging evidence from clinical neuroimaging and pre-clinical animal models implicate changes in postsynaptic dendritic spine density and functional connectivity in prefrontal cortical circuits, but it is unclear whether these effects play any causal role in transitions between depression-related behavioral states and recovery over time.

Methods: We used two-photon microscopy, in vivo calcium imaging, and novel optical probes for manipulating synapse formation to investigate how prefrontal cortical synaptic remodeling contributes to behavioral state transitions during chronic stress (N = 4–12 male and female mice) and after antidepressant-dose ketamine treatment (N = 6–15 male and female mice).

Results: We show that the induction of depression-related behavior is associated with clustered, branch-specific elimination of postsynaptic dendritic spines on prefrontal projection neurons (P = 0.004, Wilcoxon W = 15.0; median elimination rate: 18.4% vs. 10.6% in controls), mediated in part by a mineralocorticoid receptor-, transcription-dependent signaling process. Antidepressant-dose ketamine reverses these effects by selectively rescuing eliminated spines (P = 0.001, Wilcoxon W = 21.0; median restoration rate = 47.1% of lost spines vs. 12.1% in controls) and restoring coordinated activity in multicellular ensembles that predict motivated escape behavior (t = 4.34, P = 2e-5). Using behavioral interventions and a photoactivatable driver of Rac1 signaling to bidirectionally modulate the survival of newly formed spines, we show that ketamine-induced synapse formation is required for the long-term maintenance of selected antidepressant behavioral effects (P = 0.002, Wilcoxon W = 50.0), but not for their induction.

Conclusions: These results define a previously unappreciated, causal role for prefrontal synaptogenesis in sustaining antidepressant effects on selected depression-related behaviors and suggest new avenues for optimizing interventions aimed at enhancing and maintaining remission after ketamine treatment.

Disclosure: Nothing to disclose.

Panel

41. Should I Stay or Should I Go to the Ventral Pallidum

41.1 Cell Type Specific Ventral Pallidum Regulation of Cocaine Seeking

Jasper Heinsbroek

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Background: Neuronal activity in the ventral pallidum (VP) is critical for driving drug relapse, yet precisely how VP subcircuits contribute to drug seeking and taking remains unknown. The VP is densely innervated by the nucleus accumbens and is canonically considered an inhibitory relay and output structure for the ventral basal ganglia. However, VP neurons integrate information from a variety of different brain regions, and two nucleus accumbens projections to the VP (coded by dopamine D1 and D2 receptors) transmit opposing motivational information. Besides GABAergic neurons (VP-GABA), the VP contains a population of glutamatergic neurons (VP-Glu), and a unique population of GABAergic VP neurons co-expresses the neuropeptide enkephalin (VP-Penk). We propose that these VP subpopulations comprise different limbic subcircuits that drive distinct motivated states during relapse and drug use. Here we examine VP cell type specific contributions to cocaine seeking and taking, and the synaptic physiology and ensemble states of these cells during addiction related behavior.

Methods: To investigate the anatomical connectivity and behavioral role of distinct VP cell types, we used virus-based anterograde and retrograde tracing methods, chemogenetics, miniature microscope Ca2 + imaging, and slice electrophysiology in Vglut2-IRES-Cre and Vgat-IRES-Cre, and Penk-IRES-Cre lines. Mice received bilateral virus injections, GRIN lens implants, indwelling jugular vein catheters, and cocaine self-administration training. The role of distinct VP cell types on the motivation to take and seek drugs was measured using progressive ratio (PR) and cue-induced reinstatement tests.

Results: VP-Glu, VP-GABA and VP-Penk neurons receive privileged innervation from the nucleus accumbens and other limbic structures. For instance, VP-Glu neurons (30% of VP population) are preferentially innervated by the nucleus accumbens D1-pathway, and Fos activated during reinstatement. During self-administration, individual VP-Glu neurons demonstrated strong response tuning during nosepokes for cocaine, while other cell types showed less well-defined responses. Chemogenetic stimulation of all VP neurons augmented cue-induced reinstatement of cocaine seeking, while inhibiting the VP reduced reinstatement. In contrast, selectively stimulating VP-Glu neurons reduced reinstatement, and abolished the motivation to take drugs during a PR test, while inhibiting VP-Glu neurons augmented these behaviors. Stimulation of VP-GABA neurons produced mixed effects by increasing motivation to take drugs, while decreasing the motivation to seek drugs during reinstatement. Stimulating VP-Penk neurons, on the other hand, strongly augmented both drug seeking and drug taking.

Conclusions: These results affirm that the VP is a critical regulator for addictive behaviors and expand our knowledge of the addiction circuitry by showing that motivational states are differentially regulated by distinct subpopulations of VP neurons. In addition, they reveal that VP-Glu, VP-GABA and VP-Penk neurons preferentially integrate with different basal ganglia subcircuits.

Disclosure: Nothing to disclose.

41.2 Ventral Pallidal Circuits in Risky Pursuit of Food and Cocaine

Stephen Mahler

University of California, Irvine, Irvine, California, United States

Background: Drug addiction is characterized by excessive drug use despite harmful consequences, as well as an inability to maintain abstinence after one makes a decision to quit. These features of addiction are thought to result in part from sensitized motivation for drug, resulting in strong cravings that are hard to resist. However, far less attention has been paid to the other side of the motivational coin—an apparent inability of the negative

consequences of use to prevent people from appropriately limiting or ceasing their intake.

Here we examine the roles of ventral pallidum (VP) neural subpopulations play in both excessive motivation for both drug and natural rewards, as well as insufficient appreciation of risk. Based on our prior work, and that of our collaborators and fellow panel members, we examine how VP input and output connectivity, and genetically-defined neuronal subpopulations promote or inhibit pursuit of risky rewards under mixed motivational drives.

Methods: In female and male rats, DREADDs or GFP was expressed in all VP neurons, or only VP GABA neurons of GAD1:Cre rats. First, rats with VP neuron hM4Di DREADDs were trained to self-administer cocaine, then to suppress this intake in a separate context where cocaine was delivered along with increasingly intense footshocks (Marchant et al, 2013). Subsequently, they were re-introduced (without drugs or shock) to the "safe" cocaine context, or the "unsafe" punishment/cocaine context with or without discrete cues. VP neurons were inhibited with CNO during punishment training, "safe" context reinstatement, and "unsafe" context suppression of seeking. We also quantified neural activity (Fos) in histologically-defined VP subregions during behaviors.

Second, we asked how manipulating VP GABA neurons affects perception of risk/reward balance. Adapting Orsini et al (2016), rats learned to choose between a small, safe reward and a large, risky one. Over the course of each daily session, choosing the larger reward becomes increasingly, and predictably likely to result in a mild footshock, and rats learn to shift their preference to the smaller safer reward accordingly. We examined effects of inhibiting or exciting VP GABA neurons on behavior and associated neural activity in VP efferent targets.

Results: Inhibiting VP neurons suppresses the ability of a safe context to reinstate cocaine seeking and facilitates shock suppression of seeking. Context-induced Fos expression varied by VP subregion, potentially implicating distinct accumbens inputs. Manipulating VP GABA neurons during risky decision making appeared to modulate perception of reward magnitude, rather than punishment likelihood.

Conclusions: We show an essential role for VP GABA circuits in determining costs and benefits of risky reward seeking, as occurs in addiction. In addition, being essential to the lives of prey species like rats, the balance of reward and aversion is likely also relevant to humans who continue to be tempted by drugs long after they decide to quit using them.

Disclosure: Nothing to disclose.

41.3 Recruitment and Disruption of Ventral Pallidal Value Encoding in Alcohol Seeking

Jocelyn Richard

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Background: A critical area of inquiry in the neurobiology of drug and alcohol abuse is the neural mechanisms by which cues gain the ability to elicit craving and relapse. We previously showed that the activity of ventral pallidum (VP) neurons in response to a cue predicting sucrose availability encodes both the likelihood and latency of subsequent instrumental reward-seeking actions. Here, we assessed representations of cue value by activity in ensembles of VP neurons during cue-elicited alcohol seeking under Pavlovian versus instrumental behavioral contingencies, and the impact of alcohol exposure itself on the integrity of this type of signaling.

Methods: Following 8 weeks of intermittent exposure to 15% alcohol, male and female Long Evans rats underwent either Pavlovian conditioning or training in a discriminative stimulus (DS) task, with 15% alcohol or 10% sucrose as the reward. During Pavlovian conditioning an auditory tone predicts the delivery of reward to a reward port. In the DS task, an auditory cue (the DS) indicates the availability of reward from the port if the animal enters the reward port during the cue period. Rats were trained in one of these tasks until they entered the reward port on > 70% of reward cue trials (CS + or DS), and < 30% of control cue trials (CS- or NS), in which no reward delivery ever occurred. Once rats met criteria, they were implanted with drivable microwire arrays aimed at VP. We then fit linear discriminant analysis (LDA) models trained on the activity of VP single units, or ensembles of VP neurons, to assess the strength of VP encoding of cue value.

Results: While similar proportions of VP neurons (30-35%) are excited by cues predicting alcohol availability (DS) or delivery (CS +), we found that decoding of cue value (reward versus control cue) was significantly blunted for an alcohol CS +, versus an alcohol DS, as well as in comparison to a sucrose DS or sucrose CS +. VP encoding of alcohol CS + value was not simply more distributed throughout the neural population, as decoding using increasing ensemble sizes of VP neurons did not significantly improve accuracy, with ensembles of 100 units only reaching ~60% accuracy for predicting the presence of the alcohol CS + versus the control cue (versus 90% accuracy in the other tasks). We also found that home cage alcohol exposure had opposing effects on VP encoding of cue value for a sucrose DS versus a sucrose CS + at all ensemble sizes, enhancing decoding accuracy for the DS and reducing decoding accuracy for the CS +.

Conclusions: Here we find that VP encoding of cue value is bidirectionally altered by exposure to and conditioning with alcohol, depending on the associative structure of the task. Specifically, alcohol exposure enhances VP responses to an instrumental cue, which encode the vigor of subsequent reward-seeking actions but reduces VP responses to a Pavlovian cue predicting outcome value. These findings suggest that neural circuitry involving the VP is a promising neural target to address problem alcohol seeking, which may result from biased engagement of specific decision-making processes that promote pathological motivated behavior.

Disclosure: Nothing to disclose.

41.4 Dissecting Ventral Pallidal Sub-Circuitry to Treat Reward-Related Disorders

Abstract not included.

Mini Panel

42. Quo Vadis Ketamine, Enantiomers, and Metabolites?

42.1 Is (S)-Norketamine an Alternative Antidepressant for (S)-Ketamine?

Kenji Hashimoto

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Background: The N-methyl-D-aspartate receptor (NMDAR) antagonist ketamine can produce rapid-onset and sustained antidepressant effects in treatment-resistant patients with major depression and bipolar disorder. (R,S)-Ketamine (Ki = 0.5 μM for NMDA-R) is a racemic mixture containing equal parts of (S)-ketamine (esketamine: Ki = 0.30 μM) and (R)-ketamine (arketamine: Ki = 1.4 μM). Previously, we reported that (R)-ketamine showed greater potency and longer lasting antidepressant effects

than (S)-ketamine in animal models of depression. (R)-ketamine and (S)-ketamine are metabolized to (R)-norketamine (Ki = 13.0 μM for NMDAR) and (S)-norketamine (Ki = 1.7 μM for NMDAR), respectively. Finally, these are metabolized to (2 R,6 R)-hydroxynorketamine (HNK) and (2 S,6 S)-HNK, respectively. Here, we compared the antidepressant and side effects of enantiomers of ketamine metabolites in animal models of depression.

Methods: The antidepressant effects of two enantiomers of norketamine, a major metabolite of ketamine, in the rodent models of depression were examined. Furthermore, we compared the side effects profiles of (S)-norketamine and its parent compound (S)-ketamine in rodents.

Results: (S)-norketamine had more potent antidepressant effects than (R)-norketamine in chronic social defeat stress (CSDS) model. Furthermore, (S)-norketamine induced more beneficial effects on decreased dendritic spine density and synaptogenesis in the prefrontal cortex (PFC) and hippocampus compared with (R)-norketamine. Unexpectedly, AMPAR antagonists did not block the antidepressant effects of (S)-norketamine. The electrophysiological data showed that, although (S)-norketamine inhibited NMDAR-mediated synaptic currents, (S)-norketamine did not enhance AMPAR-mediated neurotransmission in hippocampal neurons. Moreover, (S)-norketamine improved the reductions in brain-derived neurotrophic factor (BDNF)–tropomyosin-related kinase B (TrkB) signaling in the PFC of CSDS susceptible mice. The TrkB antagonist ANA-12 and a mechanistic target of rapamycin (mTOR) inhibitor rapamycin blocked the antidepressant effects of (S)-norketamine. Unlike (S)-ketamine, (S)-norketamine did not cause behavioral abnormalities, such as prepulse inhibition deficits, reward effects, loss of parvalbumin immunoreactivity in the medial PFC, or baseline γ-oscillation increase.

Conclusions: Our data identified a novel AMPAR activation-independent mechanism underlying the antidepressant effects of (S)-norketamine. Therefore, (S)-norketamine and its prodrugs could be novel antidepressants without the detrimental side effects of (S)-ketamine.

Disclosure: Inventor of the patent on R-ketamine, Patent; Inventor of the patent on S-norketamine, Patent

42.2 (R,S)-Ketamine and (2 R,6 R)-Hydroxynorketamine are Efficacious as Prophylactics Against Stress-Induced Depressive-Like Behavior in Female Mice

Christine Ann Denny

Columbia University, New York, New York, United States

Background: Females are more likely than males to develop major depressive disorder (MDD) after exposure to stress, a significant risk factor for MDD. We previously reported that the administration of (R,S)-ketamine before stress can prevent depressive-like behavior in male mice but have yet to assess efficacy in female mice or for other compounds. The goal of this study was to test the prophylactic potential of ketamine and its metabolites in females.

Methods: We administered (R,S)-ketamine or its metabolites (2 R,6 R)-hydroxynorketamine ((2 R,6 R)-HNK) and (2 S,6 S)-HNK at various doses 1 week before one of a number of stressors, including contextual fear conditioning (CFC), learned helplessness (LH), and chronic immobilization stress (CIS), in male and female 129S6/SvEv mice. Prophylactic efficacy was validated using the forced swim test (FST). To examine the interaction between ovarian hormones and stress resilience, female mice also underwent ovariectomy surgery (OVX) prior to drug administration.

Results: (2 R,6 R)-HNK was prophylactic against depressive-like behavior in males but did not attenuate learned fear. (R,S)-ketamine and (2 R,6 R)-HNK, but not (2 S,6 S)-HNK, significantly decreased stress-induced depressive-like behavior in females. (R, S)-ketamine and (2 R,6 R)-HNK were effective as prophylactics at lower in females than in males. Moreover, prophylactic efficacy was dependent on ovarian-derived hormones.

Conclusions: Our results suggest that prophylactics against stress-induced depressive-like behavior can be developed in a sex-specific manner. To our knowledge, this is the first demonstration of the prophylactic efficacy of (2 R,6 R)-HNK and of pharmacologically enhanced stress resilience in females.

Disclosure: Provisional Patent

42.3 The Ketamine Metabolite (2 R,6 R)-Hydroxynorketamine (HNK) Blocks NMDA Receptors to Impacts Downstream Signaling

Abstract not included.

Panel

43. Interpreting Psychiatric Risk Genetics at a Dynamic Synapse

43.1 Modulation of PSD Phosphorylation Networks in Complex Brain Disorders

Marcelo Coba

University of Southern California, Los Angeles, California, United States

Background: The postsynaptic site of neurons is composed of more than 1600 proteins arranged in protein-protein interaction complexes, the composition of which is modulated by protein phosphorylation through the actions of complex signaling networks. Components of these networks function as key regulators of synaptic plasticity, in particular hippocampal long-term potentiation (LTP). We triggered LTP in the mouse hippocampus CA1 region and then performed large-scale analyses to identify phosphorylation-mediated events in the PSD and changes in the protein interactome. We will show how LTP modulates protein phosphorylation in the PSD signaling machinery, how mutations in highly connected nodes alter protein–protein interactions, their role in complex brain disorders and their modifications through development

Methods: De novo coding mutations on exome-sequenced parent–proband trio cohorts from SCZ, ASD, DD and ID were used for analysis, using probands from unaffected siblings and from probands diagnosed with congenital heart disease but without associated neurodevelopmental or syndromic phenotypes (CHD-NS) as controls. We also analyzed de novo protein truncating variants (PTVs).

Immunoisolation, phosphoproteomics, Mass spectrometry identification and quantitation, and PSD fractions were prepared as described in Nature Neuroscience volume 20, pages 1150–1161 (2017). Synaptic plasticity and phosphoproteomics, assays were performed as described in Li et al, Science Signaling. 2016:Vol. 9, Issue 440

Results: We will show the composition of the PSD phosphoproteome machinery. We used liquid chromatography tandem MS (LC-MS/MS) to identify changes in protein phosphorylation that occurred after the induction of LTP by high-frequency tetanic stimulation of Schaffer collateral fiber synapse in the CA1 region of the mouse hippocampus. Such high-frequency synaptic

stimulation altered the phosphorylation status of 570 sites within 222 PSD proteins. We identified 81protein kinases and 20 protein phosphates associated with the PSD from a total of 1626 mouse PSD, and we show the effect of the induction of LTP in the modulation of the PSD kinome. We determined 4000 proteins across 50 in vivo interactomes and their relationship to complex brain disorders and their spatio-temporal organization during development.

Conclusions: The dynamic organization of the PSD links glutamate receptors signaling to kinases (writer), phosphates (eraser) as well as proteins that are able to recognize phosphorylation patterns of their binding partners (reader modules). The core scaffold of this signaling machinery starts to organize at embryonic day 14. We found a differential enrichment of complex brain disorders risk factors within the core scaffold machinery of the PSD. LTP regulated phosphoproteins, represented the "PSD risk" for schizophrenia and autism spectrum disorders. These sets of proteins are more likely to be multiple-phosphorylated during LTP and are more susceptible to integrate combinatorial patterns of phosphorylation.

Disclosure: Nothing to disclose.

43.2 Identification and Validation of Candidate Spine-Regulating Protein Phosphorylation Events in Schizophrenia

Matthew MacDonald

University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania, United States

Background: Reduced dendritic spine density has been reproducibly observed in multiple brain areas in schizophrenia (Sz) and is believed to underlie deficits in cortical processing. Dendritic spine plasticity is regulated by synaptic protein network features such as protein trafficking and activity, both of which are mediated by posttranslational modifications (e.g. phosphorylation). Finally, a significant number of Sz genetic risk loci code for synaptic proteins. Here, in a cohort of 50 Sz and matched controls, we utilized parallel microscopy, proteomic, and phosphoproteomic approaches to identify protein phosphorylations highly correlated with both dendritic spine loss and synaptic protein level alterations in Sz. The effects of one candidate phosphorylation (MAP2 S426) was evaluated in HEK293 cells and mice.

Methods: Fixed and Frozen auditory cortex grey matter from 50 pairs of Sz and matched control subjects was obtained from the Pitt Brain Bank. Fixed tissue was used for microscopy studies to assess layer 3 spines, frozen for targeted mass spectrometry (MS) to quantify 400 proteins in homogenates; Targeted MS to quantify 350 proteins in synaptosomes; and Differential MS to quantify > 2000 phosphorylation events in homogenates from a subset, 34 pairs. Postmortem interval and antipsychotic effects were measured in mouse and monkey models. MAP2 S426E was knocked into C57BL/6 J mice with CRISPR/Cas9. Preliminary phenotyping has been conducted by western blot, IP-MS, microtubule binding assay, and light microcopy in mice and transfected HEK293 cells.

Results: We observed robust changes to synaptosome and phosphorylation levels of canonical postsynaptic proteins in Sz, while controlling for multiple hypothesis testing ($q < 0.05$), postmortem interval, antipsychotic drug treatment, and pH. These alterations were not explained by corresponding changes in homogenate protein levels. WGCNA and cross-network analyses observed significant correlations ($q < 0.05$) between synaptosome, phosphorylation, and dendritic spine alterations in Sz. Nine phosphorylations on eight proteins were highly correlated with both synaptosome protein level alterations and spine loss. The phosphomimetic of one such site (MAP2 S426E) decreased MAP2 binding to microtubules and cortical volume.

Conclusions: Our finding of robust alterations to synaptosome and phosphorylation levels of canonical postsynaptic proteins (while their homogenate levels were unaltered) suggests that Sz genetic risk and synaptic protein network pathology manifests in processes beyond protein expression, such as trafficking and activity, both of which are regulated by phosphorylation. Of the eight proteins with spine and synaptic protein level correlated phospho-alterations, all but one have well documented roles in vesicular trafficking of postsynaptic glutamate receptors and spine regulation via F-actin binding, indicating that they could be upstream of spine loss in Sz. Preliminary analysis of one candidate phosphorylation (MAP2 S426E) found that this single modification was capable of impairing MAP2-microtuble binding and resulted in decreased cortical volume, a well replicated finding in Sz, further supporting a role for these phosphorylations in Sz pathology.

Disclosure: Nothing to disclose.

43.3 Functional Selectivity of G Protein-Coupled Receptor Signaling in Alzheimer's Disease

Amantha Thathiah

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Background: Alzheimer's disease (AD) is one of the most significant medical and societal challenges of our time and yet no current intervention strategies can halt or modify the underlying disease course. Neuropathologically, the AD brain is characterized by the accumulation of aggregates of the amyloid-β (Aβ) peptide and neurofibrillary tangles (NFTs) composed of the hyperphosphorylated microtubule-associated protein tau. Aβ is derived from proteolysis of the β-amyloid precursor protein (APP) following sequential cleavage by the β- and γ-secretases. G protein-coupled receptors (GPCRs) are involved in key neurotransmitter systems that are disrupted in AD patients and are also associated with multiple stages of APP proteolysis, indicating an intimate association between GPCRs and the molecular pathways involved in AD. However, the true molecular nature of these relationships remains only partially understood.

Methods: RNA interference and site-directed mutagenesis approaches were utilized to determine whether β-arrestins are required for the GPCR-mediated effect on Aβ generation. Immunoprecipitation and liquid chromatography-tandem mass spectrometry (LC-MS/MS) approaches were used to determine the putative GPR3 phosphorylation sites. A CRISPR/Cas9 genome editing strategy to generate phosphorylation and βarr2-signaling deficient mouse models.

Male and female mice were used for the proposed studies. Littermate pairs were used for all behavioral experiments. In order to achieve statistical significance in repeated measures ANOVA for behavioral testing, 10-12 animals per group was required for a power of 0.8. The number of animals in each group was based on statistical power analysis using the coefficient of variation from previous studies (Thathiah et al. (2013) Nat Med.; Huang et al. (2015) Sci Transl Med.).

Results: We identified the orphan GPCR GPR3 as a key modulator of γ-secretase activity and Aβ generation. Although canonical G protein-signaling is not involved in the GPR3-mediated modulation of γ-secretase activity, β-arrestin 2 (βarr2), which belongs to a small family of multifunctional GPCR adaptor proteins, specifically interacts with the γ-secretase complex and is required for the GPR3-mediated effect on Aβ generation.

Significantly, GPR3 and βarr2 expression is elevated in the brains of sporadic AD patients.

To determine the in vivo consequence of selective disruption of β-arrestin-dependent signaling on γ-secretase function, we determined that mutagenesis of specific serine residues in the C-terminus of GPR3 differentially regulates βarr2 recruitment and Aβ generation. These studies suggest that the GPR3 phosphorylation status is critically involved in regulation of the interaction with βarr2 and modulation of γ-secretase activity. Accordingly, we determined that a specific GPR3 phosphorylation barcode regulates βarr2 functional selectivity and γ-secretase activity in vivo.

Conclusions: These studies establish the fundamental importance of GPR3 phosphorylation and abrogation of βarr2-dependent signaling versus canonical G protein-signaling in Aβ pathology and provide the molecular basis of a selectivity barcode, likely critical for triggering physiological and pathophysiological outcomes.

Disclosure: Nothing to disclose.

43.4 The Role of SynGAP Mutations in Human Cognitive Disorders

Richard Huganir

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Background: Dynamic changes in synaptic connectivity in the central nervous system underlie information processing and higher brain processes such as learning and memory. SynGAP is a key synapse associated protein that is critical for this synaptic plasticity. SynGAP is a Ras-GTPase activating protein highly enriched at excitatory synapses that regulates several downstream signaling pathways. Phosphorylation and regulation of SynGAP activity is key for the induction of long-term potentiation (LTP) of excitatory synaptic transmission, a cellular model for learning of learning and memory. Furthermore, SynGAP heterozygote knockout mice have severe deficits in synaptic plasticity and learning and memory. De novo deleterious SYNGAP mutations have recently been found to account for a significant percentage of non-syndromic intellectual disability (ID) cases. SYNGAP variants have also been associated with other neuropsychiatric disorders including autism (ASD) schizophrenia (SCZ) and bipolar disorder (BP).

Methods: We have used molecular, cellular and electrophysiological methods and mouse model systems to analyze the functional effect of human SynGAP mutations on synaptic transmission and plasticity, as well as on behavior in mouse models. We are also performing mechanism-based drug screens to target disrupted SynGAP signal transduction pathways to discover small molecules that may ameliorate synaptic and behavioral deficits in the SynGAP heterozygote knockout mice.

Results: We have found that several ID-associated SynGAP mutations severely affect synaptic structure and function and plasticity. Most ID associated mutations are result in severe loss of SynGAP and either inhibit SynGAP targeting to synapses or inhibit the regulation of SynGAP function required for LTP. In contrast the SynGAP variants associated with ASD and SCZ are less severe mutations which appear to result in hypofunctioning of SynGAP. Preliminary results suggest that rare variant associated with BP may actually be gain of function mutations of SynGAP.

Conclusions: This data supports the idea that human SynGAP mutations might alter synaptic transmission and plasticity in the brain and that the severity of the mutations may be involved in several psychiatric disorders including ID, ASN, SCZ, and BP. These studies will allow us to gain insight into mechanisms underlying SynGAP-associated diseases and may pave the way for future novel therapeutic strategies.

Disclosure: Nothing to disclose.

Panel

44. Genomic Mechanisms in the Etiology of Bipolar Disorder

44.1 Genetic Pleiotropy Between Mood Disorders, Metabolic, and Endocrine Traits in a Multigenerational Pedigree

Maja Bucan

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Background: Bipolar disorder (BD) is a mental disorder characterized by alternating periods of depression and mania. Individuals with BD have higher levels of early mortality than the general population, and a substantial proportion of this is due to increased risk for co-morbid diseases.

Methods: To identify the molecular events that underlie BD and related medical comorbidities, we generated imputed whole genome sequence data using a population specific reference panel, for an extended multigenerational Old Order Amish pedigree ($n = 394$) segregating BD and related disorders. First, we investigated all putative disease-causing variants at known Mendelian disease loci present in this pedigree. Second, we performed genomic profiling using polygenic risk scores (PRS) to establish each individual's risk for several complex diseases.

Results: We identified a set of Mendelian variants that co-occur in individuals with BD more frequently than their unaffected family members, including the R3527Q mutation in APOB associated with hypercholesterolemia. Using PRS, we demonstrated that BD individuals from this pedigree were enriched for the same common risk-alleles for BD as the general population ($\beta = 0.416$, $p = 6 \times 10\text{-}4$). Furthermore, we find evidence for a common genetic etiology between BD risk and polygenic risk for clinical autoimmune thyroid disease ($p = 1 \times 10\text{-}4$), diabetes ($p = 1 \times 10\text{-}3$), and lipid traits such as triglyceride levels ($p = 3 \times 10\text{-}4$) in the pedigree. We identify genomic regions that contribute to the differences between BD individuals and unaffected family members by calculating local genetic risk for independent LD blocks.

Conclusions: Our findings provide evidence for the extensive genetic pleiotropy that can drive epidemiological findings of comorbidities between diseases and other complex traits.

Disclosure: Nothing to disclose.

44.2 Neuronal RNA Splicing in Major Adult Psychiatric Disorders

Abstract not included.

44.3 Increasing and Interpreting Bipolar Disorder Risk Loci From GWAS

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Background: Bipolar disorder (BD) is a severe psychiatric disorder that features episodes of mania and depression. A lifetime prevalence of 1-2%, elevated morbidity and mortality, onset in

young adulthood, and a frequently chronic course make BD a major public health problem and a leading cause of the global burden of disease. BD heritability has been estimated to be more than 70% in twin studies, indicating a substantial involvement of genetic factors in the disorder. Genome-wide association studies (GWAS) have identified over 30 bipolar disorder risk loci, with strong evidence that additional studies in larger samples will yield more discoveries and biological insights into the disease. Here we describe the current state of bipolar disorder GWAS in the Psychiatric Genomics Consortium, including unpublished results with 35,000 cases.

Methods: GWAS data have been generated for new samples using the Illumina PsychArray, or Psychchip, and meta-analyzed with the PGC2-BD GWAS and several other existing GWAS for BD. All GWAS were imputed to the Haplotype Reference Consortium reference panel. Polygenic scoring and LD-score regression approaches are used to investigate the genetic relationships among bipolar, related psychiatric disorders, behavioral traits and clinical psychiatric subphenotypes. Gene-level GWAS were conducted with MAGMA, and with Predixcan to integrate with available post-mortem brain eQTL data; pathway analyses were run on these results using MAGMA.

Results: Psychchip data from 16 studies have been analyzed, totaling 11,346 cases and 18,191 controls after quality control and removal of related individuals. GWAS meta-analysis was conducted combining the Psychchip samples, additional data used in previous replication analyses, and the PGC2-BD GWAS, for a current grand total of 34,695 cases and 65,115 controls. We report novel and previously known genome-wide significant BD risk loci from this analysis. Pathway analyses, including integrating with other genomic and functional data, reveal neuronal and synaptic mechanisms and suggest energy metabolism and mitochondrial dysfunction in bipolar disorder pathogenesis. Analyses with GWAS for other psychiatric and related traits highlight shared and specific genetic causes between BD and schizophrenia, and among BD subtypes and subphenotypes.

Conclusions: We report the identification of new loci conferring risk of bipolar disorder in genome-wide association study of nearly 35,000 cases and over 65,000 controls. Annotating BD risk loci both functionally and in terms of effects on other psychiatric phenotypes, provides insights into the biology of bipolar disorder and other psychiatric disorders. We also discuss challenges and initiatives for bipolar disorder to achieve greater sample size and further success in GWAS locus discovery.

Disclosure: Nothing to disclose.

44.4 Transcriptional Mechanisms of Genetic Risk Variants for Bipolar Disorder

Peter Zandi

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Background: Although bipolar disorder (BP) is among the most heritable serious mental illnesses, it is only recently that genome-wide association studies (GWAS) conducted by the Psychiatric Genomics Consortium (PGC) have begun to identify credible genetic associations with the disorder. However, the neurobiological mechanisms by which implicated variants increase risk for BP remain unknown. The Lieber Institute, which specializes in studying the neuro-developmental origins of mental disorders, has amassed a large collection of post-mortem brain samples from psychiatric patients and non-mentally ill control subjects. These samples provide a rich resource for examining the neurobiological mechanisms by which genetic factors contribute to mental disorders directly in the primary affected tissue.

Methods: We carried out RNA sequencing with these samples in three key brain regions of the frontal cortex and limbic system thought to play a role in mood disorders: the dorso-lateral prefrontal cortex (DLPFC), the amygdala (AMYG) and the subgenual anterior cingulate cortex (sACC). The samples were processed using the RiboZero protocol and sequenced to a median depth of 132 M reads. They were also genotyped with Illumina SNP Chips and imputed using the 1000 Genomes Phase 3 reference panel. After quality control, data was available on 678 samples (268 sACC, 243 AMYG, and 167 DLPFC). To examine in detail the transcriptional effects of SNPs implicated by the PGC study of BP, we separately modelled gene, exon, junction, and transcript expression level features as a function of SNP genotypes using linear regression and controlling for diagnosis, sex, principal components of the expression data to adjust for "batch" effects, and principal components of the genotype data to adjust for population sub-structure.

Results: Of the 31 genome-wide significant loci reported by the latest PGC study of BP, significant cis ($+500$ Kb) eQTLs were identified in 18, of which 11 were observed across all 3 brain regions. In 8 of these loci, the eQTLs revealed specific transcriptional effects with a single gene (SCN2A, ZCCHC2, GRIN2A, LMAN2L, STK4, SSBP2, MMS22L, and BBX). The most significant association was with SCN2A in which the lead SNP abrogated the first junction ($p = 5.08 \times 10\text{-}29$) and led to decreased expression of transcript ENST0000636985 ($p = 8.58 \times 10\text{-}15$). Of the 550 additional suggestive loci reported by the PGC, 69 yielded significant eQTLs with a single gene, providing mechanistic evidence that these may also be true susceptibility genes.

Conclusions: These findings provide evidence that reveal the detailed transcriptional mechanisms underlying genetic associations with BP identified through GWAS. The mechanistic understanding is needed to begin developing targets for more rational treatment development.

Disclosure: Nothing to disclose.

Panel

45. Neural Circuitry Underlying Retrieval of Reward and Fear Memories

45.1 Thalamo-Striatal Pathways Regulating Fear and Reward-Seeking Responses

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Background: The ability to survive in nature depends on a balance between foraging for food and avoiding dangerous situations. Recent studies have demonstrated that neurons in the paraventricular nucleus of the thalamus (PVT) change their activities during the presentation of both food and fear-associated cues, making this region a strong candidate to regulate reward and fear responses. Here, we combined pharmacology, optogenetics and single-unit recordings in freely behaving rats to investigate which PVT circuits and neuronal subpopulations are involved in the competition between fear and food-seeking behavior.

Methods: Male Long-Evans adult rats previously implanted with guide cannulas, optical fibers or electrodes aiming at the PVT were initially trained to press a bar for sucrose in the presence of cues

(reward cues). Cat saliva was used to induce innate fear responses (predator odor). During a competition test, reward cues and predator odor were simultaneously presented, and rats needed to overcome their fear of predator cues to search for food.

Results: Rats exposed to predator odor alone showed stronger defensive behaviors characterized by increased freezing and avoidance responses, when compared to neutral odor controls (Freezing, neutral = 1.8% vs. predator odor = 38.1%, $p < 0.001$; Avoidance, neutral = 36.5% vs. predator odor = 75.7%, $p = 0.007$, Unpaired Student t test). Predator odor exposure also increased the expression of the neural activity marker cFos in the anterior portion of PVT (aPVT), compared to neutral odor ($p < 0.05$). Single-unit recordings from aPVT neurons revealed two distinct populations of neurons that changed their firing rate in response to either predator odor or reward cues, compared to baseline (Z-score > 2.54 for excitatory and < -1.96 for inhibitory responses). During the competition test, inactivation of aPVT with the GABAA agonist muscimol increased time approaching the food area and reduced time avoiding the predator odor area ($p < 0.05$). Notably, inactivation of aPVT had no effect when the predator odor or the food-seeking tests were carried out independently. Cat odor exposure also induced a suppression of food-seeking responses when the animals were tested in a neutral context. Because predator odor exposure activates the stress neuropeptide corticotropin-releasing factor (CRF), we speculated that CRF release would play a role in food-seeking attenuation. Consistent with our prediction, photoactivation of aPVT-CRF neurons reduced food-seeking and increased avoidance responses (presses/min, eYFP-Control: 18.8 ± 1.2 vs. CRF-ChR2 = 4.2 ± 1.1, $p < 0.05$). Neuroanatomical investigation of aPVT-CRF efferents revealed dense projections to the nucleus accumbens shell (NAcsh). Slice recordings from NAcsh neurons demonstrated that photoactivation of aPVT-CRF fibers in the NAcsh elicits large excitatory postsynaptic responses, which were blocked by AMPA and NMDA receptor antagonists.

Conclusions: Our results demonstrate that aPVT activity is necessary to balance food seeking with fear responses, thereby allowing the most appropriate behavior. A defined subpopulation of glutamatergic CRF-expressing neurons in the aPVT, which is sufficient to mediate anorexigenic and aversive effects, may be participating in this balance.

Disclosure: Nothing to disclose.

45.2 Two-Photon Calcium Imaging of Cell-Type Specific Neurons and Afferent Axons in the Paraventricular Thalamus During Appetitive Learning

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Background: The paraventricular nucleus of the thalamus (PVT) is innervated by cortical and subcortical structures that underlie appetitive learning and reward seeking, such as prelimbic cortex (PLc) and lateral hypothalamus (LH). Furthermore, activation of PVT neurons that project to the nucleus accumbens (PVT-NAc) can prevent consummatory behaviors, although how these neurons might function in vivo to control behavior is unknown.

Methods: Here we use gradient refractive index (GRIN) lenses to gain optical access to the PVT of mice that express the genetically-encoded calcium indicator GCaMP6s in cell bodies of PVT-NAc neurons (n = 406 neurons), in PLc axons in PVT (n = 48 axons) or LH axons in the PVT (n = 61 axons). Used in combination with two-photon microscopy, we study the activity dynamics of these distinct circuit elements during appetitive learning and reward seeking. Finally, we combine this approach with patch-clamp electrophysiology and optogenetics to determine how PLc and LH axons functionally control the encoding of behaviorally-relevant information at the level of PVT-NAc neurons.

Results: We record the activity dynamics > 400 PVT-NAc neurons across appetitive learning and find that these cells gain inhibitory responses to reward-predictive stimuli in a manner that is predictive of learning (~33% develop inhibitory responses; $p < 0.001$). In addition, we find that glutamatergic axons from the PLc acquire similar responses across learning (~54% develop inhibitory responses, $p < 0.001$), whereas GABAergic axons from LH show excitatory responses to reward consumption (~44% show excitatory responses; $p < 0.001$). Finally, optogenetic activation of PLc-PVT inputs during the presentation of a reward-predictive stimulus disrupts the expression of inhibitory responses in PVT-NAc neurons ($p < 0.001$), showing that PLc inputs control stimulus encoding in downstream PVT-NAc circuitry. Ongoing experiments are now elucidating the function of LH-PVT inputs for stimulus encoding in PVT-NAc neurons.

Conclusions: These data reveal that PVT integrates learning and feeding signals from the PLc and LH, respectively, and uses this information to drive downstream activity to the NAc for reward-seeking behaviors.

Disclosure: Nothing to disclose.

45.3 Chemogenetic Inhibition of Prelimbic Cortical Output to Paraventricular Nucleus of the Thalamus Prevents Anxiety-Related Behaviors and Attenuates Cocaine Seeking

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Background: Background A single infusion of brain-derived neurotrophic factor (BDNF) into the prelimbic (PL) cortex of rats immediately after the last cocaine self-administration session suppresses cocaine-seeking after abstinence or extinction. This effect is blocked by inhibiting NMDA receptors in the PL cortex. To dissect the contribution of different cortical efferents to the ability of BDNF to attenuate relapse, in this study we used a combinatorial viral approach to selectively express hM4Di DREADD or mCherry in PL neurons that project to the nucleus accumbens (NAc) core or the posterior paraventricular thalamic nucleus (pPVT).

Methods: Methods Male rats were implanted with a jugular catheter. The NAc core or pPVT of rats was infused bilaterally with a retrogradely transported adenovirus. Then the PL cortex was infused with AAV5-hSyn-DIO-mCherry or AAV5-hSyn-DIO-hM4Di-mCherry. Immediately after the last of 14 cocaine self-administration sessions, all rats received an injection of CNO (10 mg/kg, i.p.) followed 30 min later by an intra-PL infusion of PBS or BDNF. After 6 days of abstinence, rats underwent a post-abstinence test under extinction conditions, extinction training to criterion, and a cue-induced reinstatement test. Rats were intracardially perfused and brains were processed for mCherry, eGFP, BFP, and Fos immunoreactivity. A subset of rats underwent testing on an elevated zero maze to assess anxiety and in an operant runway task in which they received a cocaine injection in the goalbox.

Results: Results As expected, Infusion of BDNF in the mCherry-control rats suppressed cocaine-seeking after abstinence and extinction ($p < 0.01$). However, activation of hM4Di DREADD in the PrLNAc core pathway had no effect on drug-seeking in PBS-infused rats but blocked the BDNF-mediated suppression of relapse ($p < 0.01$). Interestingly, inhibition of PrLpPVT, a brain

region linked to stress and anxiety, blocked subsequent cocaine-seeking ($p < 0.01$) and an intra-PL BDNF infusion reversed this effect ($p < 0.01$). Moreover, 2 h after the last cocaine self-administration session we found that Fos-IR was significantly increased in pPVT ($p < 0.01$), suggesting that early withdrawal may engage anxiety-related structures that support subsequent relapse. To test whether the pPVT mediates anxiety-related behaviors, rats were tested in the elevated zero maze 2 h after the last cocaine self-administration session. Preliminary data indicate that activation of hM4Di DREADD in the PrLPVT pathway decreased anxiety-related behaviors compared to mCherry control rats. To strengthen the hypothesis that pPVT contributes to the aversive effect of cocaine after its acute rewarding effects disappear, we investigated whether inhibition of pPVT with GABA receptor agonists, baclofen/muscimol, would block avoidance conditioning in an operant runway task. As expected, PBS-infused rats showed a progressive increase in the run time toward the goalbox and the number of reversals in the runway, demonstrating aversion to the anxiogenic effects of cocaine. However, infusion of baclofen/muscimol into pPVT blocked the development of conditioned avoidance ($p < 0.001$).

Conclusions: Conclusions These data demonstrate that inhibition of PrLpPVT pathway decreases to cocaine-seeking and inhibition of pPVT itself prevents anxiety-like behaviors that ultimately contribute to drug-seeking.

Disclosure: Nothing to disclose.

45.4 CB1 Receptor-Mediated Limbic Plasticity During Memory Reconsolidation Shapes Cocaine Memory Strength

Rita Fuchs

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Background: The post-retrieval reconsolidation of labile context-response-cocaine associative memories into long-term memory stores is critical for the maintenance of drug context-induced motivation for cocaine over time. Hence, it has been theorized that interference with context-response-cocaine memory reconsolidation can mitigate relapse propensity. Inhibition of protein synthesis in the basolateral amygdala (BLA) during the putative time of memory reconsolidation attenuates subsequent drug context-induced cocaine-seeking behavior. Moreover, protein synthesis-dependent cocaine memory reconsolidation in the BLA is dependent on neural activity in the dorsal hippocampus (DH). We hypothesized that cannabinoid type 1 receptor (CB1R) stimulation regulates memory reconsolidation given its critical role in synaptic plasticity. Thus, we evaluated whether systemic or intra-BLA CB1R antagonism during memory reconsolidation regulates (a) plasticity-related proteins (PRP) expression/activation in the BLA or DH, (b) hypothalamic-pituitary-adrenal (HPA) axis activation, and (c) subsequent context-induced cocaine-seeking behavior.

Methods: Sprague-Dawley rats were trained to lever press for cocaine infusions in a distinct environmental context (≥ 10 days) followed by extinction training in a different context (7 days) during daily 2-hour sessions. On post-cocaine day 8, rats received CB1R antagonist (AM251; 3 mg/kg, IP or 0.3 µg/0.5 µL/hemisphere, intra-BLA) or vehicle (VEH) treatment (a) with memory reactivation (i.e., immediately after re-exposure to the drug-paired context expected to prompt memory retrieval, destabilization, and reconsolidation) or (b) without memory reactivation (i.e., with treatment administered outside of the time window of memory reconsolidation or after re-exposure to the home cage). The effects of systemic AM251 or VEH treatment were assessed on (1) immediate-early gene expression (zif268, ARC) and glutamate receptor subunit expression and activation (GluA1, GluA2, GluN2b) in the BLA and DH during the putative time of memory reconsolidation, and (2) drug context-induced cocaine-seeking behavior 72 h later. Further, the effects of intra-BLA AM251 or VEH treatment were assessed on (3) serum corticosterone concentrations and (4) cell type-specific neuronal activation in the BLA during the putative time of memory reconsolidation and on (5) drug context-induced cocaine-seeking behavior 72 h later.

Results: Systemic AM251 treatment after, but not without, memory reactivation inhibited memory reconsolidation-associated increases in PRP expression/activation in the BLA and DH, relative to VEH. Furthermore, it attenuated drug context-induced cocaine-seeking behavior and concomitant PRP expression in the BLA and DH at test, relative to VEH. In contrast, intra-BLA AM251 treatment after, but not without, memory reactivation augmented serum corticosterone concentrations and BLA neuronal activation during the putative time of memory reconsolidation and potentiated subsequent cocaine-seeking behavior at test, relative to VEH.

Conclusions: Together, these findings suggest that CB1R populations are functionally heterogeneous. CB1R populations bidirectionally control drug context-induced motivation for cocaine possibly by regulating BLA and DH cellular plasticity, HPA axis activation, and cocaine memory strength during memory reconsolidation.

Disclosure: Nothing to disclose.

Panel

46. Neuroimaging as a Novel Tool to Enhance the Development of Psychotherapeutic Strategies

46.1 Cognitive Reappraisal Strategies to Enhance Emotion Regulation in Borderline and Avoidant Personality Disorders: Insights From Neuroimaging

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Background: Borderline Personality Disorder (BPD) is the prototypical disorder of affective instability (AI). AI is associated with the high levels of emotional distress, functional and social impairment, and suicidality seen in BPD, yet it shows limited response to current pharmacologic and psychotherapeutic approaches. Building upon our previous work that showed that BPD patients showed impaired performance and anomalous prefrontal activity compared to healthy volunteers (HV) in utilizing the adaptive emotion regulatory strategy of cognitive reappraisal, we designed the current study to examine whether BPD patients could be trained to more effectively utilize reappraisal and normalize neural activity. Avoidant personality disorder (AvPD) patients were included as a psychopathogical control.

Methods: At each of six sessions over approximately 4 weeks, 19 BPD, 21 AvPD and 15 HV participants were shown negative social emotional images. Subjects were instructed in the reappraisal by psychological distancing tactic, which involves viewing stimuli as an impartial, objective observer. They were then shown a series of emotional pictures and instructed, on a picture-by-picture basis, to either reappraise or simply look without attempting to regulate. Emotion self-reports were obtained after each image presentation. Sessions 1-5 were spaced approximately two days apart and afforded focused reappraisal training through guided practice. Session 6 was a follow-up session using the same

training and task approximately 2 weeks following session 5. fMRI data were acquired at Sessions 1, 5, and 6.

Results: BPD and AvPD patients showed decreases in negative emotion self- reports during reappraisal-by-distancing over the course of the training period, with AvPD patients achieving scores comparable to HCs by session 2 and BPD patients, who rated the images significantly more negatively than HCs at sessions 2 and 3 ($p < .01$, each day), did not differ from HCs by sessions 4, 5 and at follow-up session 6. A condition (reappraise vs. look) analysis across groups on day 1 indicated left ventrolateral (VLPFC) and right dorsolateral prefrontal (DLPFC) cortices were engaged during reappraisal (both $p < .05$ FWE). In the BPD group only, training was associated with an increase in L VLPFC activation during reappraisal vs. look and this training-related change in reappraisal activation was significantly greater in BPDs compared to AvPDs ($p < .05$). A similar effect was seen at trend level in the DLPFC ($p < .08$). Moreover, in the DLPFC, increased post-training reappraisal related activity was associated with improved behavioral reappraisal success ($r = .39$, $p < .05$, one-tailed).

Conclusions: These data represent the first evidence that longitudinal training can increase reappraisal success and normalize reappraisal neural activity in any patient population and suggest a potential translational role for reappraisal training in BPD treatment.

Disclosure: Nothing to disclose.

46.2 Neural Mechanisms of Enhancing Emotion Regulation via Cognitive Reappraisal in Bereaved Spouses

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Background: Cognitive reappraisal involves reframing an emotional stimulus in a way that changes its emotional impact. Reappraisal has been shown to be effective in single sessions, eliciting prefrontal cortex (PFC) to down-regulate amygdala activity. Little is known, however, about whether one can improve over time in implementing reappraisal, and how reappraisal responses endure in both healthy and stressed populations. Bereavement upon the death of a spouse represents a profound life change stress, typically involving acute suffering and substantial dysphoria in everyday life activities often persisting for years.

Methods: Reappraisal was operationalized using two different tactics: psychological distancing and reinterpretation. Distancing involves thinking about an emotional stimulus as an impartial, objective observer. Reinterpretation, by contrast, involves imagining a better outcome, or that the situation is not as bad as it first seemed. Recently bereaved male and female spouses ($N = 19$) underwent five sessions of reappraisal training over two weeks using either distancing or reinterpretation and bereavement-specific images, with longitudinal collection of depressive symptoms, grief rumination, respiratory sinus arrhythmia (a measure of heart rate variability linked to adaptive stress responses), and neural activity via fMRI.

Results: Preliminary analyses have revealed that, in contrast to reinterpretation training, distancing training in recently bereaved spouses is associated with reduction in depressive symptoms and grief rumination ($p < 0.02$), and longitudinal engagement of dorsolateral prefrontal cortex activity ($p < 0.01$). Further, greater longitudinal decrease in depressive symptoms was associated with greater longitudinal increase in respiratory sinus arrhythmia ($p < 0.05$), which has previously been positively associated with adaptive health outcomes.

Conclusions: These results suggest that reappraisal, particularly reappraisal-by-distancing, is trainable and adaptive in bereaved spouses. Future work is warranted to assess the durability of these reappraisal training effects both in laboratory and real-world contexts.

Disclosure: Nothing to disclose.

46.3 PTSD Psychotherapy Alters Frontopolar Intrinsic Connectivity Patterns

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Background: Trauma-focused psychotherapy is a first-line, efficacious treatment for post-traumatic stress disorder (PTSD). Though various efficacious trauma-focused therapies utilizing divergent treatment techniques exist, it has been challenging to demonstrate superiority of one active treatment type over another, and to isolate individual-level characteristics rendering one form of psychotherapy better-suited to a particular individual. Efforts to improve treatment outcomes by designing better interventions and/or matching individuals to the most appropriate psychotherapy may remain unsuccessful due to the fact that such efforts are rarely informed by an understanding of the brain mechanisms by which a particular treatment exerts therapeutic benefit. A neuroscience-informed model of how or why a particular treatment works is crucial to optimizing intervention construction and delivery. Towards this end, the authors previously published findings demonstrating that prolonged exposure therapy, the gold-standard exposure-based PTSD intervention, alters frontopolar activation and connectivity during emotion regulation. Here, we report on how psychotherapy changes instinct connectivity patterns of the frontopolar cortex in PTSD during an unconstrained resting state.

Methods: Sixty-six individuals with PTSD were recruited to undergo functional magnetic resonance imaging (fMRI) during an open-eyes resting state. Individuals were then subsequently randomized to immediate treatment with prolonged exposure, delivered over 9-12 once or bi-weekly sessions by a trained clinical psychologist, or to a treatment waitlist over a comparable period of time. Individuals were then reassessed clinically and with fMRI one month following cessation of the treatment or waitlist.

Results: At post-treatment, intent-to-treat linear mixed models revealed that the frontopolar cortex displayed prominent treatment-specific shifts in intrinsic connectivity patterns at rest from pre- to post-treatment (all FDR-corrected $p's < 0.05$), characterized by decreased connectivity with regions implicated in affect and memory (brainstem, anterior hippocampus, parahippocampal gyrus, mediodorsal thalamus, posterior cingulate) and increased connectivity with regions implicated in sensory processing (visual cortex, primary sensory cortex, posterior hippocampus, and ventral middle insula).

Conclusions: Exposure-based psychotherapy for PTSD exerts prominent therapeutic effects on both emotional regulatory dynamics and intrinsic connectivity of the frontopolar cortex, the most highly-evolved region of the human brain. These findings provide evidence that frontopolar cortical function is a key mechanism of psychotherapeutic benefit and furthermore implicate functional change as being both affective domain specific as well as generalized and pervasive when the brain state is unconstrained. Enhancement or augmentation of frontopolar cortical function with brain modulation or novel interventions may provide one avenue to improving psychotherapy outcomes for trauma survivors.

Disclosure: Nothing to disclose.

46.4 Brain Network Changes Associated With Transdiagnostic Depressive Symptom Improvement Following CBT

Abstract not included.

Study Group

47. Bundling the Haystacks and Finding the Needle: Enhancing Rigor and Reproducibility in Early Life Stress Research

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Study Group Summary: Considering the vast number of children worldwide growing up under some form of chronic early life stress (ELS), the importance of understanding how ELS influences development is undeniable. Since many of the symptoms resulting from ELS often first emerge later in life, intervening variables found in clinical studies make the role that ELS plays in these diseases difficult to interpret. Animal models have therefore been helpful to clarify the causality of ELS, however they also bring new issues including the translatability to human experience, strain and species differences, and paradigm differences between laboratories. Indeed, the increasingly wide variability in species, strains, and paradigms used to model ELS has made the task of identifying underlying mechanisms akin to finding a needle in a haystack. Currently, rigor and reproducibility of ELS studies are hindered by a lack of a standard operational definition of ELS, and a lack of training opportunities to consult and establish a consensus on the appropriate design, implementation, and reporting of this work. The goals of this study group are to (1) foster discussion on the best practices to be implemented in ELS studies; (2) connect animal researchers with clinical researchers to share ideas about what is needed for effective translation; (3) establish a consensus for what ELS is (and what it isn't), as well as how experimental design should be reported.

In this session, we will compare and contrast different models of postnatal ELS, including the limited bedding paradigm, maternal separation, and maternal deprivation. The participants will address the intended and unintended consequences of various ELS paradigms, and how each of these factors can vary between laboratories. Emphasis will also be placed on methodological considerations like strain and sex differences, timing of stress exposure, reporting maternal behavior alterations, and breeding issues. Finally, presenters will discuss how the various human experiences of ELS can be best modeled, and how animal researchers and clinical researchers can report findings to each other with the most transparency.

We believe that our study group discussion will facilitate the development of best practice guidelines for ELS models in order to improve the rigor, reproducibility, and transparency of this research.

Disclosure: Nothing to disclose.

Panel

48. Intergenerational Transmission of Threat Learning in Normative and Atypically Developing Youth

48.1 Neural Mechanisms of Intergenerational Transmission of Threat Learning

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Background: Humans learn much by observing others, including what to fear and what to trust in our environment. Observational threat learning may be especially important early in life when children turn to their parents to gather information about their world. Yet, the vast majority of empirical research on threat learning in children has thus far focused on firsthand classical conditioning, which may fail to capture one of the primary means by which children acquire fears. To address this gap in the literature, the present neuroimaging study examined observational threat learning in children and adolescents.

Methods: 33 children and adolescents (age range: 6-17 years; 21 female) were scanned while watching videos of their parent and an unfamiliar adult undergo fear conditioning (acquisition phase). Participants were then presented with the CS + and CS- from the videos (test phase). Participant's liking ratings for the CS + and CS- were subjected to a repeated-measures ANOVA to examine learning that utilized participant age, model (parent vs. stranger), condition (CS + vs. CS-) and time (baseline, post-acquisition and post-test) as predictors. Activation and functional connectivity estimates in the amygdala and medial prefrontal cortex were extracted and also subjected to repeated-measures ANOVAs with participant age, model (parent vs. stranger), condition (CS + vs. CS-) and time (baseline, post-acquisition and post-test) as predictors. Parents completed the State-Trait Anxiety Inventory and also reported on their children's anxious traits (using the Screen for Child Anxiety-Related Disorders. Follow-up tests were conducted to assess the role of anxiety in learning. A subset ($n = 19$) of parents also underwent fMRI scanning while completing fear conditioning and exploratory analyses are planned to explore parent-child neural synchrony in the amygdala and medial prefrontal cortex.

Results: Children and adolescents demonstrated robust observational learning, as indicated by changes in their self-reported liking for the CS + (a geometric shape that was paired with an aversive noise 80% of the observed trials) and CS- (a geometric shape that was never paired with an aversive noise on the observed trials) over time ($F(2,30) = 5.5$, $p < .01$). Participants showed enhanced learning for their parent, as evidenced by a model x CS interaction ($F(1,31) = 4.24$, $p < .05$). Adding parent and child anxiety symptoms to the model revealed that children of high-anxiety parents learned better over time for their parents relative to strangers ($F(2,20) = 3.82$, $p < .05$). Left amygdala activation revealed a marginally significant interaction between model, time and CS condition such that parents, but not strangers, elicited greater amygdala responses to the CS + versus the CS- over time ($F(1,31 = 4.03$, $p = .05$). Parent anxiety predicted negative amygdala-mPFC functional connectivity (Beta = -.02, 95% confidence intervals = -.04, -.005) and this connectivity predicted changes in learning, even after controlling for parent anxiety (Beta = .91, 95% confidence intervals = .21, 1.62). A bootstrapped mediation analysis revealed that the indirect effect of parent anxiety on learning via amygdala-mPFC connectivity was significant, indicating that amygdala-mPFC connectivity mediated the relationship between parent anxiety and child learning (Beta = -.02, 95% confidence intervals = -.06, -.001).

Conclusions: These results suggest that youth preferentially learn about threats by observing their parents relative to unfamiliar adults. Moreover, these findings suggest that

observational learning is influenced by emotional traits in parents such as anxiety symptoms.

Disclosure: Nothing to disclose.

48.2 Psychophysiological Correlates of Observational Threat Learning in Girls and Boys

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Background: Fear conditioning paradigms have been widely used to better understand the mechanisms underlying threat and safety learning. Importantly, a number of fears can develop without being directly exposed to the aversive stimulus. Observational threat learning refers to the ability of learning an association between a neutral cue and an aversive stimulus, by observing someone else's experience. Given that multiple fears develop during childhood and that children are highly influenced by their familial environment and are sensitive to their parent's emotions, it is necessary to examine observational threat learning in families. The goal of this study was to develop and validate a novel observational threat learning paradigm in children and to compare physiological measures of fear in boys and girls learning from their parent and from a stranger adult.

Methods: Twenty-six parent-child dyads with children aged 8 to 12 were recruited. Children (14 girls and 12 boys) and parents were healthy and did not suffer from any psychopathology. The parent was first exposed to a classical fear conditioning paradigm during which two stimuli were presented, one that was partially reinforced (i.e., paired with a shock; red light, CS + Parent) and one that was not paired with a shock (yellow light, CS-). A stranger adult (same sex than the participating parent) was exposed to a similar procedure, where a different stimulus was reinforced (blue light, CS + Stranger) and the same stimulus was used as the CS- (yellow light). These procedures were filmed and were then presented to the child. After watching the videos, the shock electrodes were attached to the child's fingers. The stimuli (colors) were then presented directly to the child, while skin conductance responses (SCR) were recorded. The first two presentations of each stimulus (CS + Parent, CS + Stranger and CS-) were used to test fear acquisition in the child.

Results: Results showed a main effect of Stimulus, $F(2,48) = 3.086$, $p = 0.055$ and a main effect of Sex, $F(1,24) = 3.868$, $p = 0.061$. Overall, children exhibited higher SCR to the CS + Parent and the CS + Stranger relative to the CS-. Moreover, girls had higher SCR than boys across all stimuli. The Stimuli X Sex interaction did not reach significance, $p > 0.26$.

Conclusions: These results suggest that this novel paradigm can be used to study observational threat learning in children aged 8 to 12 years old. Moreover, our data suggest that children exhibit similar fear responses when learning from their parent than when learning from a stranger adult. Girls exhibited higher fear responses than boys to all stimuli. Therefore, these data support the importance of studying sex differences with regards to threat learning, even in pre-pubertal samples.

Disclosure: Nothing to disclose.

48.3 Parent-Child Extinction Learning in Typically Developing Youth and Pediatric PTSD: A Preliminary Study

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Background: Prior studies have documented vicarious threat learning in youth through parent observation. When operating normally, vicarious threat and extinction learning are adaptive. However, inappropriate threat transmission increases risk for anxiety in youth and may mediate the effects of stressors on youth anxiety. Yet, little is known about vicarious extinction learning in typically developing youth, how this process compares to direct extinction learning, and whether these processes are abnormal in youth with stress-related disorders such as PTSD. In preliminary studies, we explored the feasibility of conducting direct and vicarious extinction learning in non-traumatized typically developing (TD) youth and youth with PTSD.

Methods: We tested threat learning in youth ages 8-17 years and their mothers using a 3-day threat learning and extinction paradigm. In a validation study, we used a standard threat paradigm in 10 TD youth (ave. 10.8 yrs, 6 F) and their mothers in parallel (no vicarious learning). This paradigm includes 2 CS + s (paired with mild electrical stimulation), which were either directly extinguished (CS + D) or left unextinguished (CS + U). Next, we tested direct and vicarious extinction learning in 10 new TD youth (ave. 13.7 yrs, 4 F) and 6 youth with PTSD (ave. 13.3 yrs, 5 F) with their mothers. In this novel task adaptation, CS + s were either directly extinguished in youth (CS + D) or vicariously extinguished (CS + V) by watching a video of their mother complete extinction training. Skin conductance response (SCR) was used as the primary dependent measure and was analyzed using linear mixed effects models for acquisition and extinction recall, covaried for age and sex.

Results: In the standard paradigm, youth and mothers chose similar shock intensity (ave. 2.2 mA) and no participants aborted the protocol. SCR analysis revealed expected effects for youth and mothers, with no age group interaction ($p > .1$). Across all subjects, SCR was increased for CS + D/CS + U vs CS- during acquisition. On extinction recall, SCR was increased for CS + U ($p = .02$), but not CS + D ($p = .44$) vs CS-. In the vicarious extinction study, TD and PTSD youth chose similar shock intensities (ave. 1.6 and 1.8 mA), again similar to mothers. During acquisition, TD and PTSD youth showed increased SCR for CS + s vs CS- ($p < .05$) with no group differences ($p > .13$). On extinction recall, TD youth showed comparable SCR for CS + D, CS + V, and CS- ($p > .2$). However, PTSD youth showed increased SCR on extinction recall for CS + V, but not CS + D, vs CS-. Here, CS + V responses were greater than CS- ($p = .1$), CS + D ($p < .05$), and TD youth CS + V ($p < .01$).

Conclusions: These preliminary studies suggest excellent tolerability of a threat learning paradigm in youth. Furthermore, these studies suggest that TD youth equally and effectively extinguish threat associations directly or vicariously through their mother. Intriguingly, youth with PTSD show evidence of intact direct extinction recall, but impaired vicarious extinction recall. These findings suggest that parent-child transmission of extinction learning may be impaired in pediatric PTSD. Further study will be needed in a larger sample of youth to confirm and extend these results and map possible mechanisms of impaired learning in the parent-child dyad.

Disclosure: Nothing to disclose.

48.4 Threat Learning and Inhibitory Responses in Children at Risk for Trauma and PTSD

Jennifer Stevens

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Background: Learning to respond appropriately to threat is a key capacity necessary for survival. Previous findings, including work from our laboratory, indicate that the ability to distinguish threat

and safety cues increases over the ages of 8 to 12 in children. The neural circuitry supporting fear learning, including projections between the prefrontal cortex and amygdala, has been shown to change substantially around age 10 across a number of neuroimaging studies, and may mature earlier in children experiencing high levels of early life stress. We sought to determine whether a key resiliency factor, the relationship with a caregiver, might serve as a buffer against effects of early life stress on the development of fear learning and related neural circuits.

Methods: We examined fear conditioning using an acoustic startle paradigm, followed by MRI neuroimaging in children ages 8 to 13, whose families were primarily low-income and at high risk for trauma and PTSD. A total of 104 children participated in the fear-conditioning task: 62 children had their mother close in the testing room, and 42 had mother absent from the testing room. A subset of 50 children participated in MRI neuroimaging including a task with fearful and neutral face stimuli. Freesurfer analysis of T1 weighted images was used to extract subcortical volume, and SPM8 was used to analyze fMRI data for the Faces task. Children and their mothers also completed an observational interaction challenge (Etch-a-Sketch) task that was coded for positive and negative affect and reported on child's exposure to community violence (VEX-R; Fox & Leavitt, 1995).

Results: In the fear conditioning task, there was an interaction of age and mother availability, $F(1,100) = 4.87$, $p = .03$, such that children age 8-10 showed discrimination between CS + and CS- only when mother was in the testing room, whereas adolescents age 11-13 showed appropriate discrimination between threat and safety regardless of mother presence. This was observed irrespective of child's trauma exposure. In the fMRI task, violence exposure was positively associated with left amygdala reactivity to both fearful ($r = 0.36$, $p = .01$) and neutral face stimuli ($r = 0.52$, $p < .001$), irrespective of age group. Interestingly, in 8-10-year-old children, mothers' expression of positive affect in the interaction task was positively associated with habituation of child's amygdala response to repeated fearful face stimuli ($r = .67$, $p = .001$), but was not related to amygdala responses in adolescents 11-13 ($p = .80$). Mothers' expression of negative affect was not related to amygdala habituation or reactivity in either group.

Conclusions: Findings suggested that maternal presence increases younger children's ability to discriminate threat from safety and were consistent with a large existing literature showing associations between maternal presence and reduced threat reactivity. Habituation of the amygdala to a repeated social threat stimulus (fearful face), a form of flexible adaptation to the changing salience of a threat stimulus over time, was quicker in younger children whose mothers showed more positive affect in a challenging parent-child interaction, but unrelated to mothers' affect in older children. This provides further evidence that parental input enhances flexible learning about environmental threat in children but is less important in adolescents.

Disclosure: Nothing to disclose.

Panel

49. A Point of Convergence: Postsynaptic Density Molecular, Cellular and Extracellular Regulation and Pathophysiology in Schizophrenia

49.1 Subcellular Genomics: Exploration of the Neuronal Dendrite

Abstract not included.

49.2 mGluR5 Hypofunction in Schizophrenia and its Impact on GluN Signaling via Protein – Protein Interactions

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Background: Multiple lines of evidence point to the postsynaptic density (PSD) and glutamatergic signaling pathways therein as a point of convergence for a large number of schizophrenia genomic variations. How such convergence occurs will be crucial for pathophysiologic understanding of the illness; but is largely unknown. Glutamatergic signaling is critically modulated by proteins in the PSD and their interactions.

Methods: Postmortem DLPFC of schizophrenia and healthy subjects were examined for ligand induced activation of mGluR5. Activation of mGluR5 signaling was monitored by changes of mGluR5 phosphorylation and PPIs in mGluR5 complexes. The protein-protein interaction (PPI) network of mGluR5 or GluN was constructed and interrogated for enrichment of schizophrenia risk variants identified in the Psychiatric Genomic Consortium (PGC) cohort. PSD enrichment of the DLPFC of the postmortem cohort was immunoprecipitated for GluN or mGluR5 were analyzed by LC-SRM/MS with the [13C6] brain ISTD as standards for quantification.

Results: The patient group showed a striking decrease in agonist induced activation of mGluR5 signaling compared to controls matched for sex, age and PMI ($F(1,32) = 25$, $p < 0.001$, for Gq coupling). This was accompanied by altered protein associations of mGluR5 with Norbin, tamalin RGS4 and Preso1, each of which can reduce the receptor signaling ($F(1, 32) = 48$, $p < 0.01$ for RGS4 as an example). The PPI network constructed for mGluR5 or GluN was highly enriched for schizophrenia risk variants of schizophrenia found in the PGC cohort. mGluR5 agonist facilitates GluN signaling activation, which in turn increases mGluR5 signaling, while inhibition of one decreases signaling of the other by disrupting reciprocal facilitation.

Conclusions: We here present the first direct evidence for mGluR5 hypoactivity, propose a reciprocal interplay between GluN and mGluR5 hypoactivity as integral to glutamatergic dysregulation and suggest PPIs in mGluR5 – GluN complexes as potential targets for intervention in schizophrenia.

Disclosure: Nothing to disclose.

49.3 Role of Complement C4A in Developmental Synaptic Pruning

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Background: Recent GWAS showed that elevated expression of the complement C4A allele, but not the complement C4B allele, was found to be a risk factor for schizophrenia development. Complement C4, along with C1q and C3, has been implicated in microglia-mediated developmental synaptic refinement. Reduced dendritic spine density observed in schizophrenia patients suggests that synaptic pruning is a potential pathogenic mechanism. We hypothesize that the chemical differences between C4A and C4B affect their role on synaptic pruning and that C4A over expression induce excessive synapse elimination.

Methods: To address this hypothesis, we generated BAC transgenic mice expressing human C4A or C4B alleles. Expression and functional assays were performed to validate the mouse models. In order to characterize the role of C4A and C4B

expression during brain development, we measured, in both males and females, synapse density by immunohistochemistry (IHC), and microglia engulfment of synaptic material using flow cytometry and IHC. Then, complement C4A and C4B binding to synapse was tested using an in vitro synaptosome binding assay. To study the developmental effect of C4A overpexpression, mice bearing different number of C4A gene copies were used. Comparison of synapse density and microglia engulfment was performed, and mice were subjected to a battery of common behavior tests.

Results: Results: In the newly generated transgenic mice, C4A and C4B were both expressed and functionally active in peripheral immune system. In order to study their role in central nervous system development, we first used the retinogeniculate system as a model, and found that developmental synaptic pruning is dependent on C4A, but not C4B, and that C4A overexpression led to increase synaptic pruning. To explain this difference we showed, using an in vitro flow cytometry based assay, that C4A binds more efficiently than C4B to synaptosomes. We then explored other brain regions that are relevant to schizophrenia like frontal cortex. When C4A is overexpressed, microglia-mediated synapse uptake in frontal cortex is significantly increased during development (postnatal day 40) and adult mice (postnatal day 60) showed significantly reduced synapse density. These observations correlated with changes in mouse behavior, elevated C4A expression induced impaired short-term memory and social interaction, and increased anxiety phenotype.

Conclusions: Our results show that C4A and C4B have distinct functions during brain development and that C4A mediates synapse elimination in the retinogeniculate system and frontal cortex. Remarkably, C4A overexpression induces excessive microglia-mediated synaptic pruning leading to important changes in mouse behavior. Overall, these results suggest that unbalanced C4A-associated synaptic pruning mechanisms could explain schizophrenia pathogenesis.

Disclosure: Nothing to disclose.

49.4 The Tetrapartite Synapse in Schizophrenia: Interactions Between the Extracellular Matrix and Glia in Synaptic Pathology

Sabina Berretta

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Background: Converging evidence from our group and others supports the idea that synaptic pathology in schizophrenia (SZ) may be usefully investigated in the context of the 'tetrapartite synapse' concept, an entity comprised of the pre- and post-synaptic elements, glial cells and the extracellular matrix (ECM). The ECM forms organized perisynaptic aggregates and known to regulate synaptic functions and plasticity. Perineuronal nets (PNNs), the most extensively studied form of perisynaptic ECM, play a role in critical periods of development and are dynamically altered by experience, a process likely to be mediated by ECM remodeling proteases. Distinct populations of glial cells are main sources of ECM molecular components and contribute ECM remodeling proteases, such as matrix metalloproteases (MMPs) and cathepsin S. MMPs are responsible for ECM posttranslational modifications modulating ECM functions and affecting synaptic plasticity. Recent GWAS evidence show that genes encoding for MMPs represent risk factors for SZ. Our group showed marked decreases of PNNs in several brain regions from people with SZ. These decreases are accompanied by altered expression in glial cells of chondroitin sulfate proteoglycans (CSPGs), main components of the ECM/PNNs. CS-6/Glia clusters, an emerging ECM structure enriched in 6-sulfated CSPGs, were also found to be markedly decreased in SZ. With these studies, we tested the hypothesis that the expression of ECM remodeling proteases in the amygdala of people with SZ may be dysregulated, in association with PNN, CS-6/Glia cluster and glial abnormalities. In addition, studies in human and rodents assessed the relationship between CS-6 expression, glia and synapses.

Methods: Expression levels of MMP9, MMP16 protein (western blotting) and cathepsin S mRNA (QRT-PCR) were assessed in the amygdala from healthy control ($n = 16$) and SZ ($n = 20$) subjects. In the same subject cohort, numbers of CS-6/Glia clusters were measured in the mediodorsal nucleus of the thalamus (MD). Confocal and electron microscopy on tissue from mouse and healthy human amygdala were used to investigate CS-6/Glia clusters.

Results: The active form of MMP9 was significantly increased in the amygdala of people with SZ ($p = 0.02$) while inactivated form was significantly decreased ($p = 0.04$). MMP16 was significantly increased in SZ ($p = 0.04$). Cathepsin S mRNA was significantly decreased in SZ ($p = 0.03$). Numbers of CS-6/Glia clusters were decreased in the MD of SZ ($p < 0.005$) and bipolar disorder ($p < 0.005$). Confocal and electron microscopy studies showed complex morphological interactions of CSPG/CS-6 in astrocytes and dendritic spines, suggesting that CS-6/Glia clusters are formed by astrocyte processes carrying CSPG/CS-6 and surrounding dendritic spines.

Conclusions: The results provide evidence for a novel ECM structure, CS-6/Glia clusters, shown to be decreased the amygdala and MD of SZ and bipolar disorder donors and to play a role in the regulation of synaptic plasticity. Dysregulated expression of ECM remodeling enzymes, known to play a key role in synaptic regulation, suggests a potential mechanism impacting interactions between ECM and glia and affecting synaptic functions. We suggest that these abnormalities may contribute to decreases of PNN and CS-6/Glia clusters in SZ and to synaptic pathology in this disorder.

Disclosure: Nothing to disclose.

Panel

50. In the Pipeline: Innovative Therapeutics to Address the Opioid Crisis

50.1 The Serotonin (5-HT) 5-HT2C Receptor (5-HT2CR) as a Treatment Target for Opioid Use Disorder

Abstract not included.

50.2 Synthetic Opioids and Immunopharmacotherapy

Kim Janda

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Background: Synthetic psychoactive drugs (SPDs) are substances that are specifically designed to mimic the actions of known abused drugs, yet, skirt the legal definition of an illegal substance, and can pose a public health threat. The fentanyl's are a large family of highly potent synthetic narcotic analgesics. They have all the properties of the opiates; however, they are 100-10000 times more potent than morphine. While drug control efforts will need to continue, so will efforts to treat SPD abuse/addiction. Although, antibody therapies have been examined for treating drugs of abuse such as cocaine, nicotine, methamphetamine and heroin no

antibody effort has ever been attempted with regards to an SPD. Accordingly, we will detail a series of antibodies against the class of drugs known as the fentanyl's. We will present data showing how these antibodies cannot only counter prescription fentanyl's but also the common street analogues of this synthetic opioid.

Methods: General Studies: All studies were performed in compliance with the Scripps Institutional Animal Care and Use Committee and all protocols adhered to the National Institute of Health Guide for the Care and Use of Laboratory Animals. Mice were group-housed in an AAALAC-accredited vivarium containing temperature and humidity-controlled rooms. Mouse weights were measured every week and injection site reactions were measured on the day of antinociception. Mice were euthanized within 24 h of the lethality challenge.

Antinociception Assay: Mice (equal male and female) are tested for cumulative fentanyl response to a spinal (tail flick) behavioral test. The tail flick test was administered by lightly restraining mice in a small pouch constructed from absorbent laboratory underpads and using an IITC Life Science Tail Flick Analgesia Meter to measure time of withdrawal from a heated beam of light (active intensity, 45%). Typical baseline response was 0.2 – 1.1 s and an automatic 10 s cutoff was used to prevent tissue damage. Antinociception data are transformed from time to percent maximum possible effect (%MPE). The data are fit using a log (agonist) vs. normalized response non-linear regression in GraphPad PRISM 6. The ED50 values and 95% confidence intervals are determined for each antinociception test and individual treatment groups to determine ED50 values.

Lethality Assay: A group of the control mice ($n = 8$, equal male and female) are used to determine the appropriate dosing for the study. All doses are administered as a bolus retro-orbital intravenous injection and mice monitored over 24 h for overdose. An appropriate fentanyl dose was administered to eight mice resulting in an LD50. This dose was used for the remaining eight mice in the vaccinated groups.

Results: Hapten fentanyl conjugate vaccine design coupled with FACS analysis has allowed us to isolate antibodies that bind with subnanomolar affinity and exhibit pan cross-reactivity with other fentanyl's, including carfentanil. Tail flick nociception is mediated in the central nervous system and provided a relevant model as a substitute for drug reward in the current study. For lethality studies mice receiving monoclonal antibody were protected against lethal fentanyl overdose.

Conclusions: Monoclonal antibody therapy appears to be a promising therapeutic against the class of drugs known as the fentanyl's. We also anticipate that these antibodies could also be used to prophylactically protect first responders, medical personnel, and drug enforcement agents from inadvertent acute fentanyl exposure.

Disclosure: Nothing to disclose.

50.3 Dopamine D3 Receptor-Based Medication Strategies to Combat Opioid Addiction

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Background: Opioid abuse is a mounting public health crisis with massive personal and economic costs. Prescription opioids, including oxycodone, are critical in pain management but are often misused or diverted. The development of tolerance to opioids and opioid withdrawal syndrome represent major deterrents to reducing the prevalence of opioid misuse. Identifying non-opioid-based pharmacotherapies for the treatment of opioid abuse that do not interfere with analgesia is vital. Accumulating evidence suggests that the dopamine D3 receptor (D3R) regulates opioid reward and may be a promising therapeutic target in combatting opioid addiction. Here we determined whether two highly selective and metabolically stable D3R-targeted compounds, the antagonist VK4-116 and the partial agonist VK4-40, alter gold-standard preclinical models of opioid addiction, including intravenous oxycodone self-administration and reinstatement to drug seeking, expression of oxycodone place preference and naloxone-precipitated withdrawal, and oxycodone-induced analgesia.

Methods: The impact of pre-treatment with VK4-116 (1-25 mg/kg) or VK4-40 (1-10 mg/kg) on opioid reward was evaluated using: 1) Intravenous oxycodone self-administration (0.05 mg/kg/infusion) in rats ($n = 7$-11) followed by response extinction and oxycodone-primed reinstatement (1 mg/kg, i.p.); 2) Conditioned place preferences for oxycodone (3 mg/kg, i.p.) in mice ($n = 8$/group); or 3) Optogenetic reward, in which transgenic mice ($n = 6$-12) lever-pressed to earn optogenetic excitation of dopamine neurons in the ventral tegmental area (450 nm laser, 1-100 Hz in descending order) in the presence or absence of oxycodone (0.3-3 mg/kg, i.p.). Next, the impact of D3R compounds on opioid withdrawal and analgesia was evaluated using: 1) Naloxone-induced conditioned place aversion, in which rats ($n = 8$) were treated with oxycodone (3 mg/kg, i.p.) followed by induction of naloxone-precipitated (1 mg/kg, i.p.) withdrawal in a place preference apparatus; or 2) Nociception, in which rats were treated with oxycodone (0.5-4 mg/kg, i.p.) prior to placement on a hot plate.

Results: Both VK4-116 and VK4-40 dose-dependently attenuated oxycodone self-administration, oxycodone-primed reinstatement, and oxycodone conditioned place preferences ($p < 0.01$ for 15, 25 mg/kg VK4-116 and 5 mg/kg VK4-40). While oxycodone produced leftward shifts in optogenetic response-frequency curves ($p < 0.05$ for 3 mg/kg), VK4-40 reduced the stimulation frequencies required to maintain responding and blocked oxycodone-induced shifts in responding ($p < 0.05$ for 1 and 10 mg/kg). Importantly, VK4-40 alone did not have dysphoric effects as assessed by optogenetic real-time and classic conditioned place preference experiments. In addition, VK4-116 blocked naloxone-induced conditioned place aversion ($p < 0.05$ for 5 and 15 mg/kg) and augmented oxycodone-induced analgesia ($p < 0.01$ for 25 mg/kg) in the hot plate test.

Conclusions: VK4-116 and VK4-40 attenuate multiple facets of opioid addiction in preclinical models while simultaneously mitigating opioid withdrawal and augmenting opioid-induced analgesia. Taken together, these converging findings indicate these lead compounds have therapeutic potential for the treatment of prescription opioid use disorders and support the utility of D3R-based medication strategies in combatting the ongoing opioid crisis.

Disclosure: Nothing to disclose.

50.04 Modeling Opioid Maintenance Therapy in Rats: Effects of Chronic Buprenorphine and the Biased Mu-Opioid Receptor Agonist TRV130 on Relapse to Oxycodone Seeking

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Background: High relapse rates perpetuate opioid addiction and are a major obstacle in addressing the current U.S. opioid epidemic. Maintenance therapy with opioid agonists (buprenorphine, methadone) is an effective treatment for opioid addiction. Here, we established an experimental procedure in rats trained to

self-administer the prescription opioid oxycodone to compare the efficacy of an established treatment (buprenorphine) with that of a newer biased mu-opioid receptor (MOR) agonist, TRV130.

Methods: We trained rats to self-administer oxycodone (0.1 & 0.05 mg/kg/infusion; 7 d/dose, 6-h/d) in Context A where infusions were paired with a discrete tone-light cue. We implanted Alzet osmotic pumps containing vehicle, buprenorphine (3, 6, or 9 mg/kg/d; n = 11-16), or TRV130 (3, 6, or 9 mg/kg/d; n = 13-14) and performed three tests: (1) responding for drug-paired discrete cues under extinction conditions in a non-drug context (Context B, 7 d), (2) context-induced reinstatement of oxycodone seeking in Context A after extinction in Context B, and (3) reacquisition of oxycodone self-administration in Context A.

Results: Chronic buprenorphine significantly decreased responding for drug-paired discrete cues in Context B under extinction conditions [Dose effect: $F(3,49) = 8.1$, $p < 0.001$] and reacquisition of oxycodone self-administration in Context A [$F(3,37) = 15.5$, $p < 0.001$]; chronic buprenorphine also decreased context A-induced reinstatement of oxycodone seeking but this effect did not reach statistical significance [$F(3,49) = 2.8$, $p = 0.051$]. Chronic TRV130 significantly decreased oxycodone seeking or taking on all three relapse measures: $F(3,49) = 5.3$, $p = 0.003$, $F(3,49) = 4.4$, $p = 0.008$, and $F(3,45) = 5.2$, $p = 0.003$ for extinction responding in context B, context A-induced reinstatement, and reacquisition in context A, respectively.

Conclusions: We introduce a novel rat model to study the effect of agonist-based maintenance therapy on relapse to prescription opioid seeking. We showed that chronic buprenorphine significantly decreased oxycodone seeking provoked by exposure to oxycodone-associated discrete cues and by exposure to oxycodone itself, demonstrating the predictive validity of the model. More importantly, we showed that chronic TRV130 delivery significantly decreased oxycodone seeking using multiple measures of relapse. We propose that biased MORs should be considered as a novel opioid agonist maintenance treatment for addiction to prescription opioids and heroin.

Disclosure: Nothing to disclose.

Panel

51. New Therapies for Mental Illness and the Role of the Gut-Brain Axis

51.1 Targeting Responsive Gastrointestinal and Endothelial Barrier Phenotypes for Clinical Trials Examining Probiotic Treatment of Psychiatric Disorders

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Background: Schizophrenia and bipolar disorder are debilitating health conditions that are etiologically complex and for some, ineffectually treated. Many individuals with these disorders are keenly interested in the safety and efficacy of alternative or adjunctive treatment methods including probiotics. Clinical studies of probiotics often broadly target psychiatrically healthy people, and these studies have yielded mixed results in terms of symptom improvement. Inconsistent results could be due to many reasons including a highly heterogeneous participant population. If individuals with psychiatric disorders who have gastrointestinal (GI) issues could be accurately distinguished, then it may be that the probiotic treatment is better matched to treat a phenotype that is resolvable. Further contributing to disease heterogeneity are depression and anxiety, subclinical and clinical conditions which may accompany major psychiatric disorders in subsets of individuals. Here we evaluated blood biomarkers to identify likely responsive clinical trial participants, chosen based on evidence of GI enteropathies, endothelial barrier dysfunction and comorbid depression and anxiety.

Methods: We used enzyme-linked immunosorbent assays to screen blood samples from female and male individuals with schizophrenia (n = 332), bipolar disorder (n = 106) and healthy controls (n = 324). Blood biomarker targets included GI inflammation, endothelial barrier permeability and systemic complement activation (food antigen IgG, microbial translocation, S100B, complement C4). Analysis of variance and multi-variate regression models were used to detect differences in mean levels of blood biomarkers amongst groups and associations with anxiety, depression and psychiatric symptom scores. Regression models included age, race, sex and body mass index.

Results: In these studies, comorbid depression was associated with especially pronounced elevations of gut-related markers in schizophrenia. Levels of gluten antibodies, soluble CD14, LPS-binding protein and Candida albicans IgG were all significantly elevated in the presence of comorbid depression compared to the unstratified schizophrenia group and compared to controls (p value range 0.0001-0.02). These markers were correlated with peripheral complement C4 ($p < 0.0001$), which was itself also elevated in the depression subgroup of schizophrenia compared to the unstratified schizophrenia group and controls ($p < 0.02$). We also observed an association of the glial marker, S100B, with anxiety in bipolar disorder. S100B is often used as a surrogate of blood-brain barrier permeability. In our study, S100B was significantly increased in individuals with bipolar disorder and comorbid anxiety compared to the unstratified bipolar disorder group and controls ($p < 0.03$).

Conclusions: Thus, comorbid depression seems to strongly contribute to GI issues in schizophrenia and as such represents an appropriate phenotype to target for inclusion in clinical trials of probiotics. Anxiety may be associated with endothelial insufficiencies in bipolar disorder, but larger sample sizes are required to confirm this finding. Future applications of these markers in individuals with clinical and subclinical anxiety and depression without an additional comorbid psychiatric disorder are planned. As more information is gathered to refine this prescreening process, we predict that a highly-individualized treatment approach based on microbial manipulations can be developed.

Disclosure: Nothing to disclose.

51.2 Towards Psychobiotics: The Gut Microbiota as a Key Regulator of Stress

Robert Yolken

Johns Hopkins University

Background: The importance of the gut-brain axis in regulating stress-related responses has long been appreciated. More recently, the microbiota has emerged as a key player in the control of this axis, especially during conditions of stress provoked by real or perceived homeostatic challenge. The concept of psychobiotics has also emerged; these are targeted microbiome interventions that support brain health especially during stressful situations. In tandem a growing body of work from animal studies have implicated the gut microbiome in early life in priming for healthy neurodevelopment and HPA-axis function. Thus, alterations in microbiome composition in early life may affect stress response in adulthood. Although microbiome changes have been well documented in C-section born individuals there has been limited

investigation on the long-term effects of this on psychological and physiological responses to stress.

Methods: In this study, we sought to investigate if early-life manipulation of the microbiome (by being born by C-section) could affect long-term stress responsivity. Moreover, we investigated the potential of a probiotic, Bifidobacterium longum, to positively enhance stress, mood, memory and cognition in young adult male university students was assessed. We hypothesised that the psychobiotic intervention would have a positive effect on stress response during a period of naturalistic chronic stress (exam stress), possibly via reductions in subjective stress, reduction in HPA-axis activity, and normalisation of inflammatory profile; in addition to having a positive effect on cognitive performance.

Participants completed a course of 8 weeks of Bifidobacterium longum or placebo, in the run-up to the exam period. Cognition, mood and memory were analysed before and after the intervention period using a multi-modal approach, including the Cambridge Neuropsychological Test Automated Battery (CANTAB), in addition to validated paper-based tests. Stool samples were collected for microbiota compositional analysis. Blood samples were collected for stress and inflammatory profile, and hair and saliva were collected to determine chronic and acute cortisol levels respectively.

A mouse model of C-section is also utilized to identify some of the key mechanisms underpinning the early-life microbiota disturbances on brain, physiology and behavior

Results: When comparing psychological distress levels during the Non-Stress and Exam-Stress periods, participants born by C-section reported significantly greater levels of trait anxiety and perceived stress when compared to vaginally born participants, during the Exam Stress period but not during the Non-Stress period. The anti-inflammatory cytokine IL-10 was also significantly elevated during the Exam-Stress period in C-Section participants. Similar findings are shown in a mouse model many of which can be reversed with a specific Bifidobacterium. We are currently analyzing the results of the Bifidobacterium Longum intervention study.

Conclusions: Disturbances of the microbiome in early life (delivery by C-section) results in increased psychological vulnerability to either acute or prolonged stress in human volunteers and mice. In addition, results from the Bifidobacteria longum study are poised to address the question whether the negative impact of stress and lifestyle factors, possibly via positive modulation of gut microbiota through psychobiotic supplementation.

Disclosure: 4D Pharma, Grant; Cremo, Grant; Nutricia, Grant; Dupont, Grant; Suntory Wellness, Grant; Alkermes, Grant

51.3 Improving the Course of Bipolar Disorder by Immune Modulatory Effects on the Gastrointestinal Tract

Abstract not included.

51.4 Immune Dysfunction in Autism Spectrum Disorder: From Gut to Brain

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Background: Autism Spectrum Disorders (ASD) are a group of psychiatric conditions characterized by social difficulties, communication impairments and stereotypic behavior as well as somatic comorbidities. While the etiology of ASD remains to be elucidated, interaction between genetic and environmental factors contribute to ASD. Compared to healthy controls, at least some ASD patients have been shown to exhibit gastro-intestinal (GI) distress, intestinal microbiota dysbiosis, and altered levels of both microbiota-derived metabolites in urine and immune related molecules in serum. Furthermore, preclinical studies have shown that experimental manipulations targeting either the gut microbiota or the immune system could impact behavior in mice. Based on these studies, we have made the hypothesis that an abnormal gut microbiota resulting from specific gene-environment interactions could lead to both behavior and GI symptoms in at least a subset of ASD patients.

Methods: ASD subjects without intellectual deficit and sex and age matched healthy controls were included and extensively characterized for social difficulties, stereotypic behavior, GI symptoms, gut microbiota composition, serum cytokine levels, bacteria derived metabolites in urine, Human Leukocyte Antigen (HLA) diversity and Pattern Recognition Receptors (PRRs) genes variants. Behavioral tests in mice are used to investigate how experimental manipulation of the gut microbiota could impact brain function.

Results: While the gut microbiota of ASD patients and healthy controls both contained around 6500 operational taxonomic units (OTUs), the microbiota in ASD patients was less diverse and characterized by an increased abundance of the phyla Bacteroidetes and Firmicutes. Genetic analysis revealed an association between ASD and HLA-DRB1*11-DQB1*07 (previously shown to be associated with gut-malabsorption conditions) as well as with functional variants of the Pathogen Recognition Receptor (PRR) gene DECTIN1 (previously shown to be associated with GI disorders). Preclinical studies in mice are being performed to explore possible causal relationships between gut microbiota alterations, and behavior and GI function.

Conclusions: The gut microbiota in ASD patients is less diverse and exhibit an altered composition with an increased relative abundance of two important bacterial phyla, Firmicutes and Bacteroides. This could be due, as least in part, to specific variants of HLA class II or PRRs genes. While the causal relationship between an altered microbiota and ASD symptoms remains to be elucidated, we believe that further investigation of the interactions between the gut, the brain and the immune system in both clinical studies and preclinical models could pave the way for the development of innovative diagnostic and therapeutic strategies.

Disclosure: Nothing to disclose.

Panel

52. Single-Cell Genomics: Using the Power of Single-Cell Resolution to Understand the Molecular Complexity of Neuropsychiatric Phenotypes

52.1 Single-Cell Multi-Omic Interrogation of the Human Brain

Abstract not included.

52.2 Mapping the Brain Using Single Cell Epigenomics

Abstract not included.

52.3 The Neuroendocrine Stress Response in a Single Cell Resolution

Alon Chen

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Background: Maintenance of homeostasis in the presence of real or perceived challenges requires numerous adaptive responses

involving changes in the central nervous and neuroendocrine systems. The biological system that has been most closely linked to the stress response in mammals is the neuroendocrine hypothalamic-pituitary-adrenal (HPA) axis. Perception of physical or psychological stress by an organism is followed by a series of events, which result in changes in emotional and cognitive functions, modulation of autonomic activities and the secretion of glucocorticoids from the adrenal cortex. Both activation and termination of the behavioral, autonomic and adrenocortical stress responses are critical for adaptation and survival. Dysregulation of the stress response can have severe psychological and physiological consequences and chronic hyperactivation of the HPA axis has been linked to stress-related emotional and metabolic disorders such as anxiety, anorexia nervosa and depression.

Methods: High-throughput transcriptomic techniques provide a powerful insight into the complexity of a particular organism or tissue by enabling the identification of its molecular signatures. However, previous transcriptomic studies are limited to providing data from averaged thousands of cells, which can mask, dilute or even distort signals of interest. Recent advances in the field of genomics now allow us to obtain genome-wide data from individual cells in a system. Here, using single-cell RNA sequencing we investigated the effects of chronic social defeat stress, a mouse model of depression, on the three neuroendocrine components of the HPA axis.

Results: We analyzed more than 7,500 single cells from the paraventricular nucleus of hypothalamus (PVN), the anterior pituitary, and the adrenal glands, obtained from both naïve or chronically stressed mice. Our findings reveal the first detailed molecular identities of distinct cell types in these three complex tissues.

Conclusions: This study provides an unbiased and systematic view of cell type specific signatures of chronic stress, at multiple levels of HPA activation, using single cell resolution. Furthermore, these results provide new dimensions of HPA activity and its possible relationship with stress-related disorders. Ultimately, these findings may lead to more accurate and reliable signatures to monitor disease progression and efficacy of treatment.

Disclosure: Nothing to disclose.

52.4 Detecting Cell-Specific, Differential Gene Expression in the Depressed Brain Using sNuc-Seq

Gustavo Turecki

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Background: Molecular changes are typically measured in tissue homogenates or cellular fractions containing millions of cells pooled together. However, most tissue types, particularly the brain, have heterogeneous cellular composition. Multiple neuronal and glial subtypes with specific gene expression patterns are likely to be distinctly modified in a diseased state. As such, the normal variation of gene expression between cell types can mask specific changes when obtaining molecular information from cellular homogenates. Here, we are able to detect cell specific transcriptional changes in the brains of depressed individuals using sNuc-Seq.

Methods: Using post-mortem brain tissue obtained from the Douglas Bell Canada Brain bank we explored the frontal cortex of 17 suicide completers and 17 matched healthy controls. Bulk nuclei were isolated from BA8/9 with the aim of capturing 3000 single nuclei per sample. The unfiltered gene barcode matrices were processed using Seurat in conjunction with a set of in house tools. Extensive quality control was preformed to ensure all cells were appropriately represented; doublets were removed; and clusters represented biology entities and not technological artifact. Differential expression between groups was assessed using lme4. Co-regulation of cells was investigated using Weighted Gene Co-expression Analysis (WGCNA).

Results: Unsupervised analysis identified 33 unique clusters, the largest of which was additionally subclustered resulting in a total of 26 quality-controlled clusters. Based on the top differential Bonferroni corrected cell markers, we were able to confidently annotate all clusters into their respective cell-types, for example: excitatory (SLC17A7 + $p < 4.3 \times 10\text{-}141$, SATB2 + , $p < 6.6 \times 10\text{-}144$) inhibitory (GAD1 + , $p < 3.0 \times 10\text{-}249$, GAD2 + , $p < 5.3 \times 10\text{-}75$); astrocytes (ALDH1A1 + , $p < 1.8 \times 10\text{-}115$, AQP4 + , $p < 1.5 \times 10\text{-}227$); oligodendrocytes (PLP + $p < 1.2 \times 10\text{-}23$); OPCs (PCDH15 + , $p < 4.5 \times 10\text{-}207$, PDGFRA + , $p < 0$). In the two instances where clusters were discarded due to single sample contributions, we were able to identify biological conditions from their life histories that reflect the overrepresentation of the gene marker(s) that defined these clusters (Astros_1 (GFAP + , $p < 2.4 \times 10\text{-}255$), Inhib_4_SST (FOS + , $p < 3.7 \times 10\text{-}252$, NPAS4 + , $p < 3.7 \times 10\text{-}252$). Our data also produced numerous oligodendrocyte clusters which could be ordered into a marker- based pseudo time line of developmental cell lineage. Of the 26 clusters, 15 showed some level of differential expression between groups. In general, more genes were downregulation than upregulation per cell types in cases vs controls (FDR < 0.1).

Conclusions: Using archived brain tissue, we have been able to distinguish 26 different, highly quality controlled neural cell types. We found that gene dysregulation was specific to certain cell types that in most instances, the changes resulted in decreased expression in case. sNuc-seq has allowed us to uncover previously undetectable changes in the brains of people with depression. The use of this cell-specific information can also give us greater insight into the interactions between brain cells and how they might be involved in the manifestation of depression.

Disclosure: Nothing to disclose.

Panel

53. New Approaches to Exploring the Involvement of the Cholinergic System in Psychiatric Disorders and Cognitive Decline

53.1 Cell-Type Selective Targeting of Cholinergic Neurons and Their Receptors in Anxiety- and Depression-Like Behaviors

Marina Picciotto

Yale University, New Haven, Connecticut, United States

Background: The cholinergic system has been implicated in adaptive and maladaptive behavioral responses to stress. Work in human subjects has shown that acetycholine (ACh) levels are elevated when individuals are actively depressed, and work in our laboratory has shown that ACh signaling in the basolateral amygdala (BLA) is important for unconditioned responses to stress. We have demonstrated that manipulations of ACh signaling through nicotinic ACh receptors (nAChRs) in BLA can recapitulate effects of systemic modulation on stress-induced behaviors in mice. Here we begin to identify the cell types and microcircuits in BLA involved in nAChR-mediated changes in anxiety-like behaviors.

Methods: We have used a novel, cell-type selective method to decrease expression of proteins of interest in targeted brain regions of adult animals. The method involves delivery of Cre-

dependent small hairpin RNAs (shRNA) using adeno associated virus type 2 (AAV2) into brain area(s) of interest in Cre recombinase-expressing mice. This has allowed us to identify cell types in BLA essential for cholinergic circuits that determine thresholds for responding in behavioral tests of stress response. We are also using in vivo optical recording by fiber photometry to determine the effect of ACh manipulations on activity of different neuronal subtypes in BLA.

Results: shRNA-mediated knockdown of one of the primary mediators of ACh response, alpha7 nAChRs, specifically on GABAergic cells of the BLA in GAD-Cre mice results in increased exploration of a stressful environment (light-dark test, n = 8-12/group, ANOVA followed by LSD posthoc tests). Knockdown of the alpha7 nAChR subunit in BLA glutamatergic cells in either CaMKII-Cre or vGlut2-Cre mice is not sufficient to alter stress-induced behaviors (n = 8-12/group). GABAergic interneurons in the BLA are heterogeneous, with each subtype contributing uniquely to shape stress-related behaviors. Indeed, activation of distinct GABA neuron subtypes can even exert completely opposite effects on fear conditioning. Thus, we further determined whether knockdown of the alpha7 nAChR subtype in parvalbumin- (PV) or somatostatin (SOM) cells of the BLA could recapitulate the effects of knockdown in GAD-Cre mice. Preliminary experiments suggest that alpha7 nAChRs in both GABA neuron subtypes are involved in anxiety-like behavior. Finally, preliminary studies show that pharmacological antagonism of nAChR signaling decreases overall activity of neurons in the BLA.

Conclusions: ACh signaling is known to be important for stress responses, but the brain areas and receptor subtypes critical for its effects remain an important area of study. Taken together, the data presented here confirm that ACh signaling in the BLA is critical for mediating behavioral responses to stressful stimuli. The current data suggest that alpha 7 nAChR signaling in BLA GABA neurons is important for regulation of adaptive behaviors under stressful conditions. In particular, alpha 7 nAChRs on both PV and SOM GABAergic interneurons in BLA are important for basal activity of the structure. The ability of ACh to modulate BLA activity is complex, and elucidation of the microcircuits through which ACh signaling alters BLA output will be critical in understanding how ACh signaling may contribute to stress-related disorders.

Disclosure: Nothing to disclose.

53.2 Muscarinic Acetylcholine Receptor Regulation of Substance Abuse and Mood Disorder-Related Behavior in a Rodent Model: Role for M5 Receptors

Nii Addy

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Background: There is renewed interest in the role of the cholinergic system in neuropsychiatric disorders. Preclinical and clinical evidence suggests that muscarinic acetylcholine receptors (mAChRs) may serve as effective therapeutic targets for substance abuse disorders (SUDs) and mood disorders. We previously found that ventral tegmental area (VTA) infusion of the mAChR antagonist, scopolamine, attenuates phasic dopamine signaling in the nucleus accumbens (NAc) and cue-induced cocaine-seeking in rats. However, there is a need for greater understanding of receptor subtype-specific and brain pathway-specific muscarinic mechanisms mediating SUD and mood-disorder related behaviors.

Methods: Procedures were performed in male and female Sprague-Dawley rats. We used in vivo fast scan cyclic voltammetry to examine VTA-evoked phasic dopamine release in the NAc core of anesthetized rats. For behavior, rats received 10 days of intravenous cocaine self-administration training (FR1 schedule, ~0.5 mg/kg/cocaine infusion with a tone + light cue), followed by 14 days of forced abstinence. The forced swim test (FST) and elevated plus maze (EPM) were used to examine depression-related and anxiety-related behavior. An effort-based decision task was used to examine motivation to work for a highly palatable reward (FR5 schedule lever pressing for a 45 mg sucrose pellet) versus freely available rat chow. For drug infusion, VTA cannula were implanted by stereotaxic surgery 5 to 7 days prior to behavioral testing, and drugs were subsequently infused immediately before behavioral examination.

Results: VTA infusion of the M5-selective negative allosteric modulator (NAM), VU6000181 (30 uM infusion, 6.78 ng), attenuated phasic DA signaling in the NAc that is known to play a role in drug-seeking and responses to stress. In males, cocaine self-administration and 2 week forced abstinence induced an anxiogenic-like phenotype, as reflected by decreased open arm time in the EPM ($p < 0.05$ vs. i.v. saline trained rats). VTA infusion of the non-selective mAChR antagonist, scopolamine (2.4 or 24 micro g /side) reversed the anxiogenic effect of cocaine. In naïve male rats, VTA scopolamine (0, 2.4, 24 ug /side) led to a dose-dependent anxiolytic effect, as reflected by increased open arm time ($F2,23 = 6.502$, $p < 0.01$). In males, VTA infusion of the M5 NAM also produced antidepressant-like effects in the FST ($F2,22 = 7.594$, $p < 0.01$), with no effect on EPM behavior. In contrast, VTA M5 NAM infusion (6.78 ng) was sufficient to reverse the anxiogenic effects induced by VTA infusion of the acetylcholinesterase inhibitor, physostigmine (2 ug/side). In ongoing experiments, we are also examining these effects in female rats. In effort-related choice experiments in male and females, physostigmine administration, either systemically (0.125 mg/kg) or intra VTA (2 ug/side), decreased lever pressing for a highly palatable reward, but did not alter free chow consumption. In ongoing experiments, we will specifically examine the role of mAChR mechanisms in these processes.

Conclusions: Our findings thus far suggest that VTA mAChRs regulate multiple behaviors associated with exposure to drugs of abuse as well as mood disorder-related behaviors. Blockade of VTA mAChRs, and specifically M5 mAChRs, decreases dopamine signaling, induces antidepressant-like and anxiolytic-like effects, and reverse the anxiogenic effects of cocaine. In ongoing experiments, we will continue to examine the ability of M5 mAChRs to modulate dopamine signaling and behaviors associated SUD and mood disorders.

Disclosure: Nothing to disclose.

53.3 Sex Differences in Stress Regulation of the Cholinergic Attention System

Debra Bangasser

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Background: Chronic stress exacerbates symptoms of schizophrenia and attention deficit hyperactivity disorder, which are characterized by deficits in sustained attention. These disorders also occur more often in men than women, and men report more cognitive disruptions. As sustained attention subserves higher order cognition, sex differences in stress regulation of attention could contribute to these sex biases. Yet how stress regulates attention and sex differences therein remain unexplored. Previous work from our lab found that a 6-day chronic variable stressor (CVS) disrupted aspects of sustained attention mediated by cholinergic neurons the nucleus basalis of Meynert (NBM) to a

greater degree in male than female rats. Here we identify the changes in attention circuits that drive this effect.

Methods: We first assessed if CVS altered NBM cholinergic dendritic morphology in male and female rats (n = 22-27 dendrites from 4-5 rats per group). We developed a novel approach to label these neurons by injecting a Cre-dependent virus with a fluorescent marker into the NBM of rats genetically modified to express Cre-recombinase under control of the choline acetyltransferase promoter. Visualized dendrites were assessed using Sholl analysis, which places concentric 20 µm circles from the cell body to reveal dendritic complexity (i.e., Sholl intersections). We next used amperometry to test if CVS altered acetylcholine (ACh) release into the medial prefrontal cortex (mPFC), an effect required for sustained attention, of male and female rats (n = 6-7 per group). Choline clearance kinetics were also assessed.

Results: A mixed factors 2 × 2 × 13 ANOVA (sex × stress × Sholl distance) revealed a sex × Sholl interaction [$F(12,1152) = 12.15$, $p < .001$, η_partial^2 = .11]: female NBM cholinergic dendrites made fewer intersections with Sholls closest to the cell body than male dendrites ($p < .001$). This analysis also revealed a main effect of CVS [$F(1,96) = 4.18$, $p = .04$, η_partial^2 = .04], such that stress increased intersections. Amperometric recordings of mPFC ACh release and choline clearance analyzed with 2 × 2 ANOVAs revealed significant interactions between sex and CVS [$F(1, 21) = 6.37$, $p = .02$, η_partial^2 = .23] and [$F(1, 21) = 9.08$, $p = .01$, η_partial^2 = .30], respectively. Post-hoc tests revealed CVS impaired mPFC ACh release ($p = .01$) and choline clearance rate ($p < .001$) in male, but not female rats ($p > .05$).

Conclusions: These studies reveal how stress alters NMB cholinergic neurons that mediate sustained attention. We are the first to assess NMB cholinergic morphology in both sexes and female dendrites are less complex near the cell body. Despite this baseline sex difference, stress induced dendritic hypertrophy similarly in both sexes, which would alter the way these neurons process inputs. Unlike the structural plasticity results, CVS only reduced ACh release from NBM cholinergic neurons into the mPFC of male rats. ACh release is regulated by the ability of choline to be cleared from the extracellular space for ACh synthesis, and there was a stress-induced reduction in choline clearance in males, but not females. The proper release of ACh into the mPFC is critical for sustained attention, so the reduced ACh release in stressed males can account for their greater attentional impairment relative to stressed females. Importantly, male vulnerability to chronic stress-induced attention deficits may bias them towards cognitive deficits that characterize certain psychiatric disorders.

Disclosure: Nothing to disclose.

53.4 Development of Novel Cholinergic M1PAMS for Cognitive Enhancement: Phase 1 Tests of Target Engagement

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Background: M1 is the primary muscarinic acetylcholine receptor (mAChR) subtype involved in regulating domains of cognitive function that are impaired in Alzheimer's disease (AD). Selective activation of M1 potentiates prefrontal cortical (PFC) and hippocampal NMDAR currents and enhances both PFC- and hippocampal-mediated associative learning and memory functions. Previous orthosteric and allosteric agonists developed to activate M1 mAChRs have failed in clinical development due to a lack of true specificity for M1 resulting in dose-limiting adverse effects associated with activation of other mAChR subtypes, most notably peripheral M2 and M3 mAChRs. To circumvent this problem, we have focused on allosteric sites on the receptor that are less highly conserved. Our clinical lead (VU319) significantly potentiates the response of the M1 receptor to acetylcholine (ACh), thereby enhancing activity-dependent signaling and has robust M1-mediated effects on hippocampal synaptic plasticity, excitatory drive to the prefrontal cortex, and basal ganglia function and positive effects on domains of both hippocampal and prefrontal cortical-dependent cognitive function in animal models that reflect cognitive domains impaired in AD. Thus, this compound was advanced to first-in-human testing (Phase 1).

Methods: Completed toxicology in two species out to 28 days of daily administration showed no significant toxicity. We have now conducted a double-blind Phase 1 human Single Ascending Dose (SAD) tolerability and dose ranging studies in both sexes (n = 52). Dosing was based on NOAEL levels in 2 species. This study combined utilized typical safety and PK assessment with novel behavioral and brain-based measures targeting cognitive processes and sensitive to cholinergic drug effects including the effects of cholinergic stimulation on electrophysiologic measures of memory using event-related potentials (ERP) that we developed for a study of cholinergic enhancement in aging adults with Down syndrome (DS), a single-gene model of AD.

Results: Results of the SAD have shown excellent tolerability with no dose limiting side effects and no significant adverse events consistent with off target muscarinic receptor stimulation throughout the full range of single doses tested. PK showed excellent oral absorption and bioavailability with a half-life consistent with once daily dosing. We also have evidence that VU319 induces effects consistent with the potentiation of M1 activity in the CNS by altering cortical ERP amplitudes to novel vs repeated stimuli and changes in cognitive performance at doses that do not produce typical muscarinic side effects.

Conclusions: M1PAM compounds induce effects consistent with the potentiation of M1 activity in the CNS and can alter cognitive performance and brain activity at doses that do not produce typical muscarinic side effects. After the multiple ascending dose study, we will advance to a Phase 2a POC study to assess modulation of the functional integrity of cortical networks in Mild Cognitive Impairment. We will utilize fMRI and EEG to assess resting and task-based connectivity of the default mode and cognitive control networks involved in the regulation of episodic and working memory, attention, and inhibition of irrelevant information, as well as how M1PAMS influence global network topologies. The goal will be to validate that effects of VU319 are secondary to network connectivity alterations and help in selecting measures for larger Phase 2 and 3 trials.

Disclosure: Nothing to disclose.

Panel

54. Prefrontal Postnatal Circuit Development: Implications for Disorders Related to Cognition, Anxiety, and Emotional Dysregulation

54.1 Time-Lines for Postnatal Myelin Development Differ Both Between Prefrontal Cortical Areas and Across Cortical Layers With Each Area

Suzanne Haber

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Background: Compelling evidence points to neurodevelopmental origins of many psychiatric disorders, including schizophrenia,

bipolar disorder, major depression, post-traumatic stress disorder (PTSD) obsessive-compulsive disorder, and addiction. A key element of postnatal maturation is the development of myelin, which continues through adolescence. The general progression of myelination of white matter bundles through postnatal development has been described and progresses from with sensory systems, motor control areas, and lastly associative cortical regions. However, myelinated fibers invade the grey matter and, in adults, is particularly dense in layers 6 and 5, but also penetrates layers 4, 3, 2, and superficial 1. Indeed, myeloarchitectonic studies show the variation of myelin distribution in PFC areas and in different layers. PFC areas are closely associated with particular functions and often linked to abnormalities in specific psychiatric illnesses. Since these diseases may reflect circuit dysfunction during development, our studies focused on myelin development. We hypothesized is that areas within the PFC do not develop at a uniform rate and that this variation occurs not only between areas, but between cortical layers.

Methods: PFC tissue from nonhuman primates was collected at 6 ages: 3wk., 3mon. 6mon. 1 yr., 3 yr., and adult and stained for myelin with adjacent sections stained for NeuN to identify cortical layers. Optical density measures were used to compare across grey matter areas, layers, and ages. All experiments were carried out in accordance with the Institute of Laboratory Animal Resources Guide for the Care and Use of Laboratory Animals and approved by the University Committee on Animal Resources.

Results: The results demonstrate variation of myelination across different PFC areas at the different ages. For example, the highest levels of myelin in the cortical grey matter in the adult animals were found in the dorsomedial PFC. However, in the 3wk. old animal, the highest levels were found in lateral orbito-PFC. Furthermore, distributions also vary across cortical layers. For example, layers 4-6 showed the highest levels across all PFC areas in the adult animal. However, in the 3 wk. only animal, only lateral orbito-prefrontal cortex PFC showed had the highest levels across layers 4-6, with vlPFC and dorsomedial PFC showing dense levels in only layer 6. At this age layers 1-3 showed minimal myelin density in all PFC areas. However, in the 6mon. old animal the variability across areas and layers differed from the 3wk. old animals. Importantly, the variation in distribution patterns across areas and layers in the adult animal could not predicted by the pattern in either the 3wk. or 6mon. old animals.

Conclusions: The data demonstrates that there are different rates of development both across PFC areas and within the layers of these regions. These results, which show normative data on the variability of myelin development areas (and layers) of the PFC, provides the foundation for determining where and how abnormalities during specific times in neurodevelopment may impact on various PFC pathways.

Disclosure: Nothing to disclose.

54.2 White Matter Connectomes at Birth Predict Cognitive Abilities at Age 2

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Background: Intelligence is an important predictor of mental health outcomes that is influenced by neurodevelopment. The foundational wiring of the human brain appears to be in place by birth, and the white matter (WM) connectome supports developing brain function. It is not known how the WM connectome at birth supports emergent cognition.

Methods: A deep learning model was trained using cross-validation to classify full-term infants ($n = 78$) as scoring above, at, or below average at age 2 using WM connectomes generated from diffusion magnetic resonance images at birth. Results from this model were used to predict individual cognitive scores. Findings were replicated in a set of preterm infants ($n = 37$). WM regions and connections important for classification were identified.

Results: WM connectomes at birth predicted 2-year cognitive score group with high accuracy in both full-term (80%) and preterm (75%) infants. Scores predicted by the model were strongly correlated with actual scores ($r = 0.95$ and $r = 0.97$ for full-term and preterm, respectively). Regions in the frontal lobe and right insula were important for predicting future cognition, along with connections between language, sensory, and motor regions.

Conclusions: WM connectomes at birth accurately predict a child's 2-year cognitive group and individual score in full-term and preterm infants. The WM connectome at birth appears to be a useful neuroimaging biomarker of subsequent cognitive development that deserves further study.

Disclosure: Nothing to disclose.

54.3 Developmental Changes in Amygdala Functional Connectivity During Emotion Processing in Pediatric PTSD

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Background: Pediatric post-traumatic stress disorder (PTSD) in youth is characterized by abnormal emotion processing related to trauma-exposure and has been associated with abnormal amygdala-prefrontal coupling in cross-sectional studies. However, little is known about the developmental changes in amygdala-prefrontal coupling over time in youth with PTSD, nor to what extent these changes are associated with symptom persistence and recovery. In this naturalistic longitudinal study, we examined changes in amygdala functional connectivity over time in youth with PTSD compared to non-traumatized healthy comparison (HC) youth.

Methods: In this longitudinal neuroimaging study, we recruited 22 medication-free youth with PTSD (average age = 14.04 years at baseline, 13 F) and 21 HC youth (average age = 13.84 years at baseline, 16 F), each completing psychiatric assessments and fMRI at baseline and one-year follow-up. At each time point, youth completed an implicit emotion processing task consisting of dynamic emotional facial expressions relative to a morphing shape control. Using linear mixed-effects regression, we examined group and time main effects, as well as group by time interactions, in left and right amygdala functional connectivity, correcting for multiple comparisons using cluster-based thresholding. In FDR-corrected post-hoc analyses, we related changes in amygdala connectivity with symptom change within PTSD youth. All results were adjusted for sex and age at baseline.

Results: In group by time interactions, the right amygdala showed abnormal functional coupling with the left amygdala/anterior hippocampus ($t20 = 5.00$; $p < 0.01$), bilateral dorsal anterior cingulate cortex (dACC; $t20 = 4.03$, $p < 0.01$), and dorsomedial prefrontal cortex/pre-supplementary motor area (dmPFC/pre-SMA; $t20 = 4.11$, $p < 0.01$) for across emotional faces relative to shape. Within all circuits, youth with PTSD displayed increased coupling over time, while HC youth showed decreased coupling over time. Further interrogation showed the inter-amygdala circuit was driven by the centromedial nucleus, while

the amygdala-prefrontal circuits were driven by the basolateral and superficial nuclei. Finally, all three circuits were inversely related to PTSD symptom expression.

Conclusions: These findings indicate abnormal functional development of inter-amygdala and amygdala-prefrontal circuitry over time in pediatric PTSD. Increased coupling in these circuits may reflect improved salience processing (amygdala, dACC) as well as cognitive control of emotion (dmPFC) over time in afflicted youth. Notably, increased coupling in these circuits was associated with illness improvement, suggesting a potential neural substrate for symptom reduction in the context of development.

Disclosure: Nothing to disclose.

54.4 Multimodal Neuroimaging of Prefrontal Cortical Networks in Youth at Risk for Bipolar Disorder

Mary Phillips

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Background: We previously reported in adults with Bipolar Disorder (BD) abnormally elevated activity in the left ventrolateral prefrontal cortex (vlPFC) during uncertain reward expectancy, and abnormal left vlPFC white matter connectivity, likely reflecting underlying abnormalities in reward-related decision-making. Using multimodal imaging, we determined the extent to which left vlPFC structural, functional and connectivity abnormalities characterized BD at-risk youth versus youth at risk for psychiatric disorders in general, and whether these neural measures predicted future worsening of hypo/mania, pathognomonic symptoms of BD, in all at-risk youth.

Methods: We performed three studies: Study 1: A tract-profile probabilistic tractography approach examined 18 major white matter tracts in 38 offspring of BD parents at genetic risk for BD (OBP), 36 offspring of comparison parents with non-BD psychopathology at risk for psychiatric disorders in general (OCP), and 41 offspring of healthy parents (OHP) (13.7-14.0 (SD = 2.0-2.6) years; 45 female). Study 2: We measured resting state functional connectivity among left vlPFC and the salience network in 24 OBP, 20 OCP, and 27 OHP (13.8-14.3 years (SD = 1.9-2.3); 32 female). Study 3: In 41 OBP and OCP (13.9 years (SD = 2.28); 19 female), regularized regression determined which clinical, demographic, reward and emotion processing-related neural activity, and cortical gray matter thickness, variables predicted future mixed/mania vs. future irritability, and anxiety/depression symptoms (child affective lability scale (CALS) and affective lability scale (ALS) factors) 29 months post scan. Predictor models were tested in an independent group of 51 BD at-risk, emotionally dysregulated youth (13.7 years (SD = 1.9); 25 female) at 24.8 months post scan.

Results: Study 1. Healthy and non-BD affected OBP and OCP showed significantly lower fractional anisotropy (FA) in left-sided tracts (especially in the cingulum; also in inferior longitudinal fasciculus, forceps minor), and significantly greater FA in right-sided tracts (uncinate fasciculus, inferior longitudinal fasciculus), vs. OHP ($p < 0.05$). Study 2. Across all youth, mood lability, a risk factor for BD, correlated negatively with rsFC between left vlPFC and left insula ($p = .0007$) and middle cingulate gyrus ($p = .0002$). Study 3: Only mixed/mania symptom severity was predicted by left vlPFC thickness. Specifically, greater future mixed/mania was predicted by greater left vlPFC thickness, as well as lower bilateral parietal cortical and right transverse temporal cortex thickness, and greater self-reported depression and affective lability severity and age at scan (penalized regression coefficients: -0.67-0.45). This pattern of predictors of future mixed/mania was replicated for all variables except parietal cortical thickness in the independent youth sample.

Conclusions: Left prefrontal cortical white matter connectivity abnormalities and abnormally reduced left vlPFC resting state functional connectivity are associated with risk for psychiatric disorders, but especially BD, in youth; and greater left vlPFC cortical thickness predicts greater future mixed/mania symptom severity in these youth. These data parallel findings showing aberrant left vlPFC activity and white matter connectivity in adults with BD and suggest that abnormal development of the left vlPFC may predispose to BD in youth and adults.

Disclosure: Nothing to disclose.

Study Group

55. Drug Development in Psychiatry: Bridging Industry, Academia, and Government

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Study Group Summary: Despite the ever-growing need for novel treatments, only few large pharmaceutical companies continue to pursue neuropsychiatric treatment trials. Challenges exist throughout all stages of the development process. The early stage discovery of targeted treatments is hindered by imperfect animal and computer models, heterogeneity of pathophysiology of neuropsychiatric disease, and unintended safety issues (despite promising preclinical data from mechanistic hypotheses). The challenges of late stage development largely relate to failures to overcome placebo response in large multicenter clinical trials, despite initial excitement from small pilot studies. Particularly in neuropsychiatric disease, clinical trials face the well-known challenge of subject selection—in part, due to the inexactness of psychiatric diagnosis and lack of diagnostic biomarkers. In addition to scientific challenges faced by drug development, all stages of discovery are associated with tremendous financial and time costs. Failures can result in a myriad of consequences, including halting entire development programs.

Sponsored by the ACNP Liaison Committee, this study group (comprised of individuals from industry, academia, and government) will discuss these challenges faced by drug and device developers through shared perspectives and experience, including lessons learned from past trials. The panelists will discuss the process of choosing therapeutic areas from both neuroscience and general industry perspectives. Innovative models of collaborations across industry, academia, and government (FDA and NIMH) will be discussed with the goal of moving towards cohesiveness and efficacy in neuroscience drug development.

Disclosure: Janssen Pharmaceuticals, Employee

Panel

56. The Multi-Center Social Processes Initiative in Neurobiology of the Schizophrenia(s) (SPINS) Study: Large 'N', Computationally-Driven and Replicable Brain-Behavior Relationships

56.1 Lower- And Higher-Level Social Cognitive Factors Across Individuals With Schizophrenia Spectrum Disorders and Healthy Controls: Relationship With Neurocognition and Functional Outcome

Abstract not included.

56.2 Individual Brain Networks of Structural Similarity and Their Relation to Social and General Cognition Across Schizophrenia Spectrum Disorders

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Background: Cognitive abilities including neurocognition and social cognition are tightly related to an efficient network organization of the human connectome. Deficits are important factors in poor functional outcome in schizophrenia spectrum disorders (SSDs) which have long been considered disorders of dysconnectivity in the human brain. Here we test how aberrant individual network architecture contributes to cognitive deficits in the general and the social domain.

Methods: We used cortical network mapping based on the similarity of inter-regional morphometric parameters measured by multimodal MRI (diffusion weighted imaging and structural MRI) to compute similarity networks in each of 180 patients with schizophrenia spectrum disorder and 122 healthy controls. Using graph theory, we derived metrics describing network organization and efficiency as well as variation in network nodes and entered them in a partial least squares regression to predict variation in general and social cognition.

Results: In a preliminary analysis we found that deficits in cognitive reasoning were predicted by lower rich club organization, i.e., the connectedness of high-degree hubs with each other ($r = 0.32$, $P < 0.001$), whereas deficits in social cognition were predicted by increased network segregation ($r = -0.29$, $P < 0.001$). In addition, the relationship between nodal degree (the number of edges connecting a node to the rest of the network) and verbal intelligence was significantly weaker in SSDs ($r = 0.11$, $P = 0.14$) compared to healthy controls ($r = 0.39$, $P < 0.001$; difference: Fisher's $z = 2.54$, $P = 0.01$). Finally, patients with SSDs showed increased clustering and decreased network integration compared to controls.

Conclusions: Individual modeling of brain network characteristics provides the means to elucidate the association between cognition and brain organization across diagnostic groups. Specifically, these results suggest that distinct features of aberrant cortical network architecture contribute to deficits in social as well as general cognition across the schizophrenia spectrum.

Disclosure: Nothing to disclose.

56.3 Separable and Replicable Patterns of Social Brain Function in People With and Without Serious Mental Illness

Robert Buchanan

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Background: The case-control study design and disease heterogeneity may be major limiting factors impeding biomarker discovery in brain disorders, including serious mental illness, such as schizophrenia or bipolar disorder. We used a data-driven approach to examine whether fMRI data collected during the performance of the imitate/observe task identified unique subgroups, and whether these subgroups differed in their performance on social cognitive and neurocognitive tests. We hypothesized that differences in the patterns of mentalizing circuit activation across the identified subgroups would be differentially associated social cognitive and neurocognitive test performance.

Methods: Participants were recruited from three sites ($N = 179$; 109 with a schizophrenia spectrum disorder (SSD) and 70 healthy controls (HC)) and underwent a fMRI scan while performing the observe/imitate task. A hierarchical clustering (Ward's method) data-driven approach was used to examine whether the fMRI data collected during task performance identified unique subgroups. We compared Euclidean distance among participants by clustering membership, diagnosis, and site. We then examined whether the new data-driven groups of participants demonstrated performance differences on social and neurocognitive tests completed out of the scanner. In an independent sample comprised of participants from one site ($N = 108$; SSD, euthymic bipolar disorder, or HC), we then attempted to replicate the cluster structure observed in the discovery sample.

Results: Three clusters with distinct patterns of neural activity were found. Participants in these clusters showed substantially shorter Euclidean distance to diagnosis or site. The largest cluster represented 'typical activators', with activity in the canonical 'mirroring' network (mentalizing circuit). The other clusters represented a 'diffuse/inefficient' activating group, and an 'efficient/deactivating' group. There was a significant difference among the three clusters in cognitive test performance ($F(2,174) = 4.04$, $p = .019$), with the efficient/deactivating cluster performing better than the other two clusters (post-hoc t-tests $p < 0.05$). Cluster membership was not related to diagnosis or scan site. The replication sample identified the same three cluster patterns.

Conclusions: In independently collected samples, our findings demonstrate different patterns of neural activity among individuals during a socio-emotional task independent of DSM-diagnosis or scan site. Our findings may provide objective neuroimaging endpoints (or biomarkers) for subgroups of individuals in target engagement research aimed at enhancing cognitive performance regardless of diagnostic category.

Disclosure: Avanir, Advisory Board; Astellas, Advisory Board; Roche, Advisory Board; Takeda, Consultant

56.4 Functional MRI Biomarkers of Cognitive Performance and Functional Outcome Using Machine Learning Classification Approaches: New Data From the Multi-Center Social Processes in Neurobiology of the Schizophrenia(s) (SPINS) Study

Background: Deficits in neurocognition and social cognition are drivers of reduced functioning in schizophrenia spectrum disorders (SSDs), with potentially shared neurobiological underpinnings. Many studies have sought to identify brain-based biomarkers of these clinical variables using a priori dichotomies (i.e., good vs. poor cognition, deficit vs. nondeficit syndrome). Using our multi-center design from the Social Processes Initiative in Neurobiology of the Schizophrenia(s) study, we report early data from a large sample of people with schizophrenia spectrum disorders (SSDs) and healthy control participants.

Methods: We evaluated a fully data-driven approach to do the same by building and validating a brain-connectivity-based biomarker of social cognitive and neurocognitive performance in a sample using resting state and task-based fMRI, including the facial observe/imitate task and the empathic accuracy task ($n = 75/133$ healthy controls/SSDs, 188 total, both men and women). We used canonical correlation analysis followed by clustering to

identify a functional connectivity signature of normal and poorly-performing social cognitive and neurocognitive performance. We then attempted to validate our findings using structural neuroimaging measures of cortical thickness and white matter fractional anisotropy. We also attempted a replication of our findings in an independent sample (n = 75).

Results: Those with poor social cognitive and neurocognitive performance were differentiated from those with normal performance by greater resting-state connectivity in the mirror neuron and mentalizing systems. Resting state connectivity was superior to connectivity from either of the task-based measures in classifying cognitive performance (AUC = 0.88), and combinations of imaging approaches did not improve the classifier's performance. We validated our findings by showing that poor-performers also scored lower on functional outcome measures not included in the original analysis and demonstrating neuroanatomical differences between the normal and poorly-performing groups (cortical thinning in bilateral frontal and temporal cortex $p = 8.0 \times 10\text{-}3$) and greater mean diffusivity in bilateral external capsule, internal capsule, and fornix). We used a support vector machine classifier to demonstrate that functional connectivity alone is enough to distinguish normal and poorly-performing subjects and replicated our findings in an independent sample (n = 75 total) (AUC = 0.84). the correlation between the connections that passed FDR correction in both samples was high ($r = 0.95$, $p = 5.9 \times 10\text{-}19$), suggesting that both procedures captured similar connectivity features related to cognitive performance. The majority of the significant group differences in both samples represented over-connectivity of the mirror network regions in the poorly performing group.

Conclusions: In a large, multi-center sample, we demonstrated that fMRI features from resting state, and not social cognitive task paradigms, were best at finding cognitively-different groupings in the data, including functional outcome with a high level of accuracy. A brief fMRI scan may ultimately be useful in future studies aimed at characterizing long term illness trajectories, and treatments that target specific brain circuitry in those with impaired cognition and function.

Disclosure: Nothing to disclose.

Study Group

57. Ethical Aspects of Genetic Testing in Psychiatry

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Study Group Summary: With the rapidly accelerating discovery of robust genetic variants associated with psychiatric disorders, interest on the part of patients, families and clinicians in the prospects for genetic testing is likely to increase. Given the substantial burden of morbidity, disability and even mortality associated with major mental illness, genetic tests for predicting or diagnosing psychiatric disorders could be highly impactful. This Study Group, sponsored by the ACNP Ethics Committee, will provide a concise overview of the ethical challenges associated with growing interest in genetic testing in psychiatry. Dr. Smoller will begin by discussing the current status of genetic testing relevant to psychiatric disorders and the challenges raised by the growing number of marketed pharmacogenetic and direct-to-consumer genetics tests. Since genetic testing in child psychiatry often raises questions of the test's predictive value, Dr. Cook will focus on the limits of prediction of conditions such as autism and about the dangers of excessive enthusiasm about the value of personalized medicine in psychiatry (and other areas of medicine). Given the current limits of prediction, Dr. Austin, a pioneer in psychiatric genetic counseling, will describe the ways in which existing knowledge of the heritability of psychiatric disorders can be used to provide meaningful guidance to patients and families even in the absence of definitive predictive testing. One of the most contentious ethical issues in genetics is evoked by the ability of many genetic testing technologies to produce unexpected findings unconnected to the purpose of the testing. Dr. Lazaro, based on data from qualitative interviews with 39 psychiatric genetics researchers from different countries, will provide an insider's view of their decision-making around which results should be returned and why, semantic disputes about what "counts" as clinically valid or relevant, and contextual contingencies for return of results, including social, psychological and ethical impacts on participants and researchers alike. An important driver of uptake of psychiatric genetic testing will be coverage by health insurers. Dr. Esser, who helps to make coverage decisions for Blue Cross of Nebraska, will point to the variables that guide those determinations. Finally, since psychiatric genetic testing may have uses outside of medicine that create ethical concerns, Dr. Appelbaum will describe the ways in which the legal system has embraced genetic data in both criminal and civil cases, the future applications of genetic evidence that are likely to arise, and the ethical challenges that creates for genetic experts and the courts. These brief presentations will be followed by interaction with the attendees regarding their views of the ethical challenges of psychiatric genetic testing and how they should be addressed.

Disclosure: Nothing to disclose.

Panel

58. Challenges and Solutions to Elucidating Psychiatric Disease Biology From Genomic Association Using Human Induced Pluripotent Stem Cell-Based Assays

58.1 Functional Validation of the Impact of Rare and Common Variants to Schizophrenia Risk

Kristen Brennand

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Background: Schizophrenia (SZ) is a debilitating psychiatric disorder for which the complex genetic mechanisms underlying the disease state remain unclear. Whereas highly penetrant variants have proven well-suited to human induced pluripotent stem cell (hiPSC)-based models, the power of hiPSC-based studies to resolve the much smaller effects of common variants within the size of cohorts that can be realistically assembled remains uncertain. We consider the successes and limitations in applying human induced pluripotent stem cell (hiPSC)-based models to study the impact of rare and common variants in SZ risk.

Methods: We reprogrammed fibroblasts from patients and controls into hiPSCs and subsequently differentiated these disorder-specific hiPSCs into neural progenitor cells (NPCs) and neurons, with the objective of better understanding how both rare and common variants contribute to SZ risk. To facilitate isogenic analyses of the impact and penetrance of rare and common variants across genetic backgrounds, we integrated CRISPR-mediated gene editing, activation and repression technologies with our hiPSC-based neural platform, developing a scalable

system to test the effect of manipulating the growing number of SZ-associated variants and genes in NPCs, neurons and astrocytes.

Results: First, we investigated the relationship between heterozygous 2p16.3 (NRXN1 + /-) deletions, alternative splicing of NRXN1, and perturbations in neuronal function. We identified 102 high-confidence NRXN1α isoforms in control hiPSC neurons from two donors; 16 of the 32 detected control isoforms in patient-derived NRXN1 + /- hiPSC neurons showed > 2-fold decreased expression. NRXN1 + /- hiPSC neurons from four cases show decreased neuronal activity ($p < 0.05$), which can be ameliorated by overexpression of a single NRXN1 isoform ($p < 0.05$).

Second, we present a genetics-driven hiPSC-based approach for the functional validation of common variants and genes associated with SZ, evaluating one putative causal SZ SNP (FURIN rs4702) and two SZ-associated genes (SNAP91 and TSNARE1). Allelic conversion of FURIN rs4702 from AA to GG decreases FURIN levels in hiPSC-neurons by nearly 30% ($p < 0.03$), whereas reciprocal activation (CRISPRa) and repression (CRISPRi) of endogenous SNAP91 increases ($p < 0.0001$) and decreases ($p < 0.0001$) spontaneous excitatory post-synaptic currents, respectively.

Conclusions: We demonstrate a systematic and scalable strategy to interpret and evaluate the growing number of SZ-associated variants and genes across neural cell types and genetic backgrounds. Altogether, our objective is to dissect the genetic origins of SZ while developing a precision medicine approach to screen for novel therapeutics with which to prevent or reverse disease course.

Disclosure: Nothing to disclose.

58.2 Landscape of Allelic Chromatin Accessibility in hiPSC Models of Neuropsychiatric Disorders

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Background: Understanding the disease biology underlying the genome-wide association study (GWAS) findings of schizophrenia (SZ) is imperative for their clinical translation. Because each GWAS locus often spans multiple genes/variants equivalently associated with SZ due to linkage disequilibrium, it is challenging to identify which are the actual causal variants/genes. Given that most SZ GWAS risk variants are noncoding or regulatory and chromatin accessibility strongly influences gene expression, we hypothesize that SZ relevant noncoding sequences likely overlap with cell type-specific open chromatin regions (OCRs). Using human excitatory neurons derived from induced pluripotent stem cells (iPSCs) as a cellular model, we have recently demonstrated that neuronal OCRs can help prioritize putatively functional SZ risk variants (Forrest M. et al., Cell Stem Cell 2017). However, the landscape of genetic variants affecting open chromatin in different cell types relevant to SZ remains unknown. Considering the allele-specific open chromatin (ASoC) as a functional readout, we aim to systematically identify regulatory SZ GWAS risk variants.

Methods: Open chromatin peaks are notoriously noisy, which makes the direct comparison of case-control differential chromatin accessibility challenging. In contrast, ASoC assay directly compares the chromatin accessibility of the two alleles of a heterozygous SNP within the same sample, thus substantially reducing the variation. We carried out OCR mapping by ATAC-seq in 20 hiPSCs enriched for heterozygous SNPs at ~70 SZ GWAS loci. We assayed both ATAC-seq and RNA-seq in hiPSCs, neuronal progenitor cells (NPCs), induced glutamatergic (iN-Glut), GABAergic (iN-GABA), and dopaminergic (iN-DA) neurons. We analyzed ASoC for all the 20 subjects (> 80% power to detect ASoC) in NPCs and iN-Glut. The target genes of the putative regulatory SZ GWAS risk variants were further examined by CRISPR editing.

Results: With ATAC-seq data of 5 cell types of 8 individuals, we identified ~580 K non-overlapping open chromatin peaks, mostly overlapping with putative enhancers or promoters. Integrative analysis of ATAC-sea and RNA-seq data revealed a moderate correlation between local chromatin accessibility with cell-specific gene expression. With iN-Glut of 20 lines, we identified ~100 K heterozygous SNPs, of which ~6,000 showed ASoC. 25 SZ GWAS risk SNPs at 21 risk loci showed ASoC, representing a 6-fold enrichment ($p = 6 \times 10\text{-}11$). Furthermore, the SZ GWAS SNPs showing ASoC are enriched for brain eQTLs (2-fold), methylation-QTLs (3.6-fold) and histone acetylation-QTLs (2.6-fold). Further CRISPR/Cas9 genome/epigenome editing of the ASoC sequences identified their cis-regulated genes. We found that the transcriptional effects of individual GWAS risk SNPs may be small and genetic-background-dependent.

Conclusions: Our study suggests that chromatin accessibility in human iPSC models is an effective functional readout that predicts likely causal noncoding GWAS risk variants/genes of neuropsychiatric disorders.

Disclosure: Nothing to disclose.

58.3 From Genetic Variant to Function in Psychiatric Disease Using Human Stem Cell Models

Kevin Eggan

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Background: It has been extremely challenging to make iPSC models of nervous systems disorders sensitive and reproducible enough to measure the small effects of the common genetic variants that are implicated in Schizophrenia other psychiatric disorders. We have developed a new approach for measuring transcription and functional phenotypes of iPSC-derived cell types in large pools, allowing as many as 100 individuals to be simultaneously measured with droplet-based approaches. This system greatly reduces experimental variance and enables as a result we have been able to measure the impact of these variants successfully through expression quantitative trait locus (EQTL) identification. We will present on these approaches, which we call "Dropulation Genetics" and "Census-Seq."

Methods: We will present on our progress in using these novel techniques to analyze genetic effects on transcription and function of human neurons from more than 100 individuals in single experiment. Our overall goal to expand such studies to monitor as many as 1000 individuals at a time. We will report on our progress towards that goal. These studies will include analysis of a cohort of IPS cells lines from the California Institute for Regenerative Medicine that will eventually scale to 3000. These studies will include expression quantitative trait locus identification and in vitro GWAS experiments that will include both analysis of individuals of both sexes.

Results: Using these techniques we have found that variation in measure and technical aspects of human neurons experiments derived from stem cells are dramatically and significantly reduced. For instance, we found that the mean coefficient of variation in gene expression when analyzing 32 stem cell derived cultures was 0.2. This was reduced to 0.14 when the same samples were combined immediately before sequencing and this variance was further reduced to 0.08 when the cell lines were cultured together for two days prior to analysis. These improvements in noise

reduction have allowed us to identify expression quantitative trait loci in human neurons that are driven by precisely the same SNPS found in haplotypes associated with Schizophrenia. Through these efforts we have taken haplotypes of unclear functional importance associated with Schizophrenia and identified the gene which in them that are likely impacted, the direction of expression change and information on the cell type in which the effect is found. In particular, I will take a deep dive into how this approach has revealed the functional consequence of harboring variants of differing association with Schizophrenia clustered around Nicotinic Acetylcholine Receptor genes where the adjusted P value for the EQTL governing expression is < 0.0004. In fact, the correlation between significance of association for Schizophrenia and this EQTL is greater than 0.85 for more than 75 SNPs across the locus.

Conclusions: Improvements in single cell sequencing technologies, the availability of large numbers of iPS cell lines and advances in computation have provided a major breakthrough that will allow substantial advances understanding of how genetic variants implicated in Schizophrenia and other conditions are changing gene function.

Disclosure: Nothing to disclose.

58.4 Integrative Multi-Omics Analyses of Ipsc-Derived Brain Organoids Identify Early Determinants of Human Cortical Development

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Background: Risk variants for complex developmental disorders are often located in noncoding regions of the genome, containing putative gene regulatory elements. The mapping and functional analysis of these elements, such as enhancers, is challenging. First, the location of enhancers is often located far away from genes; second, enhancer's activity is development- and tissue-specific and polymorphic across individuals.

Methods: To examine the location and activity of gene regulatory elements (regulome) in early cortical development, we performed longitudinal analyses in human induced pluripotent stem cells (hiPSC)-derived cerebral cortical organoids with an integrated set of experimental approaches: ChIP-seq for 3 histone marks and segmentation of the chromatin into putative enhancers, promoters and repressed regions; chromatin conformation analyses (Hi-C) and mapping of putative enhancers to their target genes; and RNA-seq, to assess the effect of differential enhancer activity on gene expression.

Results: Comparative transcriptome analyses of organoids with isogenic human brain tissue placed organoids at very early stages of cortical development, before 16 weeks post-conception. Globally, a total of 96,375 active enhancers were found to be associated with 22,835 protein-coding or lincRNA target genes. There were 83,608 and 46,735 active enhancers in organoids and fetal cortex, respectively, and about 50% of the enhancers active in organoids were no longer active in fetal cortex. In organoids, more enhancers changed activities during the transition from neural stem cells to progenitors, as opposed to the transition from progenitors to neurons (15,485 vs 4,871; p-value < 0.0001 by Chi-square test). Networks of correlated gene and enhancer modules could be assembled by K-means clustering of eigengene matrices into six and four global patterns of expression/activity across time. A pattern with progressive downregulation was enriched with enhancers whose activity was increased in human evolution (p-value < 2.2e-16 by Fisher's exact test compared with non-human gained enhancers). Using WGS data of 242 families with autism spectrum disorder (ASD) from the SSC, we found that early enhancers, expressed in organoids, were significantly enriched in inherited low allele frequency SNPs found in probands relative to their siblings (t-test, p-value < 0.003, 95% CI).

Conclusions: hiPSC-derived organoids model embryonic to early fetal human brain development, stages that are difficult to study using postmortem tissue. The organoid system promises to unravel genes and regulatory elements driving the onset of neurodevelopmental disorders.

Disclosure: Nothing to disclose.