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Poster Session III
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W1. Rs362691 Polymorphism in RELN Gene Modulates the Detrimental Effect of Alzheimer's Disease Risk Genes on Hippocampal Function

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Background: Late Onset Alzheimer's Disease (LOAD) is one of the most common debilitating causes of dementia worldwide with heritability estimates ranging from 50 – 70%. Genome-wide association studies (GWAS) have identified more than 20 genetic loci in addition to APOEε4 that are associated with increased risk for LOAD. While most of these genes have weak effects, using a polygenic risk profile score (RPS) approach – a method that allows exploration of the influence of the cumulative effect of risk alleles - we and others have shown the negative influence of LOAD risk genes on brain structure (Chauhan et al., 2015) and function (Xiao et al., 2015 HBM) even in healthy volunteers. Identifying mechanisms, particularly genetic mechanisms that confer resilience to the detrimental effect of LOAD related risk genes on brain structure and function could provide a viable avenue to identify novel therapeutic targets for LOAD. To that end, in the current study, we explored the role of polymorphisms in the gene encoding Reelin (RELN), a glycoprotein that has been shown to be critical for neuronal development and synaptic plasticity (Kramer et al. 2011), on the detrimental effect of LOAD RPS on hippocampal function. Studies have shown that normal RELN levels are necessary to prevent abnormal phosphorylation of tau (Ohkubo et al., 2003) and beta-amyloid-induced suppression of long term potentiation and NMDA receptors (Durakoglugil et al., 2009).

Methods: BOLD functional MRI images (GE 3 T MRI scanner, TR/TE = 2000/28ms, flip angle = 90 deg, FOV = 64x64, 24 axial slices, 170 volumes) were collected for 265 right-handed Caucasian healthy volunteers (116 male, 149 female) from the age of 18 to 86 years (SD = 14.17) while they performed a simple declarative memory task (SDMT). Images were motion-corrected, normalized to MNI space, and spatially smoothed (8mm FWHM) using SPM5. Odd's ratios of 22 independent SNPs, with $P < 1 \times 10-5$ in Hollingworth's meta-analysis1 comprising four Alzheimer's disease GWAS datasets (GERAD1, EADI1, TGEN1, ADNI), spanning the regions of ABCA7, APOC4, APOE, BCAM, BCL3, BIN1, C16orf88, CDK1, CEACAM1E, CLPTMI, CLU, CNTN5, CR1, CR2, CUX2, EXOC3L2, IQCK, LRRC68, MS4A4A, MS4A4E, MS4A6A, PICALM, PVR, PVRL2, and TOMM40 genes, were used to calculate the RPS for each individual subject using the approach described by Purcell et al.3. Association between RPS and hippocampal activation during the neutral encoding phase of the SDMT was tested using SPM12. To control for population stratification, 5 MDS components based on 8M SNP genotypes from a GWAS analysis extracted with EIGENSOFT5.01 were included in the analysis as covariates along with age, gender, SNAV, and genotyping batch labels. A region of interest analysis was performed using bilateral hippo-parahippocampal masks from the Anatomical Automatic Labeling Atlas. Influence of RELN on association between LOAD related AD RPS and hippocampal activation was examined separately for five independent Reelin polymorphisms (rs736707, rs362691, rs7341475, rs6943822, and rs4298437.) previously implicated in Alzheimer's disease or Autism Spectrum Disease using flexible factorial analysis in SPM12.

Results: fMRI analysis showed a significant negative correlation between LOAD RPS and hippocampal activation (left: PFWE_corrected = 0.005, MNI coordinates x = -39, y = -24, z = -12, right: PFWE_corrected = 0.139, Puncorrected < 0.001, MNI coordinates x = 39, y = -18, z = -18) during the neutral encoding phase of SDMT. There were no significant positive correlations. In addition, there was a significant interactive effect (left: PFWE_corrected = 0.076, MNI coordinates x = -30, y = -30, z = -6, right: PFWE_corrected = 0.368, Puncorrected = 0.002, MNI coordinates x = 27, y = -33, z = -9) of rs362691 genotype (a G-C missense variant) and LOAD RPS on activation. Furthermore, in the left hippocampus, minor allele C carriers (N = 56) showed a significant negative relationship (r = -0.47, p = 0.0002, post-hoc analysis in R) between RPS and hippocampal activation, while the major allele G homozygotes (N = 208) showed no such relationship (r = 0.0071, p = 0.9186). None of the other RELN polymorphisms tested showed a significant effect.

Conclusions: Our results, while showing a cumulative deleterious effect of several LOAD related risk genes on hippocampal function in healthy volunteers, also illustrate that this relationship is modulated by a missense SNP (rs362691) in the RELN gene. In particular, only the minor allele C carriers show a significant negative relationship between RPS and hippocampal function suggesting that homozygosity for the G allele in this polymorphism could potentially confer a protective effect.

Keywords: Polygenetic Risk Score, Alzheimer's Disease, fMRI/imaging genetics, Hippocampal Function, Cognitive Resilience

Disclosures: Nothing to disclose.

W2. Subtypes of Prefrontal Cortical NMDA Receptors in Working Memory and Normal Aging

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Background: Working memory involves the ability to briefly maintain context-specific information in mind and

to use this representational knowledge to guide current and future action. This form of short-term memory is supported by the prefrontal cortex (PFC) and is believed to rely on the ability of selectively tuned pyramidal neuron networks to persist in firing even after a to-be-remembered stimulus is removed from the environment. Ionotropic glutamate receptors of the NMDA subtype are expressed on pyramidal neurons in the PFC, and altered activity at these receptors has been implicated in working memory deficits associated with both psychiatric disorders and normal aging. NMDA receptors are biochemically diverse heterotetramers comprised of an obligate NR1 subunit and variable NR2A or NR2B subunits, the latter of which can influence both the channel kinetics and localization of the receptor to synaptic versus extrasynaptic sites. These receptor properties conferred by the NR2 subunit suggest that NMDA receptor subtypes may differentially influence normal working memory and the decline of working memory abilities across a variety of pathological conditions. The current study was designed to assess the individual contribution of PFC NR2A- and NR2B-containing NMDA receptor subtypes to working memory abilities, and, further, to determine which subunit is most relevant to the well-characterized decline of working memory abilities in normal aging.

Methods: Subjects in all experiments were young (4-6 mo.) and aged (22-24 mo.) male F344 rats. In Experiment 1, acute slices from young rats were prepared and patch clamp electrophysiological methods were used to assess the relative contributions of NR2A- and NR2B-containing NMDA receptors to evoked NMDA currents recorded from layer II/III PFC pyramidal neurons. In Experiment 2, four cohorts of young rats (n = 6-8 rats/cohort) were surgically implanted with guide cannulae directed at the medial PFC (mPFC) and trained to perform a mPFC-dependent delayed response working memory task. After acquisition of stable baseline performance, rats in each cohort received acute intra-mPFC microinjections of drugs that preferentially target either NR2A (NVP-AM077 or TCN-201) or NR2B (ifenprodil or Ro25-6981) subunits. Three doses of each drug and vehicle were administered immediately prior to testing in the delayed response task, using a randomized, within-subjects design with a 48 h washout period between successive doses. In Experiment 3, Western blot methods were used to determine expression of NMDA receptor subunits (NR1, NR2A, and NR2B) in mPFC homogenates prepared from young (n = 8) and aged (n = 15) rats that were first characterized on the delayed response task. Finally, in Experiment 4, using similar methods as in Experiment 2, the effects of NMDA receptor modulation on aged rat working memory abilities were assessed using two different drugs (D-cycloserine (DCS) and 3-methylpyrazole-5-carboxylic acid (MPC)) that target the NMDA receptor co-agonist serine.

Results: In Experiment 1, blockade of both NR2A- and NR2B-containing receptors attenuated evoked NMDA receptor currents recorded from pyramidal neurons in young rat mPFC; however, the contribution of NR2A to the total evoked current was significantly greater than that of NR2B. Consistent with this more predominant role of NR2A in NMDA-mediated currents on pyramidal neurons, both of the NR2A-preferring antagonists administered into the

mPFC of young rats in Experiment 2 significantly attenuated working memory performance. In contrast, neither NR2B-specific antagonist affected young rats' working memory performance. In Experiment 3, Western blot analysis revealed a significant reduction in expression of all NMDA receptor subunits in aged rat mPFC, but only loss of the NR2A subunit strongly predicted working memory decline. Data from Experiments 1-3 suggest that NR2A-containing NMDA receptors in PFC are particularly critical for supporting working memory abilities. The preferred role of NR2A-containing receptors in working memory may be attributable to the fact that these receptors preferentially comprise the synaptic pool of NMDA receptors that enable the persistent firing of pyramidal neurons. In Experiment 4, intra-mPFC microinjection of the well-known NMDA receptor co-agonist d-cycloserine, which non-selectively targets both synaptic and extrasynaptic NMDA receptors, did not improve working memory in aged rats. In contrast, 3-methylpyrazole-5-carboxylic acid, a d-amino acid oxidase inhibitor that more selectively enhances activity at synaptic NMDA receptors by preventing degradation of endogenous serine, significantly improved aged rats' working memory performance.

Conclusions: The present experiments specifically implicate NR2A-containing NMDA receptors in normal working memory. The results further suggest that a decline in NR2A receptor expression may be a causal factor for working memory impairments in aging. Our working hypothesis is that NR2A-containing NMDA receptors are particularly important for working memory given that these receptors are preferentially localized to synaptic sites whereas NR2B-containing receptors are preferentially localized extrasynaptically. Consistent with this hypothesis, pharmacological modulation of synaptic NMDA receptors (presumptive NR2A-containing receptors) can significantly improve working memory in aged rats.

Keywords: aging, NMDA Receptor, prefrontal cortex, working memory

Disclosures: Nothing to disclose.

W3. A Mitochondrial Role of SV2A Protein in Alzheimer's Disease: Studies with Levetiracetam

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Background: Hyperexcitability of cortical and hippocampal structures has recently been identified as one of the mechanisms of cognitive deficits in human aging, Alzheimer's disease (AD) as well as in animals Models of AD. As underlying cause dysfunction of inhibitory interneurons rather than general neuronal overactivity has been proposed probably associated with mitochondrial dysfunction. In line with these findings, out of several antiepileptics which all reduce neuronal excitability only levetiracetam was able to reduce neuronal hyperexcitability and to improve the associated cognitive defects

Methods: Because of its close relationship to the metabolic enhancer piracetam we speculated that levetiracetam

similarly improves neuronal function by enhancing mitochondrial function in a cell model of aging, AD, and the interplay of both conditions.

Results: Our findings are fully in line with these assumptions as levetiracetam showed substantial improvement of disturbed mitochondrial paraments as ATP production, mitochondrial dynamics (fission and fusion balance), mPTP pore opening, as well as impaired neuritogenesis due to mitochondrial defects. While these data are quite clear, we still were puzzled in respect of a possible mechanism of action of levetiracetam for these effects, as its primary target, the SV2A protein, seems to be closely related to brain synaptic membranes. However, we could clearly identify substantial levels of this protein also at the mitochondrial level.

Conclusions: As SV2A works in the brain as a regulator of vesicle fusion we speculated on the basis of our findings that levetiracetam reduces mPTP opening that it might be involved in the complex mechanisms regulating mPTP function which also involve complex fusion mechanisms at the level of the mitochondrial membrane

Keywords: levetiracetam, mitochondrial function, SV2A protein, aging and dementia

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W4. Positive Allosteric Modulation of Metabotropic Glutamate Receptor 5 Reverses Deficits in Hippocampal Synaptic Plasticity and Cognitive Function in a Mouse Model of Alzheimer's Disease

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Background: Alzheimer's disease (AD) is the most common form of dementia and is characterized by the progressive decline in cognitive function, with deficits in hippocampal-mediated learning and memory. In recent years, a proline-directed serine/threonine kinase termed cyclin-dependent kinase 5 (Cdk5) that is activated by the p25 protein has been increasingly implicated in AD and other neurodegenerative disorders and p25 overexpression in forebrain neurons (CK-p25 mice) induces neuropathology and cognitive impairments that are strikingly similar to those observed in patients suffering from AD and other neurodegenerative disorders. Resembling the human condition, CK-p25 mice demonstrate decreased brain mass, decreased neuronal density, neurodegeneration, increased tau phosphorylation and aggregation, accumulation of neurofibrillary tangles, as well as elevated levels of A-beta protein (Aβ) and the amyloid precursor protein (APP) processing enzyme, β-secretase (BACE1). These neuropathological changes are associated with striking impairments in synaptic plasticity in the hippocampus, and behavioral assays demonstrate severe impairments in learning and memory in CK-p25 mice. This provides an excellent model for identification of novel approaches that could be used to reverse deficits in synaptic plasticity and cognitive function that occur in neurodegenerative disorders associated with these hallmark neuropathological changes that occur in patients suffering from AD and related disorders.

Glutamate is the primary excitatory neurotransmitter in the CNS and plasticity at glutamatergic synapses is thought to be critical for learning, memory and cognition. Over the past decade, highly selective positive allosteric modulators (PAMs) of the metabotropic glutamate receptor subtype 5 (mGlu5) have emerged as a promising new approach for improving cognitive function in schizophrenia and other non-degenerative CNS disorders. As opposed to direct activation of mGlu5, PAMs dramatically potentiate the response of the receptor to its endogenous ligand, glutamate, and offer high selectivity while avoiding unwanted side-effects seen with direct activation of the receptor. Interestingly, mGlu5 PAMs enhance specific forms of synaptic plasticity in the hippocampus, as well as hippocampal-dependent forms of learning and memory in wild-type animals that are impaired in CK-p25 mice. In addition, previous studies suggest that mGlu5 activation can increase synaptogenesis in forebrain regions in which synaptic density is reduced in AD patients and in CK-p25 mice. Finally, recent studies suggest that proteins important for mGlu5 function are lost and that mGlu5 signaling is impaired in tissues from AD patients and some preclinical animal models of AD. Taken together, these studies raise the exciting possibility that mGlu5 PAMs may offer an exciting new therapeutic strategy to enhance cognitive function in patients suffering from AD and other age-related cognitive deficits.

Methods: All animal studies were approved by the Vanderbilt University Medical Center Institutional Animal Care and Use Committee and were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. CK-p25 mice were maintained on doxycycline diet for 6 weeks following birth to maintain normal p25 levels in the forebrain during development. Subsequently, CK-p25 mice were switched to normal chow to allow p25 induction for 6 weeks. A subset of littermate control mice were maintained continually on doxycycline diet throughout the studies to inhibit p25 overexpression. At 12 weeks of age, mice underwent electrophysiology studies assessing hippocampal synaptic plasticity at the SC-CA1 synapse or behavioral studies evaluating cognitive function in the presence or absence of mGlu5 PAMs.

Results: Hippocampal long term potentiation (LTP) is a form of synaptic plasticity essential for learning and memory. CK-p25 mice displayed significant deficits in LTP at the SC-CA1 synapse. In addition, profound hippocampal- and cortical-mediated cognitive deficits were observed in these mice.

Interestingly, bath application of mGlu5 PAMs restored deficits in LTP in CK-p25 mice to levels similar to control mice. Moreover, mGlu5 PAMs demonstrated dose-dependent reversal of cognitive deficits in contextual fear conditioning, novel object recognition task, as well as the pairwise discrimination task using touch screen technology in the CK-p25 mouse model of AD.

Conclusions: Utilizing the CK-p25 mouse model of AD, these studies demonstrate that selective potentiation of mGlu5 can reverse not only deficits in hippocampal neurotransmission but also severe cognitive deficits. These

results provide critical data in validating the potential utility of mGlu5 PAMs as a novel therapeutic approach for treatment of cognitive impairments associated with AD and other neurodegenerative disorders.

Keywords: Alzheimer's Disease, mGlu5-PAM, Cognition, Synaptic Plasticity

Disclosures: Nothing to disclose.

W5. Deconstructing Serotonin Circuits that Mediate Aversion

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Background: Serotonin has long been thought to be involved in a variety of behaviors, however given the complexity of this system, it has been difficult to clearly delineate the circuits involved in various aspects of behavior. This is critical, as understand the circuits involved in the aversive actions of 5HT can lead to more effective drug treatment.

Methods: Genetically modified mice were used in combination with a variety of cutting edge tools to probe 5HT circuits in the brain.

Results: Using in vivo recordings during fear conditioning, we found that fluoxetine exposure during fear learning lead to alterations in neuronal firing in the BNST. This supported previously published results that the BNST is a critical site of action for the negative effects of acute SSRI. We next explored the impact of both optically evoked and bath applied 5HT on BNST neurons in a ex vivo slice preparation. Interestingly, we found that 5HT lead to recruitment of a local inhibitory circuit that suppressed outputs to both the hypothalamus and the ventral tegmental area. We then performed a series of in vivo experiments using chemogenetic and optogenetic approaches to demonstrate that this circuit is required for 5HT induced aversion.

Conclusions: These results define the circuitry required for acute SSRI induced potentiation of fear. Moreover, we provide a pharmacological mechanism for mitigating this effect, suggesting new treatment modalities to minimize side effects of SSRI.

Keywords: Serotonin, Fear conditioning, CRF

Disclosures: Nothing to disclose.

W6. Corticotropin-Releasing Factor Activates Different Circuits in Male and Female Rats

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Background: Stress-related psychiatric disorders, such as post-traumatic stress disorder and major depression, occur twice as frequently in women as men. Corticotropin-releasing factor (CRF) orchestrates the stress response and is hypersecreted in these disorders. Thus, sex differences in responses to CRF could contribute to the sex bias in disease prevalence. We previously identified sex differences in CRF1 receptor signaling and trafficking in the locus coeruleus (LC) that render LC neuronal responses to CRF greater in female than male rodents. However, the extent of sex differences in CRF sensitivity has not been systematically explored. Here we begin to address this question by examining how the central administration of CRF differentially activates stress-related brain regions in adult male and female rats.

Methods: Adult male and female Sprague-Dawley rats were surgically implanted with cannulas aimed at the lateral ventricle and allowed at least one week to recover during which vaginal cytology was collected to identify estrous cycle stage in females. Either vehicle (artificial cerebral spinal fluid) or ovine CRF (0.3 µg) was administered to females in the proestrus phase of the estrous cycle (higher estrogen and progesterone levels), females in the diestrus phase (lower estrogen and progesterone levels), and gonadally intact males. One hour later, tissue was collected and processed using standard immunohistochemical approaches to visualize the immediate early gene, cFOS. cFOS was quantified by a rater blind to the condition in regions known for their high levels of CRF receptor expression and/or their involvement in stress responses.

Results: ANOVAs were used to reveal the effects of infusion and hormonal condition on the number of cFOS positive profiles in select brain regions. In several areas, including the paraventricular nucleus of the hypothalamus, medial prefrontal cortex, septum, dorsal hippocampus, and anterior-dorsal nuclei of the bed nucleus of the stria terminalis (BNST), the central CRF infusion increased the number of cFOS profiles compared to vehicle, regardless of sex $\{[F(1,49) = 13.7, \ p = 0.001], \ [F(1,42) = 36.5, \ p < 0.001], \ [F(1,46) = 79.7, \ p < 0.001], \ [F(1,35) = 54.1, \ p < 0.001], \ \text{and} \ [F(1,39) = 80.6, \ p < 0.001], \text{respectively}\}$. In comparison, a sex difference was observed for the locus coeruleus and oval nucleus of the BNST, such that CRF increased cFOS profiles in females relative to males $\{[F(1,25) = 6.7, \ p < 0.013] \ \text{and} \ [F(1,25) = 4.3, \ p < 0.048], \text{respectively}\}$. Surprisingly, in several brain regions, cFOS activation was altered in different estrous cycle phases in females and, in particular, diestrus females were distinguished from other groups. Specifically, diestrus females were the only group with increased cFOS profiles following CRF administration in the periaqueductal gray, ventral medial dorsal raphe, and lateral dorsal tegmental area $\{[F(2,39) = 6.0, \ p = 0.005], \ [F(2,45) = 4.4, \ p = 0.021], \ \text{and} \ [F(2,48) = 3.73, \ p = 0.031], \text{respectively}\}$. Conversely, in the nucleus basalis of meynert and nucleus accumbens, diestrus females were the only group with cFOS profiles unaffected by central CRF administration $\{[F(2,52) = 5.9, \ p < 0.01], \ [F(2,49) = 3.7, \ p = .03], \text{respectively}\}$.

Conclusions: Collectively, these findings indicate that central CRF administration activates different circuits in males and females, and that differences are further revealed across the female estrous cycle. Surprisingly, although cFOS has been widely used, previous studies were almost exclusively conducted with male rats. Thus, sex differences in the activation of brain circuits by stressors, neuropep-

tides, and other stimuli, may be an important, but under-explored, determinant of sex differences in behavior and perhaps even pathology.
Keywords: stress, sex difference, anxiety
Disclosures: Nothing to disclose.

W7. Effects Δ9-Tetrahydrocannabinol (THC) on Brain and Behavior During Fear Extinction Learning in Humans: A Combined Psychophysiological-fMRI Study

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Background: Cannabinoid agonists such as Δ9-tetrahydro-cannabinol (THC) may facilitate context-mediated learning and retention of fear extinction memories via their actions on hippocampal function. However, studies in humans have exclusively examined the effects of THC on responding to discrete fear cues (i.e., conditioned stimuli; CS) and it is unknown whether THC's target effect is on the memory for discrete cues and/or the environment that surround those cues (e.g., visual-spatial context). This question of specificity is important, especially in light of evidence suggesting that animals, including humans, rely heavily on context when appraising potential sources of danger.
Methods: The current study examined the effects of THC on brain (i.e., blood-oxygen-level dependent [BOLD] signal) and behavioral fear responses (i.e., skin conductance response [SCR]) during a standard fear acquisition and extinction paradigm involving discrete conditioned fear cues (i.e., CS) viewed within a visual-spatial/environmental context (colored lights in front of a school or forest). Forty healthy adult volunteers completed four experimental sessions over the course of 10 days. On Day 1, subjects completed a fear acquisition (ACQ) paradigm within a specified visual-spatial context (Context A/school). On Day 2 (24-hours later), conditioned fear was extinguished (EXT) in a novel visual-spatial context (Context B/forest). Two hours prior to EXT, participants randomly received an oral dose of THC (n = 18) or placebo (n = 22). Recall of the extinction memory was tested 24-hours (RECALL1; Day 3) and 1-week (RECALL2; Day 10) after EXT in visual-spatial Context B. BOLD signal (assessed via functional magnetic resonance imaging [fMRI]) and SCRs were simultaneously collected during presentation of the visual-spatial context and CS across all four sessions. Self-reported expectancy of the unconditioned stimulus (i.e., US; brief, mild electric shock) was collected after presentation of every CS.
Results: We confirmed that participants expected the US to a greater extent during presentation of the CS+ compared with the CS-, and during ACQ compared with EXT, RECALL 1, and RECALL 2. Thus, participants were able to accurately discriminate between the CS+ and CS-, and shock versus no-shock sessions. There were no effects of drug on US expectancy ratings. As for the CS+, results indicated that although there was a main effect of session for right amygdala activation (MNI peak: [28, 0, -24], Z = 3.83, k = 1640mm3, p < 0.05; ACQ > EXT = RECALL 1 = RECALL 2), there were no main effects of drug or drug by session interactions for fMRI BOLD or SCRs. For the visual-spatial context, however, there was a main effect of session and a main effect of drug on bilateral hippocampal activation that was qualified by a significant drug x session interaction relatively specific to the hippocampus (right: MNI peak [20, -34, 6], Z = 3.40, k = 1472mm3, p < 0.05, corrected; left: MNI peak[-14, -34, 10], Z = 3.69, k = 1072 mm3, p < 0.05, corrected). During EXT and both recall phases, the THC group exhibited greater hippocampal activation relative to the placebo group (ts > 2.5, p < 0.05). Behaviorally, results also indicated an effect of drug on SCRs to the visual-spatial context such that individuals in the THC group exhibited lower SCRs to Context B during EXT and RECALL1 (ts > 2.1, p < 0.05), and RECALL 2 at a trend-level, compared with the placebo group.
Conclusions: Administration of THC did not impact responding to discrete cues but did enhance hippocampal response while dampening SCRs to repeated presentations of the visual-spatial context during EXT, RECALL 1, and RECALL 2 when the previously feared cue was re-represented in the absence of aversive stimuli (i.e., shock). This adds to a growing literature implicating the hippocampus in contextual memory processes and more importantly could suggest that THC, and potentially other CB1 agonists, facilitates and sustains the appraisal and recognition of safe environments via enhancement of hippocampal engagement. This mechanism holds promise for the treatment of individuals with anxiety and stress-related disorders as CB1 agonists may prove to be a useful cognitive enhancer adjunct to behavioral therapies that rely on extinction learning (to effectively discriminate danger from safety) and its retention across contexts.
Keywords: Fear extinction, THC, Context, conditioned cue
Disclosures: Nothing to disclose.

W8. Effects of Insulin and Diet-Induced-Obesity on Glutamatergic Transmission in the Nucleus Accumbens and Anxiety-Like Behaviors

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Background: Epidemiological data suggest that body mass index and obesity are strong risk factors for depression and anxiety. In rodents, diet-induced obesity produces depression-like behaviors in the forced swim, and sucrose preference tests, but the mechanism underlying this effect is poorly understood. Alterations in glutamatergic prefrontal cortical and amygdalar inputs to the nucleus accumbens (NAc) are thought to contribute to depression and anxiety. Furthermore, obesity is accompanied by elevations in circulating insulin, and insulin receptor activation decreases excitatory glutamatergic transmission in the hippocampus and the ventral tegmental area. However, no studies have examined the effect of insulin or the development of insulin resistance on NAc glutamatergic transmission, nor has the role of insulin-resistance in anxiety or depression been examined. Thus, we have begun a series of experiments to

determine whether NAc glutamatergic transmission is affected by insulin, and to determine whether development of insulin-resistance contributes to depression- and anxiety-like behaviors.

Methods: Whole-cell patch clamp recordings were made from medium spiny neurons in the NAc core during bath application of insulin (25, 50, 100, and 500 nM) and evoked excitatory post-synaptic potentials (eEPSCs) and spontaneous EPSCs (sEPSCs; 100 nM) were measured. The contribution of insulin receptors and insulin-like growth factor receptors (IGFR) to insulin's effects on EPSCs were determined using pharmacological approaches. For behavioral studies, male and female selectively bred obesity-prone and obesity-resistant rats were used. Anxiety-like behaviors were measured after spontaneous development of obesity in prone rats and after consumption of a high-fat diet in obesity-resistant and obesity-prone rats (60% high fat, 3 or 6 weeks). Food intake and weight gain were monitored throughout. Depressive-like behaviors were evaluated using the forced swim test at two different water depths after spontaneous weight gain in obesity-prone rats. Fasted plasma levels of insulin were used to determine metabolic dysfunction and body composition was determined using NMR.

Results: Electrophysiological data showed that insulin reduced the eEPSC amplitude in medium spiny neurons of the NAc core. This effect was completely reversed after insulin wash out. Furthermore, insulin produced a marked reduction in sEPCS frequency, suggesting that its effects are mediated by reducing presynaptic glutamate release. In addition, when the IGFR antagonist picropodophyllotoxin (0.5 µM) was co-applied with insulin, the eEPSC amplitude increased. This suggests that reductions in glutamatergic transmission induced by insulin are mediated by the IGFR and that insulin receptor activation may produce opposing effects on excitatory transmission in the NAc. Ongoing studies are examining these effects of insulin after diet-induced obesity and the development of insulin resistance. In behavioral studies, obesity-prone rats gained substantially more weight and fat mass than obesity-resistant rats when maintained on standard lab chow, as expected. Anxiety-like behaviors in the elevated plus maze and open field tests were enhanced in obese male and female rats. Furthermore, the magnitude of these anxiety-like behaviors was positively correlated with weight gain. No pronounced differences in depressive-like behaviors were found between groups. Consumption of a 60% high-fat diet produced weight gain in obesity-resistant rats that was comparable to that of obesity-prone rats fed standard chow. Preliminary results suggest that diet-induced obesity may enhance anxiety-like behaviors even in obesity-resistant rats.

Conclusions: Current results suggest that insulin bi-directionally influences glutamatergic transmission in the NAc, with IGFR activation producing reductions in excitatory transmission and insulin receptor activation producing increases. These effects of insulin were reversed by insulin wash out, suggesting that insulin can dynamically regulate excitatory transmission in the NAc. This is in contrast to the VTA where insulin produces longer lasting reductions in excitatory transmission. Anxiety-like behaviors increased with the development of obesity in obesity-prone and obesity-resistant rats. Ongoing studies are examining the relationship between insulin-resistance and enhanced anxiety.

Keywords: nucleus accumbens, glutamatergic transmission, anxiety, obesity, insulin
Disclosures: Nothing to disclose.

W9. An Avoidance-Based Rodent Model of Exposure with Response Prevention Therapy

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Background: Obsessive-compulsive disorder (OCD) is treated with exposure with response prevention (ERP) therapy, in which patients are repeatedly exposed to compulsive triggers but prevented from expressing their compulsions. Many compulsions are an attempt to avoid perceived dangers, and the intent of ERP is to extinguish compulsions. Patients failing ERP therapy are candidates for deep brain stimulation (DBS) of the ventral capsule/ventral striatum (VC/VS), which facilitates patients' response to ERP therapy. An animal model of ERP would be useful for understanding neural mechanisms.

Methods: Using a platform-mediated signaled avoidance task, we developed a rodent model of ERP called "extinction with response prevention ("Ext-RP"), in which rats are given extinction trials while blocking access to the avoidance platform. Following 3 days of Ext-RP, rats were tested with the platform unblocked to evaluate persistent avoidance. We then assessed if pharmacological inactivation of lateral orbitofrontal cortex (lOFC) or DBS of the ventral striatum reduced persistent avoidance.

Results: Following Ext-RP training, most rats showed reduced avoidance at test (Ext-RP success), but a subset persisted in their avoidance (Ext-RP failure). Pharmacological inactivation of lOFC eliminated persistent avoidance, as did DBS applied to the VS during Ext-RP.

Conclusions: DBS of VS has been previously shown to inhibit OFC activity. Thus lOFC, which is known to be hyperactive in OCD, may be responsible for impairing patients' response to ERP therapy. This rodent model may be useful for understanding the neurobiological mechanisms of ERP therapy.

Keywords: OCD, Fear extinction, compulsion, DBS, orbitofrontal cortex
Disclosures: Nothing to disclose.

W10. Anxious Temperament Related mRNA Expression Revealed by Sequencing RNA from the Central Nucleus of the Amygdala of 46 Non-Human Primates

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Background: Anxious temperament (AT) during childhood is a risk factor for the later development of anxiety, depression, and comorbid substance abuse disorders.

Children with an extremely anxious temperament (AT), react to novelty with increased behavioral inhibition and increased levels of physiological arousal. Our group has extensively validated a nonhuman primate model of early-life AT. We found that AT is stable over time, across contexts, and predicts inhibited social behavior naturalistic settings. Here, we combined RNA sequencing of targeted brain tissue, with brain imaging and behavioral assessments to investigate the molecular underpinnings of AT in the primate.

The non-human primate is an ideal model to investigate the molecular underpinnings of AT, because it allows researchers to leverage techniques that are possible in rodents alongside techniques that are more commonly used in humans. Our large-scale FDG-PET imaging studies of 592 young rhesus monkeys revealed a brain-wide network of regions to be associated with AT, which include the central nucleus of the amygdala (Ce). The Ce is of particular interest because mechanistic studies demonstrate that damage to the Ce is sufficient to decrease the expression of AT. Moreover, as part of the extended amygdala, the Ce is connected, directly and indirectly, with regulatory and evaluative cortical regions, as well as the regions required to initiate defensive behavioral and physiological responses.

Methods: To understand the molecular processes in the Ce that give rise to extreme AT and associated neural alterations, we assessed gene expression by sequencing the RNA from the Ce of 46 periadolescent male rhesus monkeys (mean age = 3.3 years old). Gene expression is an ideal method for examining the molecular underpinnings of AT because it reflects the combination of genetic and environmental influences that underlie an anxious brain.

We examined Ce gene expression using RNA-seq in combination with assessments of behavior, physiology, and multi-modal brain imaging. AT was assessed in response to the potentially threatening no-eye-contact (NEC) condition of the human intruder paradigm, using a composite of increased behavioral inhibition, decreased vocalizations, and increased cortisol. Brain function and structure were assessed using NEC-related 18FDG-PET, rsfMRI and DTI. RNA-Seq was performed using NuGEN Ovation RNA-Seq v2 libraries on Illumina DNA sequencers with ~30 million 100bp reads per animal. Using regression techniques, we examined variation in Ce mRNA expression in relation to individual differences in AT, as well as structural and functional imaging measures. Building on our previous work, in this study we examined transcript features, such as exons, introns, and splice junctions in relation to AT. To optimize the reliability of our analyses, initial analyses were restricted to features where we mapped an average of at least 50 reads with at least one read in each animal.

Results: Results demonstrated that AT was associated with Ce expression levels of 142 features at a p < .005 threshold, where we mapped over 50 reads. These features represent 119 different genes, where expression of all or part of the transcript was associated with individual differences in AT. Consistent with our prior work suggesting an important role for Ce neuroplasticity in AT, AT-related transcripts encoded molecules involved in mechanisms associated with neuroplasticity and synaptic restructuring (e.g. MAP kinase

pathway). For example, we found that a feature of ribosomal protein S6 kinase, 90kDa, polypeptide 3 (RPS6KA3, also known as RSK2), as well as multiple features of amyloid beta (A4) precursor protein (APP) and tropomyosin 3 (TPM3), all of which are involved in synaptic remodeling, were negatively associated with AT. Interestingly, additional analyses revealed differential relationships between the levels of these transcripts and metabolism throughout the AT-neural network. For example, increased TPM3 feature expression was negatively associated with AT-related brain metabolism in the brainstem (p < .005), whereas increased APP feature expression was negatively associated with AT-related prefrontal/insular metabolism and intra-amygdala functional connectivity as assessed with rsfMRI (p's < .005).

Conclusions: Understanding the molecular functions that give rise to heightened dispositional anxiety is an important step toward developing novel behavioral and pharmacological treatments for early-life anxiety. In particular, insights into the Ce molecules that give rise to dispositional anxiety and its associated brain alterations will provide novel information about both the cellular composition and behavior of neurons within the Ce of highly anxious individuals. The current data provide evidence for novel mechanisms underlying neuroplastic processes within the Ce that may mediate the early-life risk to develop anxiety, depression, and comorbid substance abuse disorders. A refined understanding of these mechanisms will provide a framework for developing novel treatments aimed at preventing children with an extreme dispositional anxiety from the life-long suffering that is associated with stress-related psychopathology.

Keywords: Anxiety, RNA-seq, Positron emission tomography, fMRI resting state, Nonhuman Primates

Disclosures: Nothing to disclose.

W11. Atypical Salience-Default Mode Network Interactions in Pediatric Obsessive Compulsive Disorder: A Compensatory Role?

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Background: The dorsal anterior cingulate cortex (dACC) and bilateral anterior insula (aI), represent a key nodes within a salience network that activates in response to cognitively relevant events (e.g., interference between competing response options), but remains connected even at rest. Previous functional neuroimaging work in pediatric and adult OCD has found atypical interactions between the salience network and the ventral medial prefrontal cortex (vmPFC), a region known to mediate the affective valuation of external stimuli that is incorporated within a widely distribute network for internal mentation or "default mode" function. At present, however, it remains unknown whether atypical interaction between salience and default mode networks represents a marker for pathology (e.g., etiologic) or compensation (e.g., adaptive) in OCD. To address this gap in knowledge, we tested the relationship of OCD

symptom severity with connectivity between salience network and vmPFC. We reasoned that a pathologic role would be supported if OCD severity was positively associated with atypical salience network-vmPFC interactions, whereas a compensatory role would be supported if atypical connectivity and OCD severity were negatively associated.

Methods: Functional magnetic resonance imaging (fMRI) data was collected in 69 patients with pediatric (13.9 +/- 2.8 years, range 8-19) during the Multi-Source Interference Task. Correct incongruent and correct congruent trials were contrasted to derive functionally defined seeds in dACC (12 mm sphere centered at -3, 8, 49) and bilateral aI (12 mm spheres centered at 45, 17, -5 on right, -30, 17, 4 on left). To examine intrinsic functional connectivity, the time series from these seeds were extracted from a general linear model that regressed out task condition, yielding a residual time series used as a covariate (as well as movement parameters and age) in a separate model that again included all task event regressors to examine positive and negative correlations between seed regions and other voxels in the brain. These correlations are described as intrinsic connectivity because this method identifies interregional coupling that is independent of and linearly superimposed upon event-related activity. Finally, OCD severity, as rated on the Child Yale-Brown Obsessive Compulsive Scale (CYBOCS), was regressed onto connectivity maps for each seed.

Results: Consistent with prior literature, positive connectivity maps for dACC and bilateral aI seeds defined the salience network, whereas negative connectivity maps showed anti-correlations between these seeds with vmPFC and posterior cingulate cortex (i.e., default mode network). Bilateral aI connectivity with vmPFC (-6, 44, -8; k = 99, Z = 3.40) was inversely correlated with OCD severity. Inverse correlation of OCD severity and dACC connectivity showed a similarly located, but much smaller vmPFC cluster (-3, 41, -17; k = 15, Z = 3.01). There were no other inverse and no positive associations of OCD severity and SN seed connectivity with vmPFC or any other default mode network region (e.g., PCC). Of note, activation of the left aI seed region to the interference task was associated positively with aI-vmPFC connectivity (r = .25, p = .04), and inversely associated with OCD severity (r = -.24, p = .05). A Sobel test suggested aI-vmPFC connectivity to mediate the inverse relation of left aI response to interference and OCD severity (Sobel statistic 1.7 +/- 0.60 p = .09).

Conclusions: Pediatric OCD has been previously characterized by atypical interactions between the salience and default mode networks. In this sample, greater connectivity of salience network, particularly the bilateral aI, with the vmPFC default mode region was associated with lower OCD symptom severity. This finding suggests that atypical aI-vmPFC connectivity in OCD may enable young patients to more effectively resist obsessive thoughts and compulsive urges. Studies of young patients before and after treatment are needed to establish whether increases in salience-default mode network connectivity drive reduction in symptom severity.

Keywords: salience network, obsessive compulsive disorder, default mode network

Disclosures: Nothing to disclose.

W12. Heightened Sensitivity to Emotional Expressions in Generalized Anxiety Disorder Compared to Social Anxiety Disorder and Controls

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Background: Few studies have examined potential differences between social anxiety disorder (SAD) and generalized anxiety disorder (GAD) in the sensitivity to detect emotional expressions. The present study aims to compare the detection of emotional expressions in SAD and GAD.

Methods: Participants with a primary diagnosis of GAD (n = 46; Mean (SD) age = 40.5 (14.2); 45.7% (n = 21) females), SAD (n = 70; Mean (SD) age = 33.8% (12.3); 38.6% (n = 27) females), and controls (n = 118; Mean (SD) age = 40.4 (13.0); 49.2% (n = 58) females) were assessed with the Structured Clinical Interview for DSM-IV (First, 1994), and the Montgomery Asberg Depression Rating Scale (Montgomery and Asberg, 1979), and completed a morph movies task. The task presented faces expressing increasing degrees of emotional intensity, slowly changing from a neutral to a full-intensity happy (20 trials), sad (20 trials), or angry (20 trials) expression (Niedenthal, et al. 2000). Participants are instructed to use a slide bar to view the movie frames from left to right, and to stop at the first frame where they perceive an emotion. Their decision is recorded on a scale of 1-100 (1 = fully 'neutral' to 99 = fully 'emotional' expression). The frame selected thus indicates the intensity of emotion required to identify the facial expression.

Results: We conducted a mixed-model repeated measures ANOVA, with expression (angry, happy, sad) as the repeated measure, diagnosis as the between subjects factor, and the frame at which the movie was stopped as the dependent variable. Because sphericity assumptions were not met (Mauchly's W = 0.658; p < .001), Greenhouse-Geisser correction was applied. After inclusion of age, race, and depressive symptom severity as covariates, both the main effect of diagnosis, $F(2, 402) = 3.0$, p < .05, and the main effect of expression in the movie, $F(2, 402) = 623.5$, p < .001 were significant, while the interaction between the two factors was not, $F(4, 402) = 1.65$, p = .18. Bonferroni post-hoc tests revealed that all groups required the smaller number of frames to detect happy expressions, followed by angry expressions, and the greatest number of frames to detect for sad expressions. The GAD group also detected faces at a lower frame than SAD (p = .002) and controls (p = .039).

Conclusions: Because our design did not allow differentiation of sensitivity to emotion or more simple differences in facial information, we cannot rule out that participants may have rated visual changes in general (i.e., merely detected facial contrasts). However, findings suggest that individuals with GAD exhibit greater sensitivity to perception of facial emotions than those with SAD or controls.

Keywords: Emotion Perception, social anxiety, generalized anxiety disorder

Disclosures: Nothing to disclose.

W13. From Relief to Safety: Omission-Induced Activation of the Nucleus Accumbens in Fear Conditioning and Extinction

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Background: Unexpected omission of an aversive unconditioned stimulus (US) gives rise to a relief prediction error signal (RPE) that promotes safety learning, like fear extinction. Despite numerous studies on the neurocircuitry of fear extinction learning and recall in the human brain, few studies have looked at neural correlates of RPE. Based on theories that link relief to reward, we expected to find reward-related activations to unexpected US omissions during non-reinforced conditioning trials (partial reinforcement), as well as during early extinction trials (when US omission is surprising) but less so during later extinction trials (when US omission should be fully expected).
Methods: Data from healthy control participants from two separate studies were combined to examine RPE-related activations (N = 33). All participants were clinically screened for current or past psychiatric diagnosis (exclusion criterion), and underwent a well-validated fear conditioning and extinction protocol in the fMRI scanner. Conditioning comprised 10 CS+/US trials, 6 CS+/noUS trials and 16 CS-/noUS trials; the extinction phase comprised 16 CS+/noUS and 16 CS-/noUS trials. In order to detect RPE-related activity, we contrasted nonreinforced CS+ offset versus CS- offset during conditioning and early extinction (first four trials). As control comparisons in which no RPE-related activations were expected, we contrasted CS+ onset versus CS- onset during conditioning, and CS+ offset versus CS- offset during late extinction (last four trials).
Results: The CS+ offset versus CS- offset contrast revealed significant activation of the right nucleus accumbens (NAc) during nonreinforced conditioning trials and significant activation of the left NAc during early extinction trials. The control comparisons revealed no NAc activations, as predicted.
Conclusions: Our conditioning and extinction data show augmented NAc activation during unexpected US-omissions, but not during expected US-omissions nor during US-anticipations. Together, these data suggest a role for the NAc in RPE processing, a putative teaching signal for fear extinction learning. These results add to a growing body of evidence on the involvement of the reward neurocircuitry in fear extinction, both in pharmacological and lesioning studies in animals as well as in pharmacological and neuroimaging studies in humans. This spotlights the reward neurocircuitry as a potential target for interventions that are aimed at enhancing fear extinction learning, and ultimately exposure-based treatments of clinical anxiety.
Keywords: Fear conditioning, fear extinction, reward prediction error, Nucleus Accumbens
Disclosures: Nothing to disclose.

W14. Genetic Influences on Resting EEG Alpha Power in an American Indian Tribe

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Background: The electroencephalogram (EEG) records the rhythmical, electrical activity of the brain that is constantly changing depending on mental activity, relaxation, drowsiness and sleep. This dynamic process is therefore an index of cortical activation, cognitive function and consciousness. Resting EEG phenotypes are highly heritable, stable traits. In the healthy awake adult two rhythms, alpha (8-13 Hz) and beta (13-30 Hz) dominate the resting EEG. The alpha rhythm is maximal posteriorly under conditions of eyes-closed relaxation and mental inactivity. Higher alertness attenuates or suppresses the alpha rhythm which is then supplanted by desynchronized low voltage fast activity. Because the resting EEG is an indicator of cortical activation it may be regarded as an intermediate phenotype for complex behaviors in which arousal is implicated, such as anxiety, depression and addiction. Therefore candidate genes for the resting EEG are likely to include stress-related genes influencing arousal-related behavior.
Methods: Male and female participants were recruited from a Plains Indian tribe living in rural Oklahoma. The resting EEG was recorded from six scalp electrodes: FZ (frontal-central), P3, PZ, P4 (parietal central, left and right), and O1 and O2 (occipital left and right). All results are for eyes closed, normalized (log10 transformed) resting EEG spectral power. This study focused on alpha power recorded at the PZ electrode where it was maximal. Stress-related genes implicated in the HPA axis, GABAergic, serotonergic, dopaminergic and cannabinoid systems were selected with an emphasis on common, functional polymorphisms and haplotype analyses. Genotyping was performed using the Illumina GoldenGate platform. EEG and genotyping data was available for 312 Plains Indians.
Results: Alpha power decreased significantly with age (p < 0.005) therefore age was included as a covariate in all analyses. There were significant CRHBP SNP and haplotype effects on alpha power; minor homozygotes were associated with increased alpha power. There was no effect of CRHR1. Within the GABAergic system, the functional GPHN SNP rs3784075 predicted alpha power but the GABRA2 splice variant rs279827 did not. Analyses are underway for GAD1, GAD2, GABRG2 and GABBR1 SNPs / haplotypes. Within the serotonergic system, the functional SLC6A4 SNP rs3813034 and the functional HTR3B missense variant rs1176744 both predicted alpha power. The cannabinoid system FAAH rs324420 functional missense variant was also significantly associated with alpha power. In all cases, the minor allele / homozygote was associated with increased alpha power. These genetic variants together accounted for 13% of the variance in alpha power at PZ.
Conclusions: Our preliminary results confirm the hypothesis that variation in stress-related genes predicts resting EEG alpha power that is an indicator of cortical activation and an intermediate phenotype for arousal related behaviors.

W15. Chemogenetic Elucidation of the Role of the Orexin System in Panic-Associated Behavior and Physiology

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Background: Discovered in 1998, the neuropeptide orexin (AKA hypocretin) is critical for arousal, but orexinergic neurons are concentrated in the perifornical hypothalamus (PeF) region, which when stimulated elicits panic-associated behavior and cardiovascular responses in rodents and self reports of "panic attacks" in humans. A number of studies support a role for the orexin system in coordinating defensive behavioral and physiological stress responses. This is based on evidence in rodents that orexin neurons are highly reactive to anxiogenic stimuli; have strong projections to anxiety and panic-associated circuitry; selective pharmacological blockade or gene silencing of orexin neurotransmission block stress-induced panic-like responses; and patients with panic responses show increased CSF levels of orexin. However, no study has selectively excited this subset of orexin neurons in conscious, behaving animals while assessing panic-associated behaviors and physiology.
Methods: Here we used chemogenetic techniques to stimulate PeF orexin neurons using DREADD technology [Designer Receptor (an inert Muscarinic 3 receptor coupled to a Gq protein, Musc3Gqr) Exclusively Activated by Designer Drugs (inert clozapine-n-oxide, CNO)]. We injected an adeno-associated virus (AAV) under the orexin promotor into the PeF, which selectively induced expression of the excitatory M3GqR with a mCitrine fluorescent reporter on ∼80-90% of local orexin neurons.
Results: Using whole cell patch clamp electrophysiology we verified that bath applications of CNO induced long lasting membrane depolarization and action potentials on Musc3GqR positive neurons in the PeF. In subsequent experiments we then repeated the AAV injections into the PeF and determined that rats systemically treated with CNO displayed robust activation of flight and escape associated behaviors accompanied by panic-associated cardioexcitation. CNO treated rats also had dramatic increases in cellular c-Fos responses in orexin immunoreactive neurons and resulted in activation of key efferent targets implicated in panic and anxiety (e.g., noradrenergic locus ceruleus and serotonergic dorsal raphe neurons).
Conclusions: Chemogenetic excitation of orexin neurons mobilizes an integrative panic response, further supporting that orexin receptor antagonists could represent a novel therapeutic target for treating emotional and physiological symptoms associated with severe anxiety disorders (e.g., panic attacks).
Keywords: orexin, panic, pharmacogenetic, DREADD, Anxiety

W16. A Norepinephrine Reuptake Inhibitor, SNRI, Improves Reproductive Function in Anxious, Stress-Sensitive Female Monkeys with Stress Induced Infertility

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Background: In response to everyday life stresses, some women stop ovulating and are infertile (i.e., are stress-sensitive, SS), while others retain normal ovulatory reproductive function (i.e., are stress-resilient, SR). Female cynomolgus monkeys who have monthly menstrual cycles like women, are also SS or SR. SS animals exhibit a greater number of anxious behaviors compared to SR animals. SS animals also have lower serotonin mRNA levels and lower serotonin secretion in nonstressed conditions. As both SSRI's and SNRI's are common medications used to treat anxiety, here we examined whether the SSRI escitalopram (CIT) or the SNRI reboxetine (REB) would improve fertility. We also explored whether there were sleep differences in SS vs. SR animals and whether CIT or REB treatment would alter sleep.
Methods: Twenty-seven female cynomolgus monkeys were given daily CIT (20 mg, B.I.D.) through a non-stressed menstrual cycle and two cycles of stress exposure. The experiment was then repeated with daily REB (2 mg, B.I.D.) treatment. The stress paradigm involved the psychosocial stress of moving to a new housing room + the metabolic stresses of mild diet + mild exercise, to model the type of everyday life stresses experienced by women who display stress-induced infertility. Monkeys also wore collar-mounted activity monitors (Actical, Philips Respironics Inc., Bend, OR) to monitor sleep patterns. A standardized anxiety test, The Human Intruder Test (HIT) was performed to assess levels of two anxious behaviors: agitation and reactivity, for each monkey.
Results: Five monkeys were categorized as SS and 18 were categorized as SR, with 4 remaining monkeys showing characteristics of both categories. SS animals had significantly lower levels of the reproductive hormone, progesterone, in the luteal phase of a control menstrual cycle with no stress (SS: 0.65 ± 0.18 ng/ml; SR: 4.99 ± 0.72 ng/ml; $p = 0.01$). CIT treatment did not improve reproductive function under stress conditions. However, REB treatment led to a significant increase in progesterone secretion in control conditions of no stress (SS: 5.75 ± 1.4 ng/ml, $p = 0.036$) and prevented stress-induced suppression of progesterone secretion in SS animals (SS: control cycle vs.

stress cycle, p = 0.552). No significant differences in night-time sleep were apparent in SS vs. SR monkeys. However, there was a significant correlation (R = -0.484, p = 0.019) between reactivity and time awake before light, such that monkeys showing the characteristic of low reactivity (a trait associated with anxious characteristics) had significantly earlier morning waking, as well as a trend toward falling asleep later (R = -0.385, p = 0.07). The relationships between anxious behaviors and sleep indices were not apparent after REB treatment.

Conclusions: REB treatment is highly effective in improving reproductive hormone secretion in SS animals both in control conditions and during stress. We conclude that SNRI's hold promise for the treatment of stress-induced infertility in anxious, stress-sensitive patients.

Keywords: sleep disturbance, anxiety disorders, infertility

Disclosures: Nothing to disclose.

W17. Prospective Study of Conditioned Fear and Extinction Learning Performance as a Risk Factor for PTSD in Active Duty Marines

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Background: Posttraumatic Stress Disorder (PTSD) is a major public health concern. To develop effective prevention and treatment strategies, identifying the underlying biological mechanisms contributing to risk for PTSD and symptom development will be critical for the advancement of treatment and prevention strategies. PTSD patients exhibit disruptions in fear conditioning processes, including increased fear expression, reduced discrimination between threat cues and safety cues (safety-signal learning) and reduced fear extinction. We have previously shown that safety-signal learning and fear extinction are disrupted in participants currently endorsing PTSD symptoms but not general anxiety or depressive symptoms (Acheson et al. 2015). Whether these disruptions in learned fear processes are preexisting risk factors or develop only after trauma is still unclear. Here we tested the hypothesis that conditioned fear, safety-signal learning and fear extinction performance are pre-existing factors associated with risk of development of PTSD.

Methods: The "Marine Resiliency Study II" (MRS-II; Oct 2011-Oct 2013) Neurocognition project is a prospective, longitudinal study of behavioral and biological markers of PTSD risk in Marines deployed to Afghanistan. As part of this investigation, Marines and Navy corpsmen underwent a fear conditioning and extinction paradigm using fear potentiated startle and psychiatric symptom assessments prior to deployment and again 4-6 months after returning from a 7-9 month deployment. PTSD, general anxiety and depression were measured by the clinician administered PTSD scale (CAPS) and Beck Anxiety or Depression Indexes respectively. To test the hypothesis that safety-signal learning and fear extinction are pre-existing risk factors for development of PTSD, we limited our analyses to participants that endorsed minimal PTSD, anxiety and mood symptoms at pre-deployment (N = 694).

Results: Participants that developed PTSD symptoms after combat deployment (N = 33) had significantly less safety-signal learning at their pre-deployment assessment compared to those that did not develop PTSD symptoms after deployment (N = 644; F(2,690) = 5.79, p < .004). Participants that developed PTSD symptoms after deployment showed no differences in pre-deployment measures of fear conditioning or extinction learning compared to those that remained healthy after deployment.

Conclusions: Poor discrimination between threat and safety-signals may be a risk factor for development of PTSD symptoms after combat trauma. These findings support future research exploring training approaches to enhance safety vs. threat discrimination as a prophylactic strategy for those at risk for PTSD. Second, these prospective data are consistent with twin studies showing that decreased fear extinction and increased fear conditioning are not pre-existing risk factors for PTSD. Overall these findings indicate that there are dissociable fear-learning processes that are preexisting risk factors vs. indicators of symptom state for PTSD which can inform our understanding of the etiology of this devastating disorder.

Keywords: PTSD, Fear extinction, Fear conditioning, acoustic startle, at-risk

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W18. Decrease in Thalamic Volumes of Refractory Patients with Obsessive-Compulsive Disorder who Were Submitted to Gamma Ventral Capsulotomy

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Background: Obsessive-compulsive disorder (OCD) is a chronic condition and has a lifetime prevalence of 2% to 3% in the general population. A small proportion of patients with OCD have severe and disabling symptoms, despite all available conventional treatments. For such individuals, psychiatric neurosurgical procedures are an alternative. In particular, gamma ventral capsulotomy (GVC) has shown promising results. However, few studies have assessed whole brain morphometric changes after GVC for the treatment of refractory OCD. Thus, the aim of this study is

to describe cerebral volumetric changes using magnetic resonance imaging (MRI) data from patients before and after this procedure, using a voxel-based morphometry (VBM) approach.

Methods: Pre- and 1-year postoperative structural MRI data from 11 refractory OCD patients (mean age ± standard deviation: 32.4 years ± 9.3) submitted to GVC were collected. Structural MRI was also collected from 22 healthy controls (mean age ± standard deviation: 31.1 years ± 7.7). Gray matter volumes were assessed with a 1.5-T MRI scanner, spatially normalized, and segmented with VBM. Between and within-group statistical comparisons were performed with the general linear model. Findings in a priori predicted regions (orbitofrontal cortex, anterior cingulate gyrus, caudate/putamen and thalamus) were reported as significant if surviving family-wise error (FWE) correction for multiple comparisons ($p < 0.05$) over the respective ROI, with voxel clusters comprising at least 20 voxels. For unpredicted findings in other grey matter regions, we used the FWE-corrected $p < 0.05$ level over the whole brain. MNI gray matter voxel coordinates were transformed into the Talairach and Tournoux system.

Results: Preoperative OCD patients presented greater GM volume in the right orbitofrontal cortex compared to controls (47 voxels, peak coordinates $x = 18$, $y = 34$, $z = -20$, peak voxel $p < 0.05$, corrected for multiple comparisons over the region of interest). One-year after surgery, postoperative OCD patients showed smaller GM volume in the bilateral thalamus (438 voxels, peak coordinates $x = 4$, $y = -12$, $z = 4$, peak voxel $p = 0.028$, corrected for multiple comparisons over the entire brain). Within-group comparisons (pre- versus and 1-year postoperative) revealed that GM volume in the bilateral thalamus decreased (left thalamus: 172 voxels, peak coordinates $x = -6$, $y = -10$, $z = 6$, peak voxel $p = 0.006$; right thalamus: 208 voxels, peak coordinates $x = 8$, $y = -12$, $z = 6$, peak voxel $p = 0.001$, both corrected for multiple comparisons over the region of interest).

Conclusions: Our results support the current theory of frontal-striatal-thalamic-cortical circuitry involvement in OCD physiopathology. GVC is associated with neurobiological changes in the thalamus in refractory OCD patients.

Keywords: Human Neuroimaging, Obsessive Compulsive Disorder, Magnetic Resonance Imaging, voxel-based morphometry (VBM), neurosurgery

Disclosures: Nothing to disclose.

W19. Myo-Inositol Reduction in Medial Prefrontal Cortex of Obsessive Compulsive Disorder: A Proton Magnetic Resonance Spectroscopy Study

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Background: Recent studies indicate that patients with obsessive-compulsive disorder (OCD) present abnormal levels of glutamate (GLU), glutamine (GLN) and γ–aminobutyric acid (GABA) in frontal and striatal regions.

These abnormalities could be related to deficits in cortico-striatal circuits described in neuroimaging literature. But there is still no consensus about the role of the glutamatergic cycle in OCD, since other studies did not observe any differences in GLU (GLX) concentrations in OCD. GLU, GLN and GABA are metabolites of very difficult detection by conventional proton magnetic resonance spectroscopy (^{1}H MRS), given its low signal and partial overlap with other metabolites. In this study we propose to use a two-dimensional JPRESS ^{1}H MRS sequence, that allows the discrimination of overlapping metabolites by observing the differences in J-coupling (second dimension of the spectrum), leading to a higher accuracy of all metabolites in the brain, including GABA, GLU and GLN. The objective of this study is to use 2D-JPRESS sequence to identify the alterations of neurometabolism present in OCD.

Methods: Twenty one OCD patients (mean age 34.7y [range 19-58y]) and 14 healthy controls (HC, mean age 31.9y [range 18-47y]) were evaluated with a 3T Achieva Philips MRI scanner. Symptom severity of OCD was measured with the Yale-Brown Obsessive-Compulsive Scale (Y-BOCS). All participants also filled the Beck Depression Inventory (BDI) and Beck Anxiety Inventory (BAI) to assess symptoms of depression and anxiety, respectively. We used ProFit 2.0 Matlab program to quantify creatine (Cr), N-acetyl-aspartate, glutathione, myo-inositol (MI), lactate, GABA, GLN, and GLU. The voxel was situated in medial prefrontal cortex (mPFC) and varied from 2x2x2cm^3 to 2.5x2.5x2.5cm^3. We used FSL routines to segment voxel content into percentages of gray matter (GM%), white matter (WM%), and cerebrospinal fluid (CSF%) and metabolite concentrations were corrected for different voxel composition. Statistics were performed with SPSS: each metabolite was entered as a dependent variable in an univariate linear model with group as the fixed factor and age, and sex as covariates. For correlation analysis, we used Pearson correlation and Pearson partial correlation, controlling for CSF% (two-tailed). Comparisons of demographic data (age and sex) were performed with student t-test and chi-squared.

Results: Groups did not differ for sex (patients: 12 male / 9 female; HC: 8 male / 6 female), age, GM%, WM% or CSF% (all p-values > 0.05), but significantly differ in terms of depression and anxiety symptoms (both BDI and BAI p-values < 0.002). All patients were free of medication at the time of the scan and the mean Y-BOCS score was 27.9 + 6.1.

There were no between-group differences regarding GLU, GLN or GABA, but OCD patients showed lower levels of MI ($p = 0.006$, corrected for Bonferroni multiple comparisons correction). Moreover, we did not observe any correlation between levels of the metabolites evaluated and clinical data (Y-BOCS, BDI or BAI) in the OCD group.

Conclusions: We did not find differences in absolute values of GLU, GLN or GABA between OCD patients and HC. On the other hand, MI was decreased in the mPFC of OCD patients. Our findings are in line with other negative studies in the literature on regards to GLU, GLN and GABA levels that have used different ^{1}H MRS sequences to measure these metabolites. Even measuring isolated levels of GLU we could not find evidences that support the glutamatergic hypothesis for OCD. Regarding MI, our results also

corroborate previous investigations of the involvement of MI in the physiopathology of OCD: one study presented MI decreases in childhood and adolescent OCD in a brain region similar to ours (anterior cingulate cortex) and the other showed a negative correlation between MI levels in the orbitofrontal cortex and symptom severity. Together, these findings highlight the importance of inositol in the neurobiology of OCD. An important limitation was the relative small sample size of groups, in particular the HC group. Future studies with larger sample sizes should investigate the role of inositol in OCD, whether it could be a good treatment predictor or if it could play a role in OCD symptoms improvement.

Keywords: Obsessive Compulsive Disorder, MRS, myoinositol

Disclosures: Nothing to disclose.

W20. Early Emergence of Fear Learning in the Selectively-Bred Anxious Rat Phenotype

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Background: Anxiety disorders are the most prevalent psychiatric disorders in childhood affecting around 10% of youth (Kessler et al., 2005). Maladaptive anxiety emerges early in life and disturbs child's psychosocial functioning and development. It is generally accepted that anxiety disorders are caused by a combination of inborn/hereditary and environmental factors. One of the best studied inborn risk factors for anxiety disorders in youth is anxious temperament whereas one of the best known environmental risk factors for anxiety disorders is a history of emotional trauma in childhood. Fear conditioning is the most commonly used experimental model of emotional trauma. In fear conditioning a neutral stimulus (conditioned stimulus, CS), such as neutral sound or odor is paired with an aversive unconditioned stimulus (US), typically a mild electric shock. As a result, an organism expresses threat responses such as freezing upon subsequent exposures to the CS. Previous studies show that fear conditioning in rodents is naturally attenuated until postnatal day (PN) 10 (Landers & Sullivan, 2014). However, the ontogeny of fear learning in phenotypically anxious animals is mostly unknown.

Methods: To study the ontogeny of fear conditioning in the anxious phenotype, we used a selectively-bred anxiety-prone rat model, in which spontaneous anxiety-like behaviors emerge as early as at PN 11 (Maras et al., SFN Abstracts, 2014). Sprague-Dawley (SD) PN 4 rat pups selectively-bred for anxiety received 11 US electric shocks (0.4 mA, 1 s) to the tail, either paired with a CS peppermint odor (30 s) (Paired), or unpaired (Unpaired). Additional control group included selectively-bred anxious PN 4 pups that received 11 CS presentations (CS Only). Other controls were wild-type SD pups matched for age and experimental conditions (Paired, Unpaired and CS Only).

Results: At PN 11, all pups were re-exposed to 3 CSs and their behavior was videorecorded and scored for freezing. Analysis of variance (ANOVA) revealed no significant differences in freezing among the wild type animals ($p > 0.05$), a finding consistent with previous studies using similar training parameters (Landers & Sullivan, 2014). However, the anxiety-prone Paired pups showed significantly higher levels of freezing as compared to the control groups (Unpaired and CS Only pups expressed comparable levels of freezing) (ANOVA: $F(3,46) = 16.51$; $p < 0.0001$; post hoc: $p < 0.05$).

Conclusions: This pattern of results demonstrates that fear conditioning in phenotypically anxious pups occurs very early in life, before they spontaneously express anxious behaviors at PN 11, and before the emergence of adult-like fear conditioning in wild-type rats at PN 10. In subsequent experiments we characterized the neuroendocrine mechanisms of infant fear learning in phenotypically anxious rats. Elucidating the mechanisms of early fear learning will contribute to the development of novel preventive and treatment interventions.

Keywords: Fear conditioning, anxiety, developmental

Disclosures: Nothing to disclose.

W21. Posttraumatic Stress Disorder and Common Genetic Variants Affect Subcortical Brain Volumes in Recent Military Veterans

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Background: To date, several studies have performed whole genome association studies (GWAS) to identify genetic markers related to PTSD. Other groups have utilized GWAS to identify genetic markers that affect brain volumes in healthy subjects as well as some psychiatric groups, such as schizophrenia. However, GWAS of regional brain volumes in PTSD has not been previously examined.

Methods: Participants included 157 military veterans of recent US conflicts who were recruited from the Mid-Atlantic Mental Illness Research, Education and Clinical Center (MIRECC) Repository for the Study of Post-Deployment Mental Health. The cohort consisted of 74 non-Hispanic black (NHB) and 83 non-Hispanic white (NHW) participants. The Structured Clinical Interview for DSM-IV (SCID) was administered to assess presence of Axis I disorders. The patient group included Veterans with PTSD with or without comorbid Major Depressive Disorder (MDD). The control group consisted of Veterans with no Axis I diagnoses or with MDD alone. There were 91 subjects in the control group and 66 in the patient group. The Clinician Administered PTSD Scale for DSM-IV (CAPS) was administered to 152 participants. Exposure to childhood trauma was assessed with the Traumatic Life Events Questionnaire (TLEQ).

Structural MRI was acquired on one of two scanners: GE MR750 3T or GE EXCITE HD 3T. T1-weighted images were automatically segmented into 16 subcortical regions with Freesurfer 5.3. The protocol established by the ENIGMA

Consortium was used for processing and quality control of imaging data. GWAS data have been described previously (Ashley-Koch et al., 2015). Briefly, genotypes were assayed using one of three platforms: Illumina HumanHapMap650 Beadchip, Illumina Human1M-Duo Beadchip and Illumina HumanOmni2.5 Beadchip. The 1000 Genomes Project was used to impute missing genotypes for a concordant set of probes in the dataset. Samples were pre-phased with SHAPEIT, and IMPUTE2 was used to impute genotypes. Linear regression assuming an additive genetic model was carried out controlling for age, sex, intracranial volume (ICV), population substructure, dichotomous PTSD diagnosis, and childhood trauma as covariates.

Results: Genome-wide significance was achieved for several SNPs in four regions: right lateral ventricle, right caudate, right pallidum, and right accumbens. The right lateral ventricle was associated with SNPs in a region near translocation associated membrane protein 1-like 1 (TRAM1L1), which has previously been linked to alcohol dependence. One downstream variant within 500 base pairs of TRAM1L1 was significant (rs12152563). The right caudate was associated with intergenic SNPs on chromosome 6 as well as an intron-variant within NKAIN3 (rs34720850). The right pallidum was associated with an intron variant in Transmembrane Protein 132D (TMEM132D) on chromosome 12 (rs55685119). The right accumbens was associated with LINC01522, a non-coding RNA on chromosome 20 (rs55886168), intron variants in transmembrane protease, serine 15 (TMPRSS15) on chromosome 21 (rs2824791 and rs2824788), and intergenic SNPs on chromosomes 4, 6, 11 and 20.

There was no difference in age ($p = 0.51$), gender ($p = 0.27$), or ICV ($p = 0.96$) between the control and PTSD groups. The total categories of childhood trauma was greater in the PTSD group than the control group ($p = 0.04$) prompting its inclusion as a covariate.

Conclusions: Despite a small sample size, genome-wide significance was achieved. Intermediate phenotypes from neuroimaging may be more powerful for examining PTSD and other psychiatric diagnosis than clinically assessed diagnostic phenotypes alone. Replication of our findings is required.

Keywords: PTSD, GWAS, Human Neuroimaging, brain volumes, combat veteran

Disclosures: Nothing to disclose.

W22. A Genotype Variant in the Promoter Region of the CRH Gene Interacts with Early Rearing Experiences to Influence Anxiety-Like Behavior: A Nonhuman Primate Model

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Background: Studies show that dysregulation of HPA axis activity is associated with anxiety disorders, and that early maternal absence often leads to HPA Axis dysregulation and increased risk for anxiety disorders. Studies by Barr and colleagues show that early maternal absence interacts with genotypic variation in the CRH gene to increase this risk for HPA axis dysregulation and anxiety-like behavior. New research indicates that a single nucleotide polymorphism (SNP) in the promoter region of the rhesus macaque CRH gene, CRH-248 C > T, interacts with adverse rearing conditions, leading to dysregulation of the HPA axis. We proposed investigating this novel CRH SNP and its influence on anxiety-like behavior using rhesus monkeys reared either in species normative groups or without parents in peer-groups and genotyped for a recently characterized CRH genotype (i.e., a CRH-248 SNP).

Methods: Prior to the study, blood was obtained from 209 infant rhesus monkeys and the extracted DNA was assessed for a recently characterized CRH-248 C > T SNP—(heterozygous C/T, or wild type homozygous C/C). Subjects were raised for the first six months of life either with their mothers and fathers and same-aged peers in species normative social groups (mother-reared – MR) or without adults in peer-only rearing groups where they had constant access to 3 other same-aged peers (peer-reared – PR). All other procedures were identical between groups. At six months of age, subjects' stress reactivity was measured using a separation paradigm. Infants were separated from their mothers (MR) or peers (PR) for 4, four-day separations, and anxiety-like behaviors were recorded twice a day using 5-minute observations by trained observers.

Results: Repeated measures, mixed design ANOVAs showed significant main effects for rearing ($p < .0009$), and genotype ($p = .003$), as well as a rearing-by-genotype interaction ($p = .003$) for self-directed behavior, and a separation stressor-by-rearing-by-genotype interaction ($p < .0009$) for stereotypic behavior. MR animals exhibited essentially no self-directed behaviors and very little stereotypic behavior. PR animals exhibited significantly higher levels of both behaviors with PR C/C animals exhibiting the highest levels of self-directed behavior and PR C/T animals exhibiting the highest levels of stereotypic behavior. These effects were exaggerated by separation stress.

Conclusions: Our findings parallel a growing body of research that suggests that anxiety-like behavior is likely mediated, at least in part, by CRH. They also indicate that variants in the CRH gene modulates anxiety-like behavior. Our results also indicate that CRH effect on anxiety-like behavior was not uniform across subjects, but instead was mediated by a gene-by-environment interaction (early maternal absence/presence).

Keywords: CRH, anxiety disorders, primate model, stress

Disclosures: Nothing to disclose.

W23. Posttraumatic Stress Disorder Modulates Neural Activity during Threat-Induced Anxiety and Goal Distraction

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Background: The primary goal of this study was to investigate differences in neural activation in patients with posttraumatic stress disorder (PTSD) that are associated with maintaining cognitive control while anxiety is evoked

by conditions of impending threat. The consequences of anxiety on one's ability to stay focused can be meaningfully measured by observing neural responses to emotional stimuli under cognitively demanding conditions. This type of cognitive control is thought to be impaired in PTSD, particularly under conditions of threat. We sought to investigate the neural differences associated with PTSD while maintaining cognitive control in the context of threat-induced anxiety.

Methods: Participants (n = 46) with PTSD (n = 22) and trauma-exposed controls (n = 24) engaged in a computerized visuospatial tracking task (similar to the "Pac-Man" arcade game) while undergoing functional MRI. Across all trials, subjects manipulated a joystick that controlled an avatar. Participants attempted to maximize rewards that were accrued by capturing prey, while minimizing losses incurred by capture of the avatar by a predator. The task was performed under two conditions modeled with a block design: (1) in the threat condition, subjects were informed they may receive an electrical shock, which was randomly delivered on one-third of the threat trials; (2) in the non-threat condition, subjects were informed they would never be shocked. Tracking behavior of the subject (avatar) was monitored in both the presence and absence of threat-induced anxiety.

Results: There were significant behavioral differences between threat and non-threat conditions. In particular, participants reported greater anxiety during the threat than the non-threat condition. We found no between-group or group * condition interactions.

There was greater activation in the control compared to the PTSD group for the threat vs. non-threat contrast in the superior temporal lobe, temporal pole, right amygdala, posterior cingulate gyrus, fusiform cortex, right hippocampus, right caudate, insula, right IFG, and vmPFC. There was greater activation in the PTSD group compared to the control group for the threat vs. non-threat contrast in the right hippocampus, left orbital frontal cortex, supramarginal gyrus, and bilateral parahippocampal gyri.

Conclusions: Anxiety can be induced by the presence of threat when combined with significant cognitive demands. In the fMRI environment, this approach has enabled observation of neural changes associated with PTSD. Despite comparable levels of anxiety evoked in the PTSD and matched control subjects, we observed differential activation in neural circuits related to monitoring safety, threat, context, and conflict. Future functional connectivity analyses will provide important insights about the relevant circuit dynamics in PTSD.

Keywords: PTSD, Human Neuroimaging, fMRI, anxiety, threat, Threat of Shock, cognitive control

Disclosures: Nothing to disclose.

W24. Hoarding Behaviours among Users of Online Classified Advertisements

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Background: Hoarding disorder (HD) is a mental disorder that has been newly included in the Diagnostic and Statistical Manual of Mental Disorders, fifth edition. It is estimated that 2-6% of adults in the general population suffer from clinically significant hoarding symptoms. HD problems typically begin during adolescence and become worse as people age. The symptom burden associated with HD is broad. HD often has significant social and economic consequences to the individual, and contribute to family dysfunction. The cardinal feature of HD is persistent difficulty in discarding or parting with possessions, however, persons fulfilling the diagnostic criteria for hoarding disorder are further categorized according to additional features, or "specifiers", including excessive acquisition. This may involve taking free items, buying items in excess, or less frequently, and stealing items that are not needed or for which there is no space available. Approximately 80 to 90% of individuals with hoarding disorder engage in excessive acquisition; these people typically experience distress if they are unable to acquire items or are prevented from acquiring items. Little research has examined the excessive acquisition component of this disorder, however one study revealed that buying and obtaining free things were markers of HD severity. With the recent shift towards Internet-based media, new venues for selling goods have emerged. Classified ad networks on the web provide a way to list items for sale, often for free, with a focus on selling locally in the community. Almost half (49%) of internet users say they have used online classified sites. On any given day about a tenth of internet users (9%) visit online classified sites. HD is a condition where research is newly emerging. Many individuals who suffer from HD have limited insight into their condition and are reluctant to seek help, making prevalence estimates difficult to obtain. We elected to examine the prevalence of hoarding behaviours among users of online classified advertisements.

Methods: A link to an online survey was posted on the following Canadian classified ad sites: Kijiji, Craigslist, Locanto and postad.ca. The ads were posted in communities across Canada from June 26, 2015 to August 14, 2015. Following acknowledgment of a disclosure statement, participants were asked to complete a short demographics questionnaire and general questions regarding their use of online classified advertisements; no personal identifiers were collected. They were then provided with the 5-question Hoarding Rating Scale (HRS). If the score indicated clinically significant hoarding (score of $\geq$ 3 on items 1,4 and 5; $\geq$ 4 on item 2, and $\geq$ 2 on item 3), participants were also asked to complete the Saving Inventory-Revised and the Clutter Image Rating. After completing the entire survey, participants were provided with feedback on their hoarding behaviours (based on the HRS). Those with significant hoarding were advised that they may have problematic hoarding behaviours and that they may wish to contact a health professional for further assessment.

Results: At the time of this analysis, 284 had completed the survey. The sample was 66% female (n = 186), with a mean age of 40.5 $\pm$ 14.4 years; 53% were married, 35 % were single, 10% were divorced and 2% were widowed. Clinically significant hoarding behavior was identified in 12.7% (n = 36) using the HRS (x□ 24.5 $\pm$ 5.2 versus x□ 9.7 $\pm$ 7.2 in those without significant hoarding, p < .0001). Individuals with significant hoarding reported visiting more non-online sources of used or free items such as garage

sales: x□ 2.5 ± 1.6 sources versus x□ 1.9 ± 1.6 sources (p = .03). No significant differences were found between hoarders and non-hoarders in the amount of time spent visiting online advertising sites, with 30% of both groups reporting spending ≥ 20 hours per month visiting these sites. The hoarding group (n = 36) also had scores indicating significant hoarding on the SI-R (x□ 47.8 ± 13.7) but not on the Clutter Image Rating.

Conclusions: Individuals visiting online classified ad sites have high rates of clinically significant hoarding behaviours. The rates of hoarding in this sample were double that found in the general population. The amount of time spent on online classified sites does not appear to be associated with hoarding behaviour. Online classified ad sites may respresent a unique medium to study individuals with hoarding behaviours.

Keywords: Hoarding Disorder, prevalence, internet

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W25. High-Reactive Infant Behavior Predicts Reduced Amygdala Volume in Young Adults

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Background: About 20% of human infants at 4 months of age demonstrate a distinctive behavioral profile that is characterized by vigorous limb activity, arching of their back and crying to unfamiliar visual, olfactory, and auditory stimuli. Such infants are classified as high-reactive (HR). Low reactive (LR) infants show both low motor activity and low vocal distress to the same experimental stimuli. High-reactivity in infancy predisposes to behavioral inhibition at two years of age. Studies indicate that both a high-reactive temperament at 4 months and an inhibited temperament at two years of age are risk factors for the development of multiple anxiety and mood disorders in adolescents and young adults. Functional imaging studies in longitudinal cohorts have demonstrated that the HR infant phenotype is associated with greater amygdalar reactivity to novel faces later in life. However, the structure of amygdala in young adults who had been high reactive infants has not been examined. Although reductions in the volume of the amygdala have been reported in both anxiety and mood disorders, the origin of this reduction is not well understood. Using high resolution structural MRI, we investigated the volume of the amygdala in a longitudinal cohort of 135 young adults who had been high-reactive infants at 4 months of age.

Methods: The Massachusetts General Hospital IRB approved the experimental protocol. Informed consent was obtained after the nature and possible consequences of the study were explained. High-resolution MRI was used to determine the volume of the amygdala in 135 late adolescents (age 18.20 ± 0.07, 72 males and 63 females) enrolled in a longitudinal study who had been assessed for infant reactivity at 4 months of age. Fifty-five of the young adults (age 18.19 ± 0.1, 30 males and 25 females) had been high-reactive infants and 80 subjects (age 18.21 ± 0.08, 42 males and 38 females) had been low-reactive (LR) infants. Each subject underwent two 3D MPRAGE structural scans on a 3T Siemens TrioTim scanner (128 sagital slices; 1.3x1.3x1 mm; TR = 2530 ms; TE = 3.39 ms; flip angle 7°, bandwidth 190 Hz/Px). The two 3D MPRAGE structural scans from each subject were averaged, after motion correction, to create a single high signal-to-noise volume. This volume was analyzed using Freesurfer (www.nmr.mgh.harvard.edu/martinos) to calculate left and right amygdala volumes in cubic millimetres. Each scan was manually inspected by an investigator (PK) who was blind to the subject's infant status to ensure accurate segmentation. The effects of infant reactivity classification (HR, LR) on left and right amygdala volume in adulthood were analyzed with a MANOVA (SAS v9.4, SAS Institute Inc., Cary, NC, USA), with for age, sex, handedness, and ICV as covariates.

Results: The volume (mean ± sem) of the left amygdala in young adults who had been high-reactive infants (1652 ± 20 mm3) was smaller than in those subjects who had been low-reactive infants at 4 months of age (1720 ± 25 mm3; F(1,118) = 4.7, p = 0.03). The difference in the right amygdala was in the same direction (HR 1741 ± 24 mm3 vs LR HR 1784 ± 20 mm3) but the difference did not reach statistical significance F(1,118) = 1.99, p = 0.16).

Conclusions: In this longitudinal study, an infant phenotype observed in the first months of life predicted differences in amygdala volumes in the brains of young adults 18 years later. Reduced amygdala volumes have been reported in generalized social anxiety and major depression. Infant high-reactivity and behavioral inhibition have been shown to be risk factors that predispose to those same psychopathological outcomes. The present finding raises the possibility that the volumetric differences that have been reported in cross-sectional studies of anxiety disorders and depression could be attributable to a shared intermediate phenotype rooted in early infancy that cuts across traditional diagnostic categories.

Keywords: Brain development, infant, Amygdala, individual differences, behavioral inhibition

Disclosures: Nothing to disclose.

W26. Brain Dynamics of Ongoing Attentional Fluctuations in ADHD

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Background: Individuals with attention deficit hyperactivity disorder (ADHD) are characterized by frequent, ongoing fluctuations in attention to their external and internal environments. Moment-to-moment behavioral variability

may serve as a marker of ongoing attentional fluctuations, with periods of high variability purported to reflect attentional lapses. The brain dynamics underlying ongoing behavioral variability in ADHD have not been explored. Brain networks involved in attentional fluctuations, including the default mode network (DMN), salience network, and dorsal attention network (DAN), have previously been shown to be disrupted in ADHD. We therefore hypothesized that behavioral variability would be associated with different patterns of attentional network activity in adults with ADHD compared to healthy controls.

Methods: Adults with ADHD (n = 18) and age-, sex- and IQ-matched healthy control subjects (HCs; n = 19) underwent fMRI while they attempted to continuously tap their index finger every 600 ms. A tap variance time course was calculated, which consisted of the deviation of each inter-tap interval from the mean of all inter-tap intervals. This variance time course was entered as a regressor in a general linear model single-subject analysis in FSL. Group-level statistics were conducted to identify brain areas with activation related to stable and variable tapping as well as areas showing group differences. All analyses were conducted at the whole brain, voxel-wise corrected level (FLAME; $Z > 2.3$, cluster-based $p < 0.05$).

Results: There were no significant group differences in tapping variability. In HCs, more stable behavior ("in-the-zone") was associated with greater activation in the DMN (medial prefrontal cortex) and more variable behavior ("out-of-the-zone") was associated with greater activation of regions within the salience network and DAN, consistent with previous work (Esterman et al., 2013; 2014). In ADHD subjects, no regions showed activation associated with in-the-zone behavior, but similar networks to HCs as well as additional regions in sensorimotor network and cerebellum were associated with out-of-the-zone behavior. Group differences revealed that ADHD subjects exhibited reduced in-the-zone activation of the medial prefrontal cortex in the DMN and increased out-of-the-zone activation of regions within sensorimotor and salience networks.

Conclusions: These data show that the underlying neural systems of attentional state ("in- vs out- of the zone") are different for HC and ADHD individuals. Abnormal structural and functional organization of attention networks, as previously identified in ADHD, could drive the ongoing fluctuations in attention and associated brain dynamics identified here. In turn, frequent attentional fluctuations in daily life could maintain and/or exacerbate abnormalities in attention networks that characterize ADHD.

Keywords: ADHD, behavioral variability, fMRI

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W27. Combining Autism and Intellectual Disability Exome Data Yields Insight into both Disorders

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Background: Autism spectrum disorder (ASD) is known to have a complex genetic architecture. Recently, exome sequencing studies have enjoyed great success using de novo single nucleotide variants (SNVs) and short indels to identify risk genes. The pace of discovery has been unprecedented, with dozens of genes identified; yet calculations indicate that there are hundreds of additional genes that confer risk yet to be discovered. In many ways this is reminiscent of what is known about the genetic architecture of ID, including many genes underlying risk and recent rapid progress identifying these genes by exome sequencing. ASD and ID are known to co-occur and consequently their risk genes show considerable overlap. These findings motivate an analysis that contrasts and combines discoveries in ASD and ID.

Methods: The Autism Sequencing Consortium (ASC) has assimilated published rare variants identified via exome sequencing of 4216 ASD and 1669 ID trios, and 869 ASD

cases and 2829 ancestry-matched control samples. We used TADA (Transmission And De novo Association) to identify likely risk genes in both disorders individually. In addition, we combined the trios to find additional genes that affect risk for both disorders.

We next applied network analysis techniques to find and refine unifying threads among the growing list of ASD implicated genes. To do so we first applied the statistical algorithm DAWN (Detecting Association With Networks) to networks estimated from brain gene expression data to discover subnetworks of interacting risk genes. We limited the DAWN analysis to two previously-identified spatio-temporal windows of brain development relevant to ASD risk, the prefrontal cortex (PFC) during mid-fetal development and mediodorsal nucleus of the thalamus and cerebellar cortex region (MD-CBC) during infancy and early childhood.

Results: Our results revealed 31 and 64 risk genes (FDR < .05) associated with risk for ASD and ID, individually, and 16 additional genes when combining the data. Based on the pattern of de novo loss-of-function (dnLoF) variants, we estimated that the total number of autosomal dominant genes in which a dnLoF imparts substantial risk for ASD and ID, respectively. We estimated a 95% confidence interval for ASD of 500-950 and for ID of 185-225.

To understand the relationship of genes with phenotypes, we partitioned the genes based on 4 risk categories: the ASD.ID category includes genes with a strong signal for both disorders; the ASD category includes the remaining genes with a strong signal for ASD; the ID category includes the remaining genes with a strong signal for ID; and the MOD category includes the remaining genes with a moderate signal for at least one disorder. The mutation effects were significant for nonverbal IQ (NVIQ) in all categories with strong risk signal. Relative to the mean NVIQ in the sample, a proband with LoF mutation in one of the genes with the strongest signal for both disorders (ASD.ID) had an average drop in NVIQ of 24 points, more than one standard deviation at the population level (i.e., 15 points). Moreover, a LoF mutation in an ID-related gene produced a similar reduction in NVIQ in the ASD proband. A LoF mutation in an ASD-related gene, which was not also implicated in ID, was associated with a weaker effect on NVIQ, reducing it relative to the sample mean by almost 12 points. Mutations in genes with moderate support for risk in either disorder resulted in far smaller reduction in IQ, and the effect was not significantly different from zero.

We applied the statistical algorithm DAWN to networks estimated from brain gene expression data to discover subnetworks of interacting risk genes. We began with ASD and found that, as expected, both temporal networks were enriched for synaptic and chromatin modifier genes. More surprising was that these genes were strongly intra-connected, but not interconnected in the estimated gene network. Next, we performed network analysis of community structure, leading to discovery of critical functional components of ASD. For the PFC we identified two functional clusters enriched for chromatin modification and regulation of synaptic transmission, whereas for the MD-CBC three functional clusters were apparent, one with dominant enrichment for chromatin modification, and two synaptic/axonal synaptic clusters. We used those components to identify which targets are enriched for ID-related risk genes. Genes strongly affecting cognitive function were primarily represented in clusters involving chromatin modification, implicating gene regulation as a mechanism shared by ID and ASD.

Conclusions: Data on the mutational spectrum for ASD continue to accrue. When these data are analyzed in the context of gene expression networks a consistent theme emerges, namely that risk for ASD concentrates in early regulatory genes as well as genes involved in synaptic function. We took these results further in two ways: first, by using community detection techniques, we identified local communities of strongly interdependent genes affecting risk for ASD; then asked which of these identified communities show enrichment for ID-related genes. The distribution of rare variants in the combined data indicated that there are fewer genes with large effect on ID than ASD and that they have large impact on IQ even for ASD subjects. In contrast, genes only implicated in ASD had a minimal effect on IQ. Community detection analyses show that genes with major impact on cognitive function and ASD often play a regulatory role in early development; whereas those with a more modest impact on cognitive function tend to be synaptic.

Keywords: Autism, intellectual disability, chromatin
Disclosures: Nothing to disclose.

W28. Copy Number Variation of the 7q11.23 Williams Syndrome Chromosomal Region Affects Brain Gyrification and Skull Shape

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Background: Williams syndrome (WS), caused by hemizygous deletion of approximately 1.5 megabases on chromosome 7q11.23, is characterized by significant visuospatial construction deficits, hypersociability and mild to moderate intellectual disability with relative sparing of concrete language (Mervis 2000). Recently, duplications of 7q11.23 (Dup7) have been identified and associated with a contrasting phenotype characterized by low average IQ, speech delay, and social phobia with relative sparing of visuospatial construction (Mervis 2015).

Prior work examining brain folding in WS has produced varying results, showing both decreased cortical complexity (Fahim 2012) and increased global and lobar gyrification (Schmitt 2002). Further, WS is associated with smaller head circumference (Pankau 1994), but regional differences in head shape or skull have not been examined. Neither measure has been studied in Dup7. Exploring gene-dosage effects (one copy of affected genes in WS syndrome vs. two copies in typically developing individuals (TD) vs. three copies in Dup7) on brain and skull characteristics offers the possibility of identifying novel genetic mechanisms. Here, we tested for differences in skull shape and brain

gyrification among children in these three groups, all having intelligence in the normal range.

Methods: T1-based MEMPRAGE scans were acquired on a 3T GE MRI scanner for 22 children with WS (10.0 +/- 4.4 years old, eight males), nine children with Dup7 (11.9 +/- 3.2 years old, five males) and 38 TD children (13.2 +/-3.0 years old, 24 males), all with KBIT-2 Composite IQs in the normal range. For analysis of brain gyrification, scans were analyzed with Freesurfer software (v5.3), including processing for local gyrification index (LGI), a regional measure of gyrification computed as the ratio of pial surface area to cortical hull surface area for a sliding region over each vertex. Individual surfaces were aligned to a standard mesh composed of 198,812 vertices using SUMA tools. A template volume and average surfaces were created from an equal number of age- and sex-matched children from each group to avoid normalization biases. For the skull analysis, 3D skull surfaces were calculated for each participant and for the template (Dogdas 2006). A 9-parameter registration was performed, co-registering each individual's skull surface to the template surface, adjusting for overall head size without affecting shape. For each vertex on the skull surface, the distance between the template and each participant was determined. For both LGI and skull, vertex-wise multivariate modeling was performed using 3dMVM to test for differences across the three groups, while controlling for effects of age and sex. Post-hoc between-group t-tests were calculated to assess directionality of the underlying group differences. Reported results were significant at $p < 0.05$, FDR-corrected for multiple comparisons.

Results: For LGI, areas showing a step-wise change such that WS<TD<Dup7 included intraparietal sulcus (IPS) extending through the majority of the dorsal visual stream bilaterally, as well as left ventral visual stream, bilateral insula/operculum and bilateral subcallosal regions. Areas showing Dup7<TD<WS LGI included Wernicke's area on the left.

For skull shape, bilateral parietal bones, extending toward the frontal bone on the left, showed a stepwise WS<TD<Dup7 change. Additionally, overall head size showed a step-wise WS<TD<Dup7.

Conclusions: This work identifies brain and skull phenotypes associated with 7q11.23 CNV. We found that increasing copy number (three>two>one copies) of genes in the 7q11.23 region is associated with increased gyrification in a large portion of the visual system and the insula bilaterally. Additionally, the skull shape analysis showed a similar gene-dosage dependent pattern in the parietal bones. Our LGI results stand in support of prior examinations of cortical complexity in WS (Fahim 2012), though in contrast to global/lobar measures of gyrification (Schmitt 2002). Our results are also consistent with other structural brain analyses in WS, showing grey matter volume and sulcal depth alterations in the IPS region of the dorsal visual processing stream (Meyer-Lindenberg 2004, Kippenhan 2005, Van Essen 2006). The skull shape changes are consistent with prior findings of smaller head size in WS (Pankau 1994) and macrocephaly in Dup7 (Morris 2015). The gene-dosage effect seen with these changes may prove useful for uncovering genetic mechanisms underlying neurocognitive phenotypes in 7q11.23 CNV.

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W29. Prenatal Maternal Smoking Increases Risk for Tourette Syndrome and Chronic Tic Disorders

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Background: Prenatal maternal smoking has been associated with several neuropsychiatric disorders including attention-deficit/hyperactivity disorder (ADHD), schizophrenia and autism spectrum disorders (ASD). Previous literature on the role of prenatal maternal smoking in TS/CT and OCD is sparse and has yielded mixed results. Some studies identified prenatal maternal smoking as a risk factor for TS/CT or for tic severity among those with TS/CT while others report no association between prenatal maternal smoking and TS/CT. Prenatal maternal smoking has been more consistently identified as a risk factor for comorbid ADHD in individuals with TS. Literature on the role of smoking in OCD risk is even more limited; one study reported prenatal maternal smoking was associated with increased risk of comorbid OCD among individuals with TS and two small studies reported no association between prenatal maternal smoking and OCD. The aim of the present study was to assess the role of prenatal maternal smoking in risk for TS/CT and pediatric-onset OCD. Additionally, we investigated whether prenatal maternal smoking was differentially associated with risk for TS/CT with comorbid ADHD and other comorbid psychiatric diagnoses.

Methods: Analyzing 73,224 singleton pregnancies from the Danish National Birth Cohort, we calculated incidence rates (IR) per 1000 person-year for TS/CT and OCD. We then determined crude and adjusted hazard ratios (aHR) and 95% confidence intervals (CIs) associated with prenatal maternal smoking, considering smoking as a dichotomous (yes/no) variable and as a three level variable (no smoking, light smoking, and heavy smoking, the latter defined as

≥ 10 cigarettes/day). We performed additional analyses to examine the effect of maternal smoking on TS/CT with other comorbid psychiatric disorders.

Results: In final adjusted analyses, heavy smoking was associated with a 60% increased risk of TS/CT (aHR 1.63; 95%CI, 1.16-2.31). Moreover, the association followed a dose-response relationship, with aHR 1.02 (95%CI, 0.78-1.34) for light smoking and aHR 1.19 (95%CI, 0.94-1.49) for any smoking. Heavy smoking was associated with a twofold increased risk of TS/CT with comorbid ADHD, and both light and heavy smoking were associated with over a twofold increased risk of TS/CT with any non-ADHD psychiatric comorbidity. Our analyses of childhood-onset OCD were likely underpowered, but showed similar relationships compared to TS/CT.

Conclusions: Prenatal maternal smoking was associated with increased risk of TS/CT as well as TS/CT with comorbid psychiatric disorders, even after correction for maternal psychiatric history. Our findings may point to a pathway linking prenatal tobacco exposure and altered brain development. Further analyses of this relationship and that of smoking and OCD risk are warranted.

Keywords: Tourette syndrome, obessive-compulsive disorder, prenatal smoking, ADHD

Disclosures: Nothing to disclose.

W30. Post-Mortem Rnaseq Characterization of the Histaminergic Neurotransmitter System in Autism Spectrum Disorders

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Background: The histaminergic neurotransmitter pathway plays an important role in modulating various neurotransmitter systems and has been shown to be involved in diverse brain functions, including wakefulness, attention, and cognition. Evidence for alterations of this neurotransmitter system has been identified in neurodegenerative and neurodevelopmental diseases, such as, Alzheimer's disease, Parkinson's disease, multiple sclerosis, schizophrenia, and Tourette syndrome. A high level of comborbity and overlap of rare CNVs is shared between autism spectrum disorders (ASD) and Tourette syndrome, suggestive of common pathophysiology. A recent study of H3R antagonism by ciproxifan showed promising rescue of sociability in a mouse model of ASD. To explore whether this system is altered in ASD we characterized the expression of histaminergic genes utilizing RNA sequencing of post-mortem brain samples in cases and controls.

Methods: We evaluated RNA sequencing data of post-mortem dorsolateral prefrontal cortex (DLPFC) samples from 52 subjects (13 ASD subjects and 39 matched control subjects) using both Poly-A selection and Ribosomal RNA depletion (RiboZero) with stranded-specific library preparation. Three controls were selected for each ASD subject matching for age, gender, and ethnicity. Forty-five of the subject samples, including all ASD samples, were sequenced with both library preparations. One hundred base pair paired-end sequencing was run on the HiSeq 2000, using the Illumina Real Time Analysis (RTA) module to perform image analysis and base calling, and the BCL Converter (CASAVA v1.8.2) to generate the sequence reads. Sequencing depth was over 40-60 million in paired-end (80-120 million reads per sample). Tophat (v2.0.4) was used to align reads to that of the known transcripts of the Ensembl Build GRCh37.67 (and to enforce strandness for RiboZero). FeatureCounts (v1.4.4) was used to determine gene and exon abundance estimates, while the junction annotation module of the RseQC package was used to determine transcript abundance estimates. The influence of diagnosis status on all gene, exon, and transcript abundance estimates with an average mean $\log2$ (Reads Per Kilobase of transcript per Million mapped reads (RPKM) $+$ 1) expression value greater than .05 was then evaluating using a linear model regression covarying for age, sex, ethnicity, RNA Integrity Number (RIN), ribosomal RNA percentage of total mapped reads, and mitochondrial percentage of total mapped reads. An additional model was evaluated including the identity of the sequencing flowcell as a covariate among the data from subjects sequenced in one unique flowcell (45 total subjects, 13 ASD subjects, 32 control subjects) to check for any intrinsic batch effects. Multiple testing correction was performed using the Benjamini Hochberg method. The expression levels of HDC, HNMT, HRH1, HRH2, HRH3, and HRH4, were then evaluated among the genome wide data.

Results: Filtering for mean expression removed HRH4 from the analysis, as it was expressed at very low levels in our samples, consistent with earlier evidence that it is lowly expressed in brain tissue. Analysis of HDC, HNMT, HRH1, HRH2, and HRH3 revealed that HRH1, HRH2, and HRH3 might be differentially expressed between ASD subjects and controls. Following multiple testing correction, when excluding flowcell from the model, HRH2 (log 2 fold change $=$ -1.65, p value $=$ 2.43×10^{-05}, q value $=$ 0.002) and HRH3 (log 2 fold change $=$ -1.74, p value $=$ 0.0004, q value $=$ 0.007) were significantly differentially expressed and more abundant in cases than controls. HRH3 (log 2 fold change $=$ -1.49, p value $=$ 0.002, q value $=$ 0.01) remained significantly differentially expressed when including flowcell in the model. HRH1 was also significantly differentially expressed and more abundant in cases when accounting for flowcell (log 2 fold change $=$ -0.8, p value $=$ 0.008, qvalue $=$ 0.03). No individual exons were significantly differentially expressed among these evaluated genes. Only junctions within HNMT and HRH3 passed the mean .05 expression filter. One junction in HRH3 located at chr20:60791982 -60793546 was found to be differentially expressed and increased in cases (log 2 fold change $=$ -2.49, p value $=$ 2.8×10^{-5}, q value $=$ 0.006) only when flowcell was not included in the model.

Conclusions: Preliminary analysis suggests that the HRH1, HRH2, and HRH3 may be overexpressed in ASD subjects. We plan to further analyze these histaminergic genes among additional datasets and among data generated using the RiboZero library preparation from the same subjects. Our results may add to evidence of involvement of histaminergic signaling in ASD, potentially leading to new drug targets for treatment.

W31. Ethanol Withdrawal in Adolescent and Adult Rats

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Background: Adolescents consume more alcohol than adults, and early initiation of alcohol use is associated with increased risk of alcohol dependence in adulthood. However, the factors that contribute to this increased intake/risk of dependence are not well understood. The immediate and protracted effects of alcohol withdrawal contribute significantly to consumption of alcohol by adults. However, there the role of immediate or protracted alcohol withdrawal in adolescent alcohol consumption is poorly understood. Previous studies from this lab suggested that protracted withdrawal was milder in adolescents than adults. The present abstract investigates HPA axis function and anxiety immediately at the end of a brief (5 day) ethanol treatment to assess stress-related withdrawal symptoms during early withdrawal in adolescent and adult male and female rats. We used this brief treatment regimen in order to best detect differences between adolescents and adults.
Methods: Adolescent (PN 28) or adult (PN 70) male and female rats from Charles River Laboratories were used in all experiments. Animals received 4 days of ethanol treatment (1.5 g/kg, 3 injections daily at 3 hour intervals). Post withdrawal anxiety-like behaviors (latency to emerge into light, percent time in light, rearing) and locomotor measures (total distance traveled, distance in the dark) were assessed 18 hours after the final dose via light/dark box testing. Blood was collected at the end of behavior testing for analysis of stress-induced corticosterone release. A cohort of animals was treated with ethanol and decapitated 18 hours after the end of treatment to assess basal corticosterone release, and another received the 4 day treatment, and then received a challenge dose of ethanol (1.5 g/kg) to assess how this brief ethanol treatment affected the HPA response to an ethanol challenge. Finally, to obtain an integrated measure of HPA axis activation during treatment, a cohort of animals was treated identically with ethanol and left in the home cage, and fecal samples were collected each day of the ethanol treatment for measurement of corticosterone. A cohort of adolescent and adult animals was injected with ethanol and killed 1 hour after the 1st, 2nd or 3rd injection to measure blood alcohol content (BAC). Corticosterone was assessed by RIA and BAC with an Analox BAC analyzer. Statistics on all results were analyzed by 3 way ANOVA (age x sex x treatment) using NCSS. All experiments were approved by the Duke University IACUC and conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals.
Results: Ethanol withdrawn adults exhibited decreased time in light, enhanced latency to enter light and decreased rearing. Alcohol-withdrawn adolescents did not exhibit significant alcohol-induced anxiety-like behavior: latency to enter light and time in light were not changed by ethanol treatment. Corticosterone 18 hours after withdrawal were not affected by ethanol treatment. However, corticosterone response to ethanol was not lower after the repeated treatment. Fecal corticosterone rose significantly over the 5 days of ethanol treatment, more in adults than adolescents, suggesting that repeated ethanol treatments continued to activate the HPA axis. BAC concentrations after each of the three injections approximated a modest binge (100 ug/dl) in both adolescents and adults. No sex differences in any parameters were observed, except a tendency for higher corticosterone in females.
Conclusions: These findings suggest that HPA axis activation by ethanol is robust in both adolescents and adults during a single injection, and that this activation persists across 5 days of multiple daily injections. During withdrawal, HPA axis function trends toward suppression. In contrast to endocrine measures, anxiety-like behaviors during withdrawal were mild in adults and not significant in adolescents. Two conclusions emerge from these early studies of endocrine and behavioral manifestations of ethanol withdrawal in adolescents. First, there is a clear divergence between hypothalamic responses (indexed by corticosterone) which remain robust during this brief treatment, and anxiety-like behavior mediated by more rostral circuits, which are mild or absent after the brief treatment. These data suggest that neuronal adaptations in rostral circuits may be less following brief daily ethanol exposure in than hypothalamic responses in both adolescents and adults. Future study of these neurochemical and endocrine mechanisms in alcohol dependent adolescents may provide insight into age-selective pharmacotherapies for alcohol dependence in adolescents.

W32. Neurodevelopmental Copy Number Variants and Clinical Risk: A Pediatric Record Population Study

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Background: Chromosomal copy number variants (CNVs) associated with schizophrenia have been shown to have a broad etiological risk for neurodevelopmental disorders such as autism or intellectual deficiency and some involve other categories of disease.. Their overall penetrance more broadly defined remains unknown within pediatric populations.
Methods: Selected CNVs were screened utilizing Taqman within a population sample of 60,000 pediatric patents. CNV carriers were examined with respect to pre-scored pediatric records and compared to disorders in 5:1 matched non carrier control group for each individual CNV. Prevalence for 14 disorder categories was estimated.
Results: Five of these CNVs (MYT1L dup, 22q11 dup, 16p11 del/dup, and 15q11.3 del/dup) were detected in the pediatric

population at expected rates derived from large control population studies. Having any CNV predicted an increase for four of the 14 disease categories after Bonferroni correction: congenital deficit(P = 0.00286), surgery (P = 0.00023) and mental disorder, primarily developmental delay (P = 0.00021). MYT1L dup did not contribute to increased risk of any disorder category.

When individual CNVs were examined in relation to their matched non-carriers, significant associations were found between 22q11 duplication, and pediatric gastro intestinal reflux disorder (p = .00085) which was more likely to be present in the presence of developmental delay (interaction P = 0.0352) an interaction not seen for the control population. 16p11.2 del was associated with both mental and nervous system disorders (P = 0.00004 and 0.0022, respectively). 15q13.3 del trend for association with mental disorders. Notably, pediatric records did not indicate increased risk for subjects with MYT1L duplication.

Conclusions: A broader concept of overall clinical penetrance is of importance for both genetic counseling and understanding the pathophysiology of these disorders. This is also the first report of a CNV association (22q11 duplication) for pediatric GERD, and may represent a delay in vagal nerve maturation.

Keywords: CNV, pediatric, penetrance, 22q11

Disclosures: Nothing to disclose.

W33. De Novo Genomic Variation in Non-Autistic Motor Stereotypes

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Background: Motor stereotypies are broadly defined as involuntary, coordinated, patterned, repetitive, rhythmic, and purposeless yet seemingly goal-directed movements. Stereotypic behaviors, including movements, are required as part of the diagnostic criteria for autism spectrum disorders (ASD) and are often associated with self-injurious behaviors in children with ASD and other neurodevelopmental syndromes, intellectual disability, and sensory deprivation. The basic pathophysiologic mechanism of motor stereotypies is unknown, but an underlying neurobiological, as opposed to psychological mechanism, has been supported.

The Pediatric Movement Disorder Center at Johns Hopkins University School of Medicine, under the Directorship of Dr. Harvey Singer, currently follows more than 250 families with typically-developing children affected by CMS. The lack of developmental comorbidities in these children with persistent and severe motor symptoms suggests that these patients may represent: a) a genetically more homogenous group of individuals with motor stereotypies, and b) a resource for gaining insight into the molecular pathophysiology of repetitive behaviors in children both without and with social disability. By focusing on one core phenotype of ASD in children without developmental comorbidities, genetic analysis of sporadic cases has the potential to accomplish the overarching goal of revealing specific genes and biological pathways contributing to motor stereotypies, both in normal development and in ASD.

Methods: Whole Exome Sequencing (WES): Genomic DNA from saliva of 130 CMS trios was enriched for exonic sequences using the NimbleGen SeqCap EZ Exome v2 capture platform and sequenced on the Illumina HiSeq 2000. Alignment and variant calling of the sequencing reads followed the GATK v3 best practices guidelines. 75-bp paired-end reads were aligned using BWA MEM (v. 0.7.10) to the b37 human reference sequence with decoy sequences. Validation for all predicted DN SNVs via Sanger sequencing of all trio family members, with sequence readers blind to affected status, confirmed 94%.

Results: WES: Among 121 trios passing quality control, 135 DN SNVs were confirmed by Sanger sequencing, including 35 silent, 83 missense and 11 loss of function (LoF, nonsense, altering a canonical splice site, or disrupting a start/stop codon). Among 370 control trios, 296 DN SNVs were confirmed, including 90 silent, 188 missense, and 9 LoF. In order to ensure that batch effects influencing call rates did not confound comparisons between our cases and controls, we normalized variant rates using the rate of silent variants in each cohort as the denominator. Overall, the ratio of DN LoF to silent variants was significantly greater in cases (0.31) versus controls (0.10) (OR 3.1, p = 0.02). Two unrelated individuals harbored a DN nonsense mutation in the gene KDM5B. Using DNENRICH (http://bit.ly/dnenrich), we simulated 10,000 random permutations of DN mutations, accounting for gene size, tri-nucleotide context, mutational effect, and per-trio effective gene coverage. Our recurrent mutation in KDM5B is highly unlikely to be a chance event (p = 0.0009), as is a finding of any gene harboring two recurrent DN mutations in a cohort of our size (p = 0.005). There was no significant difference detected for ratios of DN missense to silent variants between cases (2.4) and controls (2.1) (OR 1.1, p = 0.6).

Systems Analysis: We performed gene set enrichment analyses for all DN LoF variants confirmed in our CMS cohort. Using DNENRICH and 40 gene sets obtained from various databases and literature reports, we simulated 10,000 random permutations of DN mutations, accounting for gene size, tri-nucleotide context, mutational effect, and per-trio effective gene coverage. Our mutation list shows significant enrichment in genes known to be associated with ASD (p = 0.0008) and a trend for enrichment in known FMR1 targets (p = 0.07). Next, we plotted DN LoF variants onto BrainSpan exon array gene expression data, obtaining median expression values for each variant at multiple brain regions at multiple points in development. The expression profiles for CMS differed significantly from that seen in control subjects (Figure 1). Finally, we observed a trend toward DN variants occurring in genes that are less tolerant to variation in cases versus controls, determined by RVIS scores.

Conclusions: By exome sequencing apparently simplex CMS trios, we found an increased burden of DN LoF variants in cases vs controls. Similar to other neurodevelopmental disorders, such as autism spectrum disorders, this approach can yield a fruitful path toward gene discovery. Even in a relatively small cohort, we confirmed two DN LoF variants in the same gene, KDM5B, in two unrelated individuals, pointing toward a role for histone demethylation in the etiology of motor stereotypies. Sequencing larger cohorts

has the potential to transform our understanding of disease biology and suggest new treatment targets.

Keywords: Whole exome sequencing, rare variation, motor stereotypies, de novo mutation

Disclosures: Nothing to disclose.

W34. Neural Correlates of Social Threat Processing and Modulation in Social Anxiety Disorder

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Background: Social anxiety disorder (SAD) is associated with exaggerated emotional reactivity, particularly in response to social threat. Neuroimaging studies have linked emotional reactivity in SAD to hyperactivation in brain regions associated with emotional processing, like amygdala. Hypoactivation in areas associated with emotion modulation, like medial prefrontal cortex (mPFC), has also been reported in SAD, suggesting deficits in both emotion processing and emotion modulation in response to social threat. The purpose of this study was to further investigate differences in emotion processing and emotion modulation between SAD and healthy controls (HCs) on a novel attention modulation and emotional appraisal task.

Methods: Twenty seven subjects (18 SAD, 9 HC) completed fMRI scanning during the Shifted Attention, Emotion Appraisal Task (SEAT). Subjects viewed a series of compound images consisting of affective faces (angry, neutral, fear) superimposed on indoor and outdoor scenes, as well as neutral faces and scenes individually. Cues on each trial instructed participants to: 1) identify the gender of the face (male/female), 2) identify whether the scene is indoor or outdoor (in/out), or 3) indicate whether they like or dislike the face (like/dislike). This task allowed for the assessment of neural activation associated with implicit emotional processing (male/female), attention modulation of emotion (in/out), and emotional appraisal (like/dislike). Data were analyzed using SPM for MATLAB, to examine patterns of activation on each of these tasks in SAD compared to HCs.

Results: Results revealed hyperactivation in hippocampus (30, -16, -14) and hypoactivation in mPFC (0, 41, 29; p's <.001) in SAD compared to HCs during attention modulation of emotion. During emotional appraisal, SAD subjects demonstrated hyperactivation in hippocampus (33, -13, -14) and amygdala (30, -4, -17), as well as hypoactivation in mPFC (9, 35, 37; p's < .001) compared to HCs. During implicit emotional processing, SAD subjects demonstrated hyperactivation in visual cortex, lingual gyrus, and fusiform face area, as well as hypoactivation in mPFC (-9, 26, 31; p's < .001) compared to HCs.

Conclusions: Our findings demonstrate decreased activation in brain regions involved in emotion modulation (mPFC) in SAD during both emotion appraisal and emotion modulation tasks. In addition, SAD subjects had greater activation in threat-processing limbic regions (amygdala and hippocampus) and sensory processing regions (visual cortex, fusiform gyrus) during emotion appraisal and emotion modulation tasks. These results both replicate and extend previous reports of enhanced social threat processing and poorer emotion modulation abilities in SAD. Future investigations will continue to examine neural mechanisms underlying SAD, in an effort to develop targeted treatments to normalize neurocognitive deficits associated with anxiety.

Keywords: Social Anxiety, fMRI, Attention, Emotion Modulation

Disclosures: Nothing to disclose.

W35. D1 Closes the Sensitive Period Associated with Reduced Addiction Risk

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Background: Children rarely become addicted to stimulants, and stimulant exposure during this stage can actually reduce addiction risk in select populations. In contrast, exposure to stimulants during adolescence elevates lifelong addiction four-fold relative to exposure stimulants in adulthood. A "switch" from protection to risk for addiction maybe flipped during the transition between childhood and adolescence. The dopamine D1 receptor (D1R) within prelimbic prefrontal cortex may be that switch. D1R increases environmental salience resulting in more sensation-seeking and risk-taking that is transiently found during adolescence, but expressed at low levels in juveniles. To determine whether cocaine exposure during juvenility can increase addiction propensity if the D1R "switch" is thrown (compared with a control protein [dsRed]), the enduring effects on place conditionings for cocaine and cocaine self-administration were tested.

Methods: Sprague-Dawley juvenile male rats (n = 6-8) received bilateral stereotaxic injections of an inducible lentivirus that specifically expressed either D1 or dsRed in glutamate neurons from the CamKIIα promoter (CK.D1 or CK.dsRed) into the plPFC on postnatal day 16 (P16). To test whether juvenile D1R over-expression could permanently elevate addiction risk, D1R or dsRed was transiently over-expressed between 20-35 days of age. Subjects were given cocaine (15 mg/kg; COC) or vehicle (VEH) in the presence of cues for 60 min each day. D1R and dsRed were returned to normal levels following doxycline removal at P36. Subjects were tested with unbiased place conditioning to 10 mg/kg cocaine at P60. Subjects then received a jugular catheter and trained to self-administer cocaine during a 24 hour binge.

Results: In adulthood, D1R + COC subjects had increased preferences to COC-associated contexts paired with early exposure cues, whereas dsRed + COC had place aversions to COC-associated contexts. Cocaine self-administration during a 24-hour binge period was reduced in D1R + VEH, whereas the other conditions: D1R + COC, dsRed + VEH, and dsRed + COC groups self-administered near maximal amounts.

Conclusions: These data suggest that juveniles with D1R overexpression (found in adolescence) switches drug seeking and taking behavior relative to low D1R expression

typical of childhood. Drug exposure during juvenility in typical animals (dsRed + COC) produced a place aversion, replicating our earlier findings and is consistent with clinical data that suggest that early intervention with psychostimulants reduces substance use in some high-risk populations. More importantly, exposure to a drug-free environment during this sensitive period if D1R is elevated (e.g., a high-risk population), reduces both preferences and cocaine self-administration during a 24-hour binge period. Together these data suggest that the programming of non-drug or drug cues during childhood will have enduring effects on behavior.

Keywords: cocaine addiction, anti-ADHD like action, Sensitive Period

Disclosures: Nothing to disclose.

W36. Predicting Behavioral and Emotional Dysregulation Trajectories in Symptomatic Youth from Structural Neuroimaging: A Machine Learning Approach

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Background: Behavioral and emotional dysregulation (BED) is a common denominator across various psychiatric illnesses among symptomatic youth. However, currently, it is difficult to predict how the current manifestation of symptoms will play out in the future, based on clinical assessment alone. Youth with these behaviors may be diagnosed with a variety of disorders such as bipolar spectrum disorder (BPSD), depressive disorders, attention deficit hyperactivity disorder (ADHD), and disruptive disorders, or remain undiagnosed. We measured BED using the Parent General Behavior Inventory-10-Item Mania Scale (PGBI-10M), a ten-item parental report of observed child behaviors associated with difficulty regulating positive mood and energy (n = 146). We aimed to create a predictive model using machine learning algorithms applied to structural neuroimaging to reliably predict the trajectories of symptoms in youth with BED measured using PGBI-10M ratings.

Methods: A total of 146 young participants were recruited from longitudinal assessment of manic symptom (LAMS) study - a multisite study of youth initially aged 6–12 years of age who at enrollment were seeking treatment for BED. All recruited participants (115 LAMS and 31 Controls) completed a structural MRI scan. Structural scans were preprocessed and cortical thickness measures were extracted using Freesurfer. PGBI-10M ratings were collected every six months over a span of 5 years. Longitudinal trajectory analysis of PGBI-10M scale rating was performed in Statistical Analysis System (SAS) using "traj" software package. There were three PGBI-10M trajectories in LAMS youth. We chose two trajectories: 1. with high PGBI-10M (HighLAMS; n = 20) and 2. with low PGBI-10M (LowLAMS; n = 34). Machine learning analyses were performed using "glmnet" and "caret" software packages in R. Similar analyses were done including LAMS youth with highest

PGBI-10M trajectories (HighLAMS; n = 20) and Controls (Controls; n = 31).

Results: Optimized elastic net classification model (lambda = 0.082; alpha = 0.7) for differentiating HighLAMS and LowLAMS trajectories using 10 fold cross-validation resulted in ROC of 0.85. Our first model suggested that higher rostral middle frontal, orbitofrontal, parietal lobe, lingual area, and superior temporal thickness measures were related to the high PGBI-10M trajectories, while higher postcentral, Insula, and lateral orbitofrontal thickness were related to lower PGBI-10M trajectories. In the second analysis the optimized elastic net model (lambda = 0.6; alpha = 0.1) differentiating controls with the HighLAMS trajectories using 10 fold cross validation resulted in ROC of 0.96. Our second model suggested that higher rostral middle frontal cortical thickness predicted the membership of the high ESM trajectory group, while high Insula, precuneus, entorhinal, temporal pole, lateral occipital, and cuneus cortical thickness measures predicted the membership of the control group.

Conclusions: Our findings suggest that the combined use of Freesurfer measures and machine learning-based analysis were able to identify clusters of brain regions that can accurately differentiate youth with BED in those with higher vs lower manic symptom trajectories. This approach may lead to better understanding of different pathophysiological processes underlying BED disorders and aid diagnostic accuracy and early intervention strategies in youth with BED since early stage of illness that might ultimately change the illness trajectories in these youth.

Keywords: machine learning, neuroimaging, cortical thickness, Trajectories

Disclosures: Nothing to disclose.

W37. Capturing Rdoc Reward Constructs in Adolescents with Diverse Psychiatric Symptoms

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Background: Anhedonia is a salient feature across many psychiatric conditions. While the clinical phenomenology is well understood, its underlying neurocircuitry is still not well defined. This is in part due to its heterogeneous nature, as anhedonia can reflect the same clinical outcome of different positive valence system deficits such as approach motivation (reward anticipation) and reward attainment. Here we present preliminary data using the Reward Flanker Task (RFT) from our NIH-funded RDoC study, which seeks to examine the neuroimmunological and neurochemical mechanisms underlying reward constructs such as anticipation versus attainment in adolescents displaying a range of psychiatric symptomatology.

Methods: Participants: Fifteen psychotropic medication free adolescents, ages 12-19, were assessed for psychiatric symptomatology using the Kiddie-Schedule for Affective Disorders and Schizophrenia for School-Age Children-Present and Lifetime Version (KSADS-PL) and underwent fMRI scanning during the RFT. Adolescents were included

if they displayed any current psychiatric symptomatology whether or not diagnostic criteria for DSM disorders were met (n = 12), or were healthy controls (n = 3) with no lifetime history of psychiatric diagnoses. The presence of psychosis, pervasive developmental disorder, or substance abuse disorders was exclusionary. Reward Flanker Task (RFT): During the RFT, participants made button presses and earned a reward if they correctly identified a target letter surrounded by four flanker letters. During each trial, a monetary cue was presented for 4-6 seconds. Four cues were used: low reward ("10¢"), high reward ("50¢"), no reward ("0¢"), and unknown reward ("?"). After the cue, the flanker stimuli were presented for 300 ms, followed by a response interval that was calculated for each participant based on performance during a practice session. Participants then received feedback for 2 seconds informing them of the value of the obtained or unobtained reward. A jittered inter-trial interval between 4 and 6 seconds followed the feedback interval. A total of 120 trials were presented in a pseudo-random event-related design over 4 runs, with 30 trials each of high, low, and no reward cues, and 30 unknown reward cues. FMRI Data Acquisition: T2*-weighted gradient echo multiband echo planar images (2.3 mm isotropic) were acquired with a $16 + 4$ head-neck coil on a 3T Skyra scanner. Data processing included gradient non-linearity and EPI distortion correction, motion correction, coregistration to anatomical image, normalization to standard MNI space, and spatial smoothing (6mm). Analysis: A one-way within-subjects ANOVA was carried out at the group-level (n = 15). Contrasts of interest included comparisons of cue conditions (reward anticipation) versus feedback (reward attainment) conditions. A region of interest (ROI) analysis was done such that whole-brain activation maps were FWE corrected with $p < .05$, and activation clusters restricted to a single ROI encompassing key regions known to play a role in reward processing (i.e. orbitofrontal cortex, anterior cingulate cortex, medial prefrontal cortex, and striatum).

Results: In the contrast of all known cues ($0¢$, $10¢$, $50¢$) versus all correct reward outcomes, there was robust activation in the posterior medial frontal cortex, right precentral gyrus, left mid cingulate cortex, and right ventral caudate. In the contrast of correct feedback versus all known cues ($0¢$, $10¢$, $50¢$), there was activation in the bilateral caudate, bilateral putamen, right inferior frontal gyrus (IFG), bilateral hippocampus, left middle frontal gyrus, right amygdala, and left mid cingulate cortex. In the contrast of only monetary cues worth a known gain (i.e. $10¢$ and $50¢$) versus correct feedback for those gains, activation was restricted to the right posterior medial frontal cortex and right precentral gyrus. In the contrast of correct feedback of known monetary gains (i.e. $10¢$ and $50¢$) versus cues for those trials, the right IFG, bilateral caudate, and bilateral putamen were activated. Finally, in the contrast comparing anticipated monetary gains ($10¢$, $50¢$) versus no gain ($0¢$), the superior medial frontal gyrus was activated. No other contrasts survived the conservative multiple comparisons correction (FWE, $p < .05$).

Conclusions: Our findings are consistent with past observations showing separation in the neural mechanisms underlying reward anticipation and attainment in adolescents. Regions involved in decision making behaviors were involved in processing the anticipation of a known reward, while other emotion-regulated limbic regions were involved in processing the attainment of a reward. These findings confirm the utility of the RFT in capturing the neurocircuitry underlying RDoC reward constructs, and our future work will extend this preliminary investigation to examine the specific neuroimmunological and neurochemical mechanisms that are related to deficits in anticipation versus attainment in adolescents with diverse psychiatric symptomatology.

Keywords: reward neural circuitry, Adolescents, fMRI, anhedonia

Disclosures: Nothing to disclose.

W38. Depression in Girls during Early Adolescence Predicts Altered Cortical Midline Response to Social Evaluation in Late Adolescence

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Background: Increased risk for developing depression in adolescence emerges after puberty onset, particularly among girls (Cyranowski et al., 2000; Piccinelli & Wilkinson, 2000). The value that girls place on social outcomes may underlie their increased risk for depression. Social stressors related to affiliation (e.g., end of a romantic relationship, loss of a friend) are more common for girls, whereas status-oriented stressors (e.g., loss of social status, reputation) are more common for boys (Oldehinkel et al., 2007; Stroud et al., 2002). Cognitive theories of depression would suggest that biased cognitions about social events relate to vulnerability to depression, yet little is known about the underlying neural mechanisms. One putative marker of depression-related cognitive susceptibility is perturbed focus on salient social events manifest in neural response. In the present study, we examined girls' neural responses to peer acceptance and rejection as a function of depression symptoms.

Methods: Participants were from a sample of 232 girls (67% African-American) participating in a sub-study of the Pittsburgh Girls Study. Depression symptoms were assessed at age 11 (early adolescence) and age 17 (late adolescence) with the K-SADS. At age 17, 140 participants completed the "Chatroom," a widely-used fMRI social evaluation task. Pre-scan, participants completed a selection task where they judged 60 photos of novel peers by indicating who they LIKED (selected) and DID NOT LIKE (unselected). During the scan, participants viewed the peers again along with feedback from the peer indicating whether s/he LIKED, DID NOT LIKE or DID NOT HAVE A CHANCE TO JUDGE (baseline) the participant. AFNI was used for preprocessing, 1st and 2nd level analyses with these task events as regressors: accepted by liked peers, accepted by not liked peers, rejected by liked peers and rejected by not liked peers. Motion parameters were included as regressors as well. ROI analyses were conducted to test for main and interactive effects of early depression, peer selection and feedback, controlling for concurrent symptoms, on neural response in cortical midline regions. To decompose

significant interactions, the main effects of feedback were tested within each combination of depression and peer selection. We used a median split to create low ($< = 1$ symptoms) and high ($> = 2$ symptoms) depression groups. **Results:** Significant 3-way interaction effects of depression x selection x feedback were found on activation within several cortical midline structures including medial PFC (mPFC), left middle frontal gyrus (MFG), bilateral ventrolateral prefrontal cortex (vlPFC), and right inferior frontal gyrus (IFG). For those with high symptoms, the main effect of feedback was specific to selected peers, such that greater activation to rejections vs. acceptance was found in the vlPFC bilaterally and right IFG. In contrast, for those with low symptoms, greater activation to acceptance vs. rejection from selected peers was found in right mPFC, left MFG, and right IFG. Further, we found simple main effects for depression in right mPFC and right IFG, with increasing symptoms significantly associated with decreased activation in response to being accepted by selected peers.

Conclusions: These brain-behavior relationships associated with early adolescent depression provide clues for elements to target in therapeutic interventions. Cortical midline structures are involved in self-referential processes and self-evaluation. The double dissociation of feedback and depression level may reflect depression-related differences in the integration of peer evaluations with one's own self-evaluation. Specifically, those with low symptoms had greater cortical midline responses to acceptance, while those with high symptoms showed greater responses to rejection. This pattern is especially pronounced in the right IFG. Girls may have showed greater cortical responses to the social outcome that was consistent with their self-view. Notably, this pattern was limited to selected peers, and no depression-related differences were found for unselected peers suggesting that participants were especially likely to engage in self-referential processing when motivated for social interaction.

Keywords: Adolescent Depression, social stress, ventrolateral prefrontal cortex, gender, fMRI

Disclosures: Nothing to disclose.

W39. Hepatic Insulin Sensitivity is Associated with Whole Body Adiposity Achieved during Initial Antipsychotic Exposure in Youth

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Background: Fatty liver is quickly becoming one of the most common consequences of childhood obesity and is associated with decreased hepatic insulin sensitivity, a precursor to type 2 diabetes. Previous reports have shown a significant relationship between visceral abdominal adiposity and hepatic fat content is linear. Weight gain and increased adiposity associated with antipsychotic treatment in youth has become a significant public health concern, as antipsychotic agents are associated with significant weight gain and increased rates of obesity and type 2 diabetes in this population. Thus, it is reasonable to evaluate whether

important precursors to diabetes risk, such as hepatic insulin resistance, can be linked to increases in adiposity occurring during antipsychotic treatment. In a cross sectional sample comparing chronically antipsychotic-treated youth compared with healthy controls matched on BMI, our group has shown that level of adiposity achieved, rather than antipsychotic treatment, is associated with hepatic fat content. However, little is known about whether hepatic function, specifically hepatic insulin sensitivity, is compromised by increasing adiposity in the context of initial antipsychotic treatment. We aimed to evaluate whether increasing adiposity during an initial 12 weeks of antipsychotic exposure impacts liver function, as measured by the Hepatic Insulin Sensitivity Index (HISI).

Methods: In the NIMH-funded MEAC study (Metabolic Effects of Antipsychotics in Children, PI Newcomer MH72912), an antipsychotic-naïve cohort of youth ages 6-18 were randomized to 12 weeks of antipsychotic treatment (AP) with aripiprazole, olanzapine or risperidone. Gold standard measures of insulin sensitivity and adiposity were measured at baseline and 12 weeks ($n = 114$), including hyperinsulinemic-euglycemic glucose clamps with stable isotopomer tracing to measure endogenous glucose production and insulin sensitivity at liver, adipose tissue and muscle tissue; Dual Energy X-ray Absorptiometry (DEXA) to measure whole body adiposity; and Magnetic Resonance Imaging (MRI) to determine visceral abdominal adiposity in a subset of MEAC patients ($n = 80$). The HISI was calculated by taking the ratio of 100 over the product of basal insulin and endogenous glucose production (EGP). ANOVA was used to evaluate differences between groups on change in DEXA %fat and change in HISI. A regression model evaluated the relationships between HISI and DEXA %fat and HISI and MRI visceral fat separately at baseline and endpoint. A repeated measures ANCOVA was used to evaluate the relationship between HISI and DEXA %fat and HISI and MRI visceral fat over 12 weeks of initial AP exposure, with treatment group included in the model as a 3-level factor, and with baseline HISI and baseline DEXA %fat or MRI visceral fat inserted as covariate terms.

Results: In this subset of 114 MEAC completers, the mean age was 11.56 years (SD = 2.72). The subset was comprised of 79 males (69.3%), 55 Caucasians (48.2%) and 59 non-Caucasians (51.8%). HISI decreased over the course of initial 12 weeks of AP exposure by 0.0101 IU/mL (SD = 0.0213), with mean decreases of 0.0063 (SD = 0.0173), 0.0192 (SD = 0.0282), and 0.0062 (SD = 0.0155) for aripiprazole, olanzapine, and risperidone, respectively. Treatment groups were matched at baseline on HISI (F[2,113] = 1.41, p = 0.25) and DEXA % fat (F[2,113] = 0.54, p = 0.58). The mean change in DEXA %fat in the pooled group was 2.3% points (SD = 3.1) with significant differences between treatment groups (F[2,112] = 6.17, p = 0.003). DEXA %fat increased by 1.6% points (SD = 2.7) in the aripiprazole group, 3.8% points (SD = 2.8) in the olanzapine group, and 1.6% points (SD = 3.3) in the risperidone group. HISI was significantly correlated with DEXA %fat and MRI visceral fat at baseline, respectively (F[1,113] = 13.85, p < 0.0001; F[1,78] = 13.43, p < 0.0001) as well as at 12 weeks (F[1,112] = 21.72, p < 0.0001; F[1,79] = 22.31, p < 0.0001). In the repeated measures ANCOVA model including DEXA %fat and HISI, there was a significant time by treatment

group interaction (F[2,109] = 4.15, p = 0.02), which was largely driven by treatment with olanzapine (F[1,36] = 6.49, p = 0.02), followed by aripiprazole (F[1,38] = 4.86, p = 0.03) and did not appear to be impacted by risperidone (F[1,36] = 2.88, p = 0.010). A main effect of time was observed in the repeated measures ANCOVA model including MRI visceral fat and HISI [F(1,74) = 6.33), with no interaction between time and treatment group [F(1,74) = 1.74, p = 0.18).

Conclusions: These results confirm previous results showing a significant relationship between adiposity and hepatic insulin sensitivity in adults and youth, and suggest that antipsychotic treatment contributes to worsening hepatic insulin sensitivity, a precursor to diabetes, predominantly via increases in adiposity. These results underscore the importance of monitoring weight gain during antipsychotic treatment, and suggest that addressing increasing adiposity in antipsychotic treated individuals may be an important target for mitigating increased diabetes risk in this population.

Keywords: Antipsychotic, Antipsychotic induced weight gain, Cardiometabolic Risk, Children and Adolescents

W40. Psychoeducational Group Intervention for Adolescents with Psychosis and Their Families. A Two-Year Follow-Up: The Piensa Trial

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Background: Psychoeducational programs are among the most widely studied psychosocial interventions for psychotic disorders. These programs are systematic and didactic, and consist of psychotherapeutic interventions aimed at providing information about the disease in question to patients and their relatives to foster coping skills and understanding.

Studies in adult populations with schizophrenia have shown that psychoeducational interventions can reduce the prob-ability of relapse, number of hospitalizations, and symptom severity. They can also improve social and occupational functioning and increase adherence to treatment. Additional benefits include reduced family burden, improved coping skills, and recognition and understanding of psychosis as a disease. In accordance with the stress-vulnerability model, environmental factors such as family interactions can play an important role in the continuity of the disorder. Hence, family psychoeducational programs are aimed at influencing the environment in which the patient lives by reducing anxiety and increasing family members' self-confidence and ability to react constructively to behavioral disturbances and the patient's symptoms. This result has been confirmed in recent studies that show that the relapse rate can be reduced by approximately 20% if the parents of patients with schizophrenia are included in the treatment. Strenuous efforts to engage families in the prevention of relapses are justified, because 80% to 90% of patients are living with their parents when they are referred for treatment.

To our knowledge, only 1 study has assessed the efficacy of a psychoeducational treatment program in adolescents with psychosis, although it was not a randomized controlled trial. Furthermore, despite the fact that some programs offer psychoeducational approaches for young adults with a first episode of psychosis that include parents as an important complement in the program, none of them includes a specific age range for adolescents. Other studies evaluate the efficacy of a family-focused psychoeducational approach for adolescents with mood disorders who frequently have accompanying psychotic features. Our study aimed to examine the efficacy of a parallel, structured, and specific psychoeducational group intervention (PE) for adolescent patients and their families by comparing it with a nonstructured group intervention (NS).

To the best of our knowledge, this is the first randomized controlled trial to compare a PE intervention with an NS intervention in adolescents with early-onset psychosis. We hypothesized that patients in the PE group would have fewer hospitalizations, days in hospital, and visits to the emergency department. We also hypothesized that these patients would have better clinical outcomes and more favorably perceived family environments. At the end of the group intervention, 15% of patients in the psychoeducational group and 39% patients in the nonstructured group had visited the emergency department (χ^2 = 3.62, df = 1, p = .039). The improvement in negative symptoms was more pronounced in the psychoeducational group (12.84 [7.87]) than in the nonstructured group (15.81 [6.37]) (p = .039).

We present now a study to determine whether the beneficial effects observed immediately after a structured, psychoeducational, parallel-group program for adolescents with early-onset psychosis and their families were maintained 2 years later. The long-term results of this trial have not yet been published.

Methods: The current study examines the two-year longitudinal efficacy of a randomized controlled trial (1) based on a structured, psychoeducational, problem-solving, group intervention for adolescents with early-onset psychosis and their families (PE group) compared with a non-structured group intervention (NS group). All patients and their

parents or legal guardians signed an informed consent before enrolling in the study. The study was approved by the hospital IRB. We analyzed whether the differences between the PE and NS groups found after the intervention persisted two years later. Number and duration of hospitalizations, symptoms, and functional differences between groups were also assessed.

Results: At the two-year follow-up, 89% of patients were able to be reassessed. In the PE group, 13% of patients had visited the emergency department versus 50% in the NS group (p = 0.019). However, no statistically significant differences were found between the two groups in negative symptoms or number and duration of hospitalizations. Only the PE group significantly improved in PANSS general symptoms.

Conclusions: A psychoeducational group intervention showed sustained effects represented by decreased number of visits to the emergency department two years after the intervention, which indicates that this specific psychoeducational intervention may provide patients with long-lasting resources to face crises more effectively.

Keywords: psychosis, psychoeducation, adolescents

Disclosures: Nothing to disclose.

W41. An Epigenetic Approach for the Modulation of Amyloid Precursor Protein (APP) Processing and Improvement of Memory in Alzheimer's Disease

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Background: Alzheimer's disease (AD) is the most common form of dementia in the elderly. Currently approved treatments are not efficacious and are palliative at best. These drugs are either cholinesterase inhibitors (donepezil, rivastigmine, galantamine) or NMDA receptor antagonists (memantine). The "amyloid cascade hypothesis" places beta amyloid (Aβ) at the origin of AD, causing a chain of molecular events leading to neuronal degeneration, memory loss, motor impairment, and eventually death. No FDA-approved treatment presently reduces Aβ accumulation (the main constituent of plaques) in the brain of patients. With the recent clinical trial shortcomings of Alzheimer's immunotherapy and γ-secretase inhibitors, it has become increasingly clear that mono-therapies involving a single drug target may not be sufficient in treating AD. Experts in the field agree that "drug cocktails" are desirable but present challenges in terms of clinical and regulatory hurdles. We therefore took an epigenetic approach where a single drug would simultaneously affect the expression of a number of defined AD-related targets.

Methods: First, in an AD cell model over-expressing APP with the Swedish mutation, we screened our in-house comprehensive library of epigenetic drugs to identify small molecules that are able to significantly reduce Aβ, using AlphaLISA technology (Perkin Elmer). We then tested the Hit compounds for toxicity using the Cell-Titer-Glo assay method (Promega). Hits were further confirmed with Aβ ELISAs (Life Technologies). Then, using real time quanti-

tative polymerase chain reaction (RT-QPCR) and western blots, we honed in on compounds that affect AD-related and neuro-protective genes and proteins. We further validated results in vivo in the triple transgenic (APP/PS1/tau) AD (3xTg AD) mouse model using behavioral tests such as: the open field test, the Y-maze spontaneous alternation test, and novel object recognition.

Results: We identified an atypical small molecule histone deacetylase inhibitor (HDACi), CTI-309, that reduces beta amyloid (Aβ), decreases BACE1 and tau gene expression; and increases the expression of the following genes: BDNF, α-secretase (ADAM10), Mint2, Fe65 and REST. This molecule increases the production of sAPPα, the cleavage product of ADAM10, in line with the increased gene expression observed for ADAM10. This molecule also increases levels of immature APP, supporting an effect on APP trafficking, as do the increases in Mint2 and Fe65. Treatment of the 3xTg AD mouse model with CTI-309 resulted in significant increases in spontaneous alternations compared to controls. We also observed, in these CTI-309 treated mice, significant increases in frequency and time spent with the novel object in the novel object recognition test. Interestingly, no differences were observed in the open field test.

Conclusions: Importantly, non-amyloidogenic amyloid precursor protein (APP) processing by α-secretase cleavage precludes the formation of Aβ and has been reported to reduce AD-like pathology. Increased expression of the APP binding proteins Fe65 and Mint2 as well as BDNF have been shown to be neuroprotective. Furthermore, REST has recently been identified as being important for cognitive reserve, and as a protective component against AD and ageing related dementia. Taken together, our data suggest that the newly identified epigenetic molecule CTI-309 is brain penetrant, targets the non-amyloidogenic pathway, affects APP trafficking, increases neuroprotective genes, shows less toxicity than other HDACis tested and increases memory in an AD model. Experiments are being undertaken to decipher the exact mechanism of this molecule.

Keywords: Alzheimer's Disease, Epigenetics, Amyloid Precursor Protein, Behavioral Pharmacology, HDAC inhibitors

Disclosures: Claes Wahlestedt is a consultant for Opko Health and co-founder of Epigenetix Inc.

W42. Genetic Interaction between SORL1 and BDNF Regulates Isoform-Specific SORL1 Expression with Effects on Brain Structure and Amyloid Pathology

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Background: Variants within the sortilin-like receptor (SORL1) gene are well-replicated for Alzheimer's disease (AD) risk; however, their mechanisms of effect are largely unknown. It was recently shown that the brain-derived neurotrophic factor (BDNF) up-regulates SORL1 mRNA expression in a SORL1 genotype-dependent manner. The BDNF Val66Met variant affects the cellular secretion of

BDNF and may therefore interact with SORL1 genotypes to influence SORL1 expression and downstream AD-related phenotypes. Importantly, SORL1 transcript isoforms may be preferentially affected by these interactions and play unique roles in pathological and structural brain changes.

Methods: In total, this study included data from $n = 608$ subjects from the Religious Orders Study/Memory and Aging Project (ROS/MAP), and $n = 1 285$ subjects from the Alzheimer's Disease Neuroimaging Initiative (ADNI phases 1/GO/2). In the ROS/MAP sample, 10 SORL1 transcripts were quantified as expressed vs. not expressed in prefrontal cortex of 441 post-mortem brain samples using RNA-sequencing. For all transcripts, the interaction between each common SNP within 10kb of the SORL1 locus and BDNF Val66Met was tested using logistic regression. Interactions showing significance at $P < 0.05$ after locus-wide multiple testing correction were carried forward for further analyses in imaging-genetics samples. From ROS/MAP, a subsample of 172 subjects had ante-mortem T1-imaging data from which entorhinal cortex volumes were segmented. All ADNI subjects also had estimates for entorhinal cortex volume, and a subset of 710 subjects from ADNI GO/2 had Aβ [18F]Florbetapir PET imaging data available for multiple regions of interest.

Results: In the ROS/MAP sample isoform expression analyses, 45 tests survived correction for multiple testing. All 45 models were for the same transcript, SORL1-005, a putative truncated protein-coding transcript of 1124 amino acids, and all SNPs were in high LD (top SNP rs12364988, $P = 2.54 \times 10$-6). The rs12364988 T allele reduced likelihood of SORL1-005 expression in the BDNF Val/Val homozygotes, but greatly increased likelihood of expression among Met Carriers. In both ADNI and ROS/MAP imaging samples, 5 these 45 transcript-associated interactions explained significant variance in entorhinal cortex volume (at $P < 0.05$), whereby those genotype-defined groups more likely to express SORL1-005 had increased cortical volume (ADNI top SNP rs662821, $P = 0.02$; ROS/MAP top SNP rs12364988, $P = 0.01$). Consistent with our imaging findings, 21 out of 45 significant SNPs from expression analyses had interaction effects with BDNF Val66Met ($P < 0.05$) on prefrontal Aβ (top SNP rs662821, $P = 0.02$), as measured with [18F]Florbetapir PET.

Conclusions: Our findings point toward a novel interaction between SORL1 and BDNF variants that may be important for regulating isoform-specific SORL1 expression. Intriguingly, the top interacting SORL1 SNP found in locus-wide analyses, rs12364988, is part of the same haplotype block recently found to determine BDNF-dependent upregulation of SORL1 mRNA in neurons derived from human induced pluripotent stem cells. These interactions also predicted differences in brain structure across samples that are implicated in risk for AD and therefore may be useful for AD risk assessment and potentially targetable therapies in pre-symptomatic adults. Further, the effects of this gene-gene interaction on in vivo amyloid suggest a possible mechanistic role of the SORL1-005 transcript in triggering early pathological brain changes.

Keywords: imaging genetics, RNA Sequencing, Postmortem Brain Tissue, PET Imaging, Alzheimer's Disease

Disclosures: Nothing to disclose.

W43. High Dose Donepezil Treatment of Alzheimer's Disease

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Background: Cholinesterase inhibitors (ChEIs) remain the therapeutic mainstay for patients with dementia of the Alzheimer's type (DAT) despite their small effect size, dose-limiting adverse events (AEs), and the need for prolonged dose titration. PET imaging studies indicate that ChEIs given at their maximum tolerable dose (MTD) inhibit the target enzyme in the central nervous system by only about 30%, while animal model and clinical trial results suggest that higher ChEI doses confer proportionately greater cognitive benefit.

Methods: A phase I single-blind, placebo-controlled, cross-over, dose-escalation study of donepezil to MTD or protocol limit was conducted to begin evaluation of the hypothesis that co-administration of a peripherally acting anticholinergic to attenuate dose-limiting AEs will enable the safe and tolerable use of higher ChEI doses and thus potentially improve the antidementia efficacy of drugs of this type. Dose increments of CPC-201 occurred on a daily basis. A subsequent Phase IIa trial of similar design with ADAS-cog, MMSE and CGI measures in 36 moderate DAT patients is now nearing completion.

Results: In the Phase 1 trial, oral admin of CPC-201 containing donepezil (to 40 mg/day) together with an anticholinergic (solifenacin 10 mg/day) resulted in mean AE reductions exceeding 60%, mean donepezil MTDs to increase by 1.7-fold, and mean peak plasma concentration increments of 2.6-fold ($p < .05$). There were no serious or unexpected AEs and no laboratory abnormalities. The Phase II trial has now enrolled 36 DAT patients. All had previously been treated with donepezil at a dose of 10 mg/day for at least 3 months. Donepezil dose titration (co-administered with solifenacin 15 mg/day as CPC-201) occurred on a daily or biweekly basis (at the highest doses). CPC-201 has been well tolerated at all doses. No drug-related SAEs, laboratory abnormalities, or withdrawals have occurred. As a result, both donepezil MTDs and plasma concentrations increased, on average, about 4-fold ($p < .01$). All patients successfully completing dose titration and a 3-month stable MTD maintenance period accepted to continue treatment in post-trial long-term extension. Several in this group have now completed one year at donepezil doses ranging up to 50 mg/day without difficulty.

Conclusions: The co-administration of a peripheral anticholinergic enables substantial dose increases of a ChEI such as CPC-201 in healthy volunteers and in DAT patients and should improve antidementia efficacy in those with hypocholinergic dementias.

Keywords: Alzheimer's Disease, cholinergic system, Clinical trial

Disclosures: Employee of Chase Pharmaceuticals Corporation.

W44. Cerebellar Connectivity and Glutamatergic Metabolite Concentration in ASD as Assessed by fcMRI/MRS

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Background: Cerebellar pathology has been one of the most consistent findings in ASD, with studies revealing decreased numbers of Purkinje cells in the cerebellar hemispheres. Recent work has also revealed atypical patterns of cortico-cerebellar functional connectivity in ASD. Furthermore, several lines of evidence have suggested an atypical excitatory to inhibitory (E/I) ratio in ASD, as evidenced by an imbalance between glutamate and γ-aminobutyric acid (GABA), observed in various brain regions including the cerebellum. Magnetic resonance spectroscopy (MRS) has revealed mixed results in the assessment of glutamatergic and GABAergic concentrations in the brain in ASD. Our purpose was to examine the relationship between E/I balance and functional integrity of cerebrocerebellar connections in ASD, focusing on the portion of the cerebellum most involved with language and its cortical projections.

Methods: Twelve individuals with ASD, confirmed by the Autism Diagnostic Interview-Revised or Autism Diagnostic Observation Schedule, aged 19-32, and 12 gender, age, IQ, and handedness-matched controls participated. Social communication was assessed with the Social Responsiveness Scale (SRS), maladaptive behaviors were assessed with the Aberrant Behavior Checklist (ABC), and language competence was assessed with the Test of Language Competence (TLC). MRI was performed with a Siemens 3T Trio MRI at the Brain Imaging Center of the Department of Psychological Sciences at the University of Missouri. After anatomical localizers, functional T2*-weighted images were acquired for BOLD activation (TR = 2200 ms, TE = 30 ms, Flip Angle = 90°, 35 ACPC-aligned slices at 4 mm3) during 5 minutes of passive rest in which the participant viewed a blank screen with a cross-hair fixation point and functional connectivity (FC) analyses were performed. Single-voxel (20 mm3) point-resolved spectroscopy spin-echo sequences were used to detect metabolites of interest (TR = 2000 ms, TE = 80 ms, flip angle = 90°, 128 averages, weak water suppression at bandwidth = 50 Hz, delta frequency = -2.3 ppm, bandwidth = 1200 Hz) and were repeated without water suppression to allow absolute quantification of metabolites. Single-voxel (20mm3) MEGA-PRESS (TR = 2000 ms, TE = 68 ms, flip angle = 90°, water saturation bandwidth = 35 Hz, delta frequency = -1.7 ppm, bandwidth = 2000 Hz) sequences were also used to detect GABA. Voxels were independently localized in each participant in the right postereolateral cerebellar hemisphere junction of crus I and crus II (RCere) and the left dorsolateral prefrontal cortex (LDLPFC), with reference to the standardized MNI atlas. Standard preprocessing and conservative motion correction, accounting for the potential for excess motion in ASD, were performed on the fMRI data. Metabolite levels were quantified with LCModel.

Results: As expected, ASD participants were impaired as compared to controls on aspects of the SRS, ABC, and TLC. No significant overall group differences in cortico-cerebellar connectivity were observed for the LDLPFC-RCere between ASD and controls. Within the ASD group, LDLPFC-RCere connectivity was significantly associated with listening comprehension (r = 0.588, p = 0.027). FC demonstrated a bimodal distribution within the ASD group, with a significant non-unimodal distribution according to the Dip test (D = 0.148, p = 0.004), such that two populations of ASD patients significantly differed in FC (t(12) = 9.4, p < 0.001) and the low FC group also differed from controls (t(15) = 3.4, p = 0.004). There were no differences between the ASD and control groups for any of the metabolites or for E/I (glutamate/GABA). However, glutamate levels (F(1,12) = 5.2, p = 0.049) and E/I (F(1,12) = 7.1, p = 0.026) in the RCere region was significantly higher for the high FC ASD group than for the low FC ASD group. Overall, across all groups, RCere-LFLPFC FC was significantly associated with RCere E/I, regardless of diagnosis.

Conclusions: Significant variability exists in the literature regarding FC in ASD as well as MRS of glutamate and GABA in ASD. However, our data suggests that these factors may be interrelated, and at least part of the variability in FC may be accounted for by distinct clusters occurring within the ASD population. Furthermore, this may have some relationship with behavior, as functional connectivity to the portion of the cerebellum most involved with language appeared to be related to performance on language comprehension. Future studies will need to explore whether these might serve as markers for or predictors of response to pharmacological agents targeting E/I balance in ASD.

Keywords: autism spectrum disorder, magnetic resonance spectroscopy, Resting State Functional Connectivity, glutamate GABA

Disclosures: Nothing to disclose.

W45. Dopamine Synthesis and Receptor Profile are Associated with Body Mass Index in Humans

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Background: Obesity is a worldwide epidemic with significant adverse health consequences such as diabetes, cardiovascular disease, hypertension, and stroke. While behavior clearly plays an important role, the neurobiological underpinnings of weight control require further delineation in humans. Dysfunction of the mesolimbic dopamine system, which mediates the rewarding effects of appetitive stimuli such as food, has been implicated in the development of obesity. Furthermore, striatal D2 receptor levels are decreased in obese individuals and inversely correlated with future weight gain and food seeking-behavior in rats. However, no studies to date have assessed the roles of both dopamine synthesis and receptor density in healthy humans in the normal to obese range. Characterizing the dopaminergic signature related to higher body mass index (BMI) could provide insight into an important neural mechanism underlying the obesity epidemic.

Methods: Seventy one healthy volunteers (mean age 35.7, range 18.7-58.6; mean BMI 25.6, range 17.8-36.1; 32 females) were recruited from the local community and screened by physician-administered physical examination, neurological examination, standardized clinical interview (SCID-IV), laboratory tests, and structural MRI read by a radiologist to rule out history of or current psychiatric, neurological, or major medical illness, as well as substance abuse or current medications that could affect the PET measurements. Subjects completed three PET scans on separate days to assess dopamine presynaptic synthesis capacity (with [18F]DOPA on a GE Advance scanner), and D1 and D2 receptor binding potential (with [11C]NNC112 and [18F]Fallypride, respectively on a Seimens HRRT scanner). Following a transmission scan, dynamically binned emission scans were collected for 4 hours (Fallypride) and 1.5 hours (FDOPA and NNC) after tracer injection. Caffeine and nicotine were restricted for four hours and food (for FDOPA only) for six hours before the scan, and subjects were pretreated with carbidopa 200 mg one hour prior to injection for the FDOPA scan to reduce peripheral metabolism of the tracer. BMI was calculated using height and weight measurements taken by nursing staff typically on the same day as the PET scan (an average of $15 + 77$ days from the PET scan). In addition, for forty four subjects, BMI had also been measured an average of 1.6 ± 0.7 years prior to the PET scan, and this earlier value, in comparison to the more current value, was used to calculate a rate of BMI change.

Subjects also completed a T1-weighted MRI scan used for registration and brain segmentation. MRI scans were segmented using Freesurfer and manual adjustments to generate ROIs of the basal ganglia including the putamen, caudate nucleus, ventral striatum, and midbrain. MRI images were registered to native space PET images and values were extracted for each of the ROIs. The FDOPA uptake rate (Ki) was calculated with the Patlak method and dopamine receptor D1 and D2 receptor binding potential were calculated with the SRTM method, both using a cerebellar reference region. Correlations between BMI and PET ROI values were assessed in SPSS, with a threshold of $p < 0.01$ (uncorrected). All results remained significant after controlling for age and gender.

Results: We found that BMI correlated positively with presynaptic dopamine synthesis capacity (FDOPA) in the midbrain ($r = 0.327$, $p = 0.005$) and negatively with D2 receptor binding potential (Fallypride) in the midbrain ($r = -0.388$, $p = 0.002$). In addition, presynaptic synthesis capacity (FDOPA) in the left caudate nucleus correlated negatively with the rate of BMI change before the PET scan ($r = -0.382$, $p = 0.010$). BMI did not correlate with D1 receptor binding potential (NNC) in any of the ROIs.

Conclusions: We demonstrated that BMI correlated positively with dopamine synthesis capacity and, in line with independent past work, inversely with D2 receptor binding potential in the mesostriatum of healthy human volunteers. Though the current design cannot distinguish with certainty between pre- and post-synaptic receptor binding, our results are consistent with previous findings that D2 autoreceptor deficient mice show increased food-seeking behavior. Our finding that recent weight change was negatively correlated with dopamine synthesis capacity in

the caudate nucleus suggests a potential dopaminergic correlate of dynamic weight regulation and is particularly compelling in light of previous findings that obese individuals have decreased BOLD activation in the caudate nucleus during milkshake consumption compared to lean individuals. Taken together, these data suggest that a neural signature related to BMI across the normal to obese range includes an overactive tonic dopamine system with impaired regulation by D2 receptors.

Keywords: Dopamine, Obesity, Neuroreceptor imaging
Disclosures: Nothing to disclose.

W46. Pattered Feeding Promotes Food Addicted Behaviors

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Background: Obesity and associated metabolic complications such as type-2 diabetes (T2D) represent the foremost unmet health concerns in our country. Increased body weight gain can stem from "food addicted behaviors" such as increased motivation to obtain palatable foods, hedonic food intake in the absence of caloric need and loss of behavioral control over food. Notably, it is now clear that a shared set of neural circuitry regulates the reinforcing properties of both palatable food and drugs of abuse. Binge eating disorder (BED) is a particular type of pathological feeding condition character-ized by enhanced attention to palatable food, repeated consumption of unusually large amount of food in a short period of time and loss of control over food intake. Consequently, these anatomical observations combined with aforementioned behavioral manifestations have led to the inclusion of BED as an addictive behavior. New evidence indicates that BED may be controlled by psychological factors or inherent genetic traits. We hypothesize that patterns of feeding which include caloric overconsumption and voluntary food restriction promote the emergence of food addicted behaviors that initiate and maintain pathological feeding conditions.

Methods: In the present study, we utilized a rodent model to examine the effects of patterned feeding on a variety of food addicted behaviors. Specifically we evaluated food-reinforced behavior, attention to palatable food and compulsive food seeking behavior. We also measured accompanying neuroen-docrine changes following acquisition of the patterned feeding regimen. To accomplish this we used two separate groups of male Long Evans rats: group 1 received two hour (2hr) restricted access to a nutritionally complete High Fat Diet (HFD; 4.54 kcal/gm) every day (HFD-ED), group 2 received intermittent access to HFD every third day (HFD-3D) and group 3 (Controls) received standard rodent chow (3.4kcal/gm) for a six week period. All groups had unlimited access to chow and water throughout the study. Following six weeks, all rats underwent operant conditioning for sucrose using a rodent touch screen system. This system allowed us to measure acquisition and expression of operant responding, attention for sucrose and responding under extinction conditions. We also measured compulsive food seeking

behavior using a light-dark box apparatus and plasma levels of the neuroendocrine peptides ghrelin, Glucagon-like peptide-1 (GLP-1) and glucose.

Results: Both HFD-3D and -ED rats consumed equivalent amounts of HFD during 2hr feeding sessions and notably both groups decreased chow intake in anticipation of HFD delivery. Interestingly, the HFD-3D group displayed sustained increases in total caloric intake (HFD + chow) and voluntary food restriction from chow when HFD was not present. The HFD-ED group displayed acute caloric overconsumption which was not sustained but did voluntary restrict intake from chow throughout the study. Importantly, these patterns of feeding were not associated with body weight gain or increases in body fat. When tested for food-reinforced behavior, both HFD-3D and -ED group displayed enhanced acquisition of operant responding, made fewer errors to obtain sucrose and took longer to extinguish this response. In addition, both groups were more willing to enter an aversive context to obtain HFD relative to controls. Neuroendocrine profiles indicated that plasma levels of acyl-ghrelin and glucose were elevated at multiple time points beginning 1 hour prior to HFD delivery.

Conclusions: The present data suggest that patterns of feeding that include intermittent or restricted access to a palatable food and voluntary caloric restriction from a healthy alternative promote food addicted behaviors that are accompanied by critical physiological changes. The observed increases in acyl-ghrelin, a gut-peptide known to regulate palatable food and drug intake, present a plausible mechanism regulating both the maintenance of patterned feeding and the emergence of food addicted behaviors. The fact that we also observe increased plasma glucose suggests that patterned feeding can produce dysregulated homeostatic control of blood sugar prior to body weight gain, an important consideration for the maintenance of pathological feeding conditions. Importantly, these combined data indicate that behaviors and physiological perturbations which preclude feeding pathologies can be derived from feeding itself independent from body weight gain. In this regard, T2D patients who display similar patterns of feeding to that presented here are the most likely population to develop pathological feeding conditions such as BED. Therefore the present data unveil the power of pattered feeding to provoke behavioral changes and suggest that feeding behavior could be a critical variable amenable to intervention for patients at risk for developing BED.

Keywords: Eating Disorders, Food Addiction, Binge Eating Disorder, Obesity, Type-2 Diabetes

Disclosures: Nothing to disclose.

W47. Altered Neural Processing of Aversive Interoceptive Stimuli in Adult Women Recovered from Anorexia Nervosa

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Background: A growing body of research suggests that the neural regions and circuits key to interoceptive awareness and processing may be abnormal among individuals ill with or recovered from anorexia nervosa (AN). Several functional imaging studies have reported aberrant neural responses to rewarding or pleasant stimuli in AN, yet relatively little is known about how the brains of individuals with the disorder process unpleasant interoceptive stimuli. As many behaviors specific to AN (e.g., self-starvation) result in sensations that would be unpleasant to individuals without an eating disorder, cued aversive interoceptive stimuli may improve our understanding of the pathophysiology of AN. In the present study, we examined the neural correlates of the anticipation, delivery, and termination of aversive breathing restriction in women recovered from anorexia nervosa (RAN) relative to healthy controls (HC). Only adults who were remitted from AN were studied to avoid the confounding effects of malnutrition and underweight. We hypothesized that, as we previously observed using a thermal pain paradigm (Strigo et al., 2013), RAN participants, compared with controls, would demonstrate increased activation in inhibitory control regions and the anterior insula during anticipation of the aversive stimulus, but that the RAN group would show decreased posterior insular activation and increased prefrontal activation in response to the aversive stimulus. We also predicted that the RAN group would show decreased insular and increased prefrontal activations even after the aversive stimulus was terminated.

Methods: We used fMRI to measure brain activation during an event-related, intermittent breathing load restriction paradigm in 16 RAN and 18 HC females. This task includes three conditions of interest: anticipation of breathing load, 40 cm H20/L/sec inspiratory breathing load, and termination of breathing load. Whole-brain voxelwise contrasts examined whether neural response to each condition differed between groups (cluster threshold = 31 contiguous voxels, blur = 4.2, p < 0.05 corrected using Monte Carlo simulations for multiple comparisons).

Results: The RAN group showed decreased activation in left inferior (t = -2.44) and superior (t = -2.43) frontal gyri during breathing load anticipation, but no group differences in the insula were detected during stimulus anticipation. As predicted, during the breathing load, the RAN group showed reduced activation in right posterior insula (t = -2.46) and increased activation in both left (t = 2.47) and right (t = 2.34) inferior frontal gyri. After breathing load termination, the RAN group showed increased activation compared to controls in several frontal regions: bilateral superior frontal gyrus (left t = 2.50, right t = 2.31), left dorsolateral prefrontal cortex (t = 2.28), and right anterior (t = 2.24) and posterior cingulate (t = 2.40).

Conclusions: We extended our prior findings by demonstrating that anticipation, receipt, and termination of an aversive interoceptive stimulus are associated with aberrant brain activation in RAN. We did not find evidence in RAN of an increased insular response during the anticipation of a non-painful aversive stimulus, but instead, as has been found among individuals with anxiety disorders during anticipation of aversive visual stimuli, the RAN group showed reduced prefrontal activation during breathing load anticipation. This reduced activation may contribute to well-documented elevations in harm avoidance in ill and RAN. Consistent with prior pain findings, aversive inter-

oceptive stimulus processing in RAN was associated with increased activation in inhibitory control regions and reduced activation in the posterior insula, a region implicated in visceral and sensory information processing. Whether attenuated insular activation in RAN depends upon higher-order downregulation is not yet known; nonetheless, this pattern of activation in response to unpleasant experiences may underlie the deficits in accurate body signal perception, elevated pain threshold, and behavioral inhibition characteristic of AN. Further, as prior studies have documented reduced insular activation in RAN in response to receipt of palatable solutions, our results provide additional evidence that AN is associated with altered processing of both pleasurable and aversive stimuli. Increased activation in RAN in inhibitory control and working memory regions even after an aversive stimulus has stopped may indicate cognitive processes further contributing to overcontrol in AN. These preliminary results, if replicated in ill AN, may have important implications for treatment and suggest either a trait-based response to aversive interoceptive stimuli or a brain-based abnormality resulting from AN that could contribute to relapse.

Keywords: anorexia nervosa, fMRI, Insula, interoception
Disclosures: Nothing to disclose.

W48. Modeling Anorexia Nervosa Using Human iPS Cells

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Background: Anorexia nervosa (AN) is a highly heritable, neurodevelopmental disorder affecting primarily women with post-pubertal onset. Psychiatric disorders like AN are most likely syndromes with multiple genetic contributions. Patients face consequences such as high morbidity, mortality and no treatments to reverse symptoms. Without access to the tissue of interest, cellular and molecular mechanisms underlying Anorexia have remained out of reach. Prior studies have implicated a network of risk-conferring genes involved in dopamine (DA) neurotransmission. Additionally, functional neuroimaging studies reveal a dysfunctional dopaminergic reward circuit in AN. Human induced pluripotent stem cells (hiPSCs) provide experimental access to the disease-risk conferring genetic complement facilitating both hypothesis-based and discovery-oriented studies.
Methods: In order to model this disease and to reveal cellular mechanisms in the eating disorders field, we established iPSCs from 4 AN patients and 2 healthy controls by transducing skin fibroblasts with four retroviral reprogramming vectors (Sox2, Oct4, c-Myc, Klf4). iPSCs were characterized and showed that we could successfully re-establish the pluripotent state at the molecular and cellular levels. Moreover, iPSCs differentiated into cortical neurons in the same manner as unaffected counterparts.
Results: We present results from our neuronal studies, using RT-PCR from human neurons that showed differ-

ential expression of dopamine genes in AN versus unaffected controls. However, a commercially available PCR-based screen of 84 neurotransmitter pathway genes did not provide us with any differential expression signals. To screen for novel genes, we assayed differential neuronal expression at the whole exome level using RNAseq. Clustering algorithms applied to differentially expressed genes ($p < 0.01$) segregated AN from controls. For the first time, we report differentially expressed transcripts from AN neurons and controls.
Conclusions: The establishment of AN-specific iPSCs and neural cells makes it possible to investigate the effects of risk-conferring genetic variations on molecular pathways and cellular networks in Anorexia nervosa. Differences in gene sets as well as methodology are likely contributors to the results from our PCR screens. Our study demonstrates the feasibility of applying human iPSC technology to eating disorders and offers a foothold for drug discovery.
Keywords: anorexia nervosa, Induced pluripotent stem cells (iPSCs), gene expression
Disclosures: Nothing to disclose.

W49. Activation of the Corticoaccumbens Circuit Attenuates Inherent Impulsivity and Binge Intake of High Fat Food

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Background: Eating is essential for life, but repeated consumption of large amounts of food in a brief period (i.e., bingeing) can alter the reward value of food and food-related cues and fuel binge-eating cycles. Impulsivity, a predisposition toward rapid unplanned reactions to stimuli, is one of the multifaceted determinants underlying the etiology of dysregulated eating, its pathogenesis, and treatment outcomes. The medial prefrontal cortex (mPFC) is a major neural director of reward-driven behavior, impulse control and integration of internal states with environmental cues. Compromised signaling between the mPFC and nucleus accumbens (NAc) is thought to underlie the cognitive inability to withhold prepotent responses (impulsive action) and binge intake of high fat food. We propose that direct activation of the ventral infralimbic (IL) to nucleus accumbens shell (NAcSh) pathway will suppress impulsive action and binge eating of high fat food.
Methods: We employed a dual viral vector technology that allows for the targeted and isolated modulation of IL mPFC neurons that project to the NAcSh via a Cre-loxP system. A Cre-dependent viral vector based "double-floxed" inverted open reading frame (DIO) switch system expresses an engineered Gq-DREADD which only binds clozapine-N-oxide (CNO). In the presence of Cre, the loxP sites are excised and the transgene is inverted into the sense direction and expressed from the hSyn promoter. An AAV DIO construct that contains an inverted version of Gq DREADD (hM3D)-mCherry or mCherry alone was infused into the IL mPFC. A canine adenovirus-2 (CAV)-Cre axonal retrograde viral vector was infused into the NAcSh of the

same rat; stable transgene expression in IL mPFC occurred only at the site of DIO vector infusions thus restricting expression to cortical neurons that project to the NAcSh.

Results: Activation of the circuit with DIO-hM3D-mCherry AAV in the presence of CNO significantly suppressed impulsive action in the 1-choice serial reaction time task (p<0.05); no differences in task acquisition, accuracy, omissions or additional task parameters were observed. Administration of CNO in the DIO-hM3D-mCherry-AAV rats significantly decreased binge intake for high fat food (p<0.05).

Conclusions: These data indicate that IL mPFC to NAcSh pathway serves as a 'brake' over impulsive action and binge eating. Through addressing a fundamental gap in our knowledge of how the neural aspects of impulsivity relate to binge eating, we hope to develop pharmacological strategies to minimize binge eating and enhance clinical practice for disorders of overeating.

Keywords: impulsivity, binge-eating, corticoaccumbens circuit, DREADD

Disclosures: Nothing to disclose.

W50. Kappa Opioid Receptor Activation Disrupts Behavioral Inhibition in Schedule-Controlled Tasks

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Background: Dysregulation of the dynorphin/kappa opioid receptor (KOR) system has been implicated in the pathologies observed in substance use disorders and affective disorders. In humans, pharmacological KOR activation is hallucinogenic and disrupts cognition. A fundamental component of cognitive tasks is the development and maintenance of temporal associations between stimuli to properly guide responding. The first goal of the current studies is to determine whether disruptions in cognition induced by KOR activation are due to failures in inhibitory control of behavior or temporal estimation. Specifying the nature of cognitive disruptions induced by KOR activation could benefit therapeutic interventions in substance use disorders and affective disorders. The second goal of the studies presented here is to determine whether the cognitive disruptions induced by KOR activation could be attributed to β-arrestin signaling mechanisms. Our lab has shown that the β-arrestin/G-protein receptor kinase (GRK3)/p38α mitogen activated protein kinase (MAPK) signaling pathway is required for KOR-mediated dysphoria. Understanding whether KOR-mediated cognitive disruptions are arrestin-dependent could guide the development of pharmacological interventions using KOR agonists and antagonists in a variety of clinical settings.

Methods: Male C57Bl/6 mice were trained in a differential reinforcement of low rates of responding (DRL) task or fixed interval (FI) task for food reward. In the DRL task, mice were required to withhold responding during an unsignaled, fixed time period (15s) following a reinforced response. Optimal responses in the DRL task required interresponse times (IRT) greater than 15s, and premature or 'incorrect' responses in the DRL task led to reset of the

15s wait period. Following 4-6 weeks of training in the DRL task, mice reached criterion levels of responding, where >50% of the total responses were reinforced or 'correct' responses. Mice were then tested for the effect of chronic stress or KOR activation in the DRL task. In the FI task, mice were reinforced for responses occurring 20s following the previous reinforcer, but premature responses had no programmed consequence. Once mice reached >60 reinforced responses during a session (4-6 weeks training), animals were tested for effects of KOR activation. To measure the contribution of KOR to stress-induced disruptions of DRL performance, mice received repeated forced swim stress with or without norBNI (a kappa antagonist). Mice were tested in the DRL task with U50,488 (a prototypical KOR agonist) at 5 mg/kg or Salvinorin A (a recreationally used KOR agonist) at 1 mg/kg administered intraperitoneally immediately prior to testing. Mice were tested in the FI task with U50,488 (5 mg/kg). To determine whether cognitive disruption is arrestin-dependent, GRK3 knockout mice and littermate controls were tested in FI and DRL tasks using U50,488 (5-10 mg/kg).

Results: Repeated forced swim stress significantly increased the percentage of incorrect responses in the DRL task and this effect was blocked by KOR antagonism. KOR activation with U50,488 and Salvinorin A also significantly increased the percentage of incorrect responses in the DRL task. The increase in premature response percentage was attributed to increased perseverative responses, as the majority of errors occurred during the 0-3s IRT interval. U50,488 treatment also decreased efficient response percentage in the FI task, and the effect was primarily observed in the 0-3 IRT interval. DRL performance was not affected by U50,488 in GRK3-/- mice.

Conclusions: These results demonstrate that KOR activation, via stress or pharmacological treatment, disrupts performance in the DRL and FI task. These effects are hypothesized to be specific to inhibitory control of responding, rather than time estimation, as most errors occurred in bursts of responding at the beginning of wait period. Decreased behavioral inhibition has been proposed as a mechanism underlying substance abuse disorders, and the present study indicates these cognitive disruptions may be KOR mediated. These effects may be arrestin-dependent, as GRK3 knockout animals did not show disruptions in the DRL task. Future studies will further specify the cellular and neural substrates involved in KOR-mediated disruptions in cognition.

Keywords: kappa opioid receptor, p38 MAPK, chronic stress, Behavioral Pharmacology, Inhibitory control

Disclosures: Nothing to disclose.

W51. Psychophysiological Correlates of Escalating and De-Escalating Behavior during an Aggressive Interaction

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Background: Recurrent aggressive behavior in adults has been associated with abnormal structure and function

of the frontal lobes and limbic hyperreactivity to emotional stimuli. Together, frontal and limbic structures support a variety of functions that are relevant to aggressive behavior including emotion regulation, cognitive control, and social cognition. One function of the frontal lobes is to support error monitoring, which reflects the identification of differences between a correct and incorrect response in order to adjust subsequent behavior. Error monitoring has been linked to activity in the anterior cingulate cortex and is evident in electrophysiological (EEG) studies in the error-related negativity (ERN) component of the event-related potential (ERP) that is evident approximately 50-100 ms after an error. This study examines the evidence for error monitoring during an aggressive interaction.

Methods: A well-validated standardized laboratory paradigm for simulating an aggressive interaction was adapted for use with EEG. Over the course of several trials, research participants were provoked by a (fictitious) opponent with a threatened electric shock at varying intensity levels while scalp EEG was recorded from 128 electrodes. On certain trials the participant could retaliate by setting a level of shock of their choosing. Eight healthy participants (male = 5, female = 3) with no history of psychiatric disorder and three aggressive participants (female = 3) diagnosed with intermittent explosive disorder or borderline personality disorder completed the task. Peak ERN amplitude was measured in the response-locked ERP. We compared trials in which the participant escalated against the opponent (setting a higher shock level than the opponent), de-escalated, or matched the opponent's shock level. It was hypothesized that ERN would be larger on trials in which the participant escalated and that this effect would be localized to anterior cingulate cortex.

Results: On average across subjects, the effect of escalation on ERN amplitude was marginally significant, $F[2,20] = 3.25$, $p = .06$. Escalating aggressive responding was associated with larger ERN amplitude at the Cz electrode (M = -5.5, SD = 4.7) compared to de-escalating responding (M = -3.0, SD = 2.7) and matching behavior (M = -4.5, SD = 2.2). Permutation testing of ERN amplitude across 128 channels further revealed a significant difference between the escalated and de-escalated conditions with the maximum difference around the Cz electrode ($p < .05$). Source localization using CLARA identified a source in the anterior cingulate.

Conclusions: The current finding provides evidence of error monitoring during an aggressive interaction, with participants showing possible error-related activity (ERN) following aggressive responses. Furthermore, this effect showed similar localization to previous studies of error detection. The results suggest a possible pathway by which individuals may develop recurrent, problematic aggression. Future research is needed to understand the behavioral consequences of error detection during an aggressive interaction and possible individual differences that may help to explain the tendency toward pathological aggression.

Keywords: aggression, anterior cingulate, cognitive control

Disclosures: Nothing to disclose.

W52. Early-Life Experience Reprograms Stress-Sensitive Neurons and Influences Adult Phenotype via NRSF/REST-Dependent Epigenetic Mechanisms

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Background: Human emotional phenotypes are controlled by both genetics and environment. Experience, particularly during sensitive periods early in life, leaves indelible marks on an individual's resilience or vulnerability to stress-related emotional disorders. There is evidence that early-life experiences influence the function of brain neurons involved in these crucial behaviors by modifying neuronal gene expression via epigenetic processes. However, it is not known how early-life experiences signal to specific brain cell populations and how these signals influence the orchestrated programs of gene expression that mediate phenotypic resilience or vulnerability.

Methods: Here we capitalized on observations that a resilience-promoting early-life experience–augmented maternal care—reduces the numbers and function of glutamatergic synapses onto stress-sensitive hypothalamic neurons and represses expression of the stress-sensitive gene, CRH (Korosi et al., 2010). In hypothalamus in vitro, we found that reduction of glutamatergic receptor activation sufficed to recapitulate the effects of augmented maternal care on CRH repression. This effect required enhanced expression and recruitment of the transcriptional repressor REST/NRSF to the CRH gene. NRSF binding was accompanied by epigenetic changes to histones and DNA in immature and adult rats experiencing augmented maternal care. NRSF ChIP-seq analyses identified the gene networks that, in addition to CRH, contribute to the phenotypic changes initiated by the neonatal experience.

Results: In hypothalamus in vitro, we found that reduction of glutamatergic receptor activation sufficed to recapitulate the effects of augmented maternal care on CRH repression. This effect required enhanced expression and recruitment of the transcriptional repressor REST/NRSF to the CRH gene. NRSF binding was accompanied by epigenetic changes to histones and DNA in immature and adult rats experiencing augmented maternal care. NRSF ChIP-seq analyses identified the gene networks that, in addition to CRH, contribute to the phenotypic changes initiated by the neonatal experience.

Conclusions: The current studies are the first to causally connect neonatal environmental sensory experiences, synaptic modulation and epigenetic processes within select neuronal populations. They provide a novel mechanistic pathway from early-life experience to phenotypic outcomes that govern human health and disease.

Keywords: Epigenetic, resilience, Neuroplasticity, stress, Neuronal Epigenome

Disclosures: Nothing to disclose.

W53. The Effects of Gabapentin and Pregabalin in the Consolidation and Reconsolidation of Auditory Threat Memory in Rats

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Background: Exposure to traumatic events is a common experience, with prospective studies indicating that post-traumatic stress disorder (PTSD) symptoms are almost universal in the immediate aftermath of trauma. The majority of individuals will have symptoms of re-experiencing, avoidance, and hyper-arousal initially following the trauma that will extinguish over time. For some individuals, however, their symptoms persist and cause impairment in functioning, which leads to a diagnosis of PTSD. Unfortunately, little progress has been made in identifying interventions that prevent trauma survivors from developing PTSD or ameliorate symptoms once PTSD has appeared. Clinicians still need a treatment strategy that can alleviate normal trauma reactions, decrease rates of PTSD or treat this incapacitating disorder.

In daily life, stimuli acquire significance as threats by their association with harmful events, in other words, through Pavlovian conditioning. These conditioned threats elicit automatic reactions that are called conditioned responses (CR), such as freezing behavior, increased heart rate and blood pressure, and the release of stress hormones. Threat conditioning is a good experimental paradigm by which to study the neural mechanisms engaged in the emotional learning and memory processes. In this procedure a neutral conditioned stimulus (CS), often a tone, is paired with an aversive unconditioned stimulus (US), typically a footshock. This paradigm is widely used as a model system for understanding how the brain forms and stores information about aversive emotional experiences. Moreover, alterations of fear learning, processing and memory seem to account for the appearance of PTSD, hence drugs that prevent the consolidation of threat memories are potential targets for preventing PTSD.

Clinical epidemiologists has recently suggested expanding the notion of translational research to include bidirectional bedside to bench translational epidemiology in order to inform more tractable intervention approaches. These epidemiologic methods have determined which medications with strong theoretical support in animal models for the secondary prevention of PTSD, are already in widespread use in trauma center settings. The most highly used drugs are the opiate and the non-opiate analgesics. Opiates have been identified as potent anxiolytics in animal models, and pain responses appear to be regulated in part by centrally mediated catecholamine metabolism. Also, opiates may prevent memory consolidation through a beta-adrenergic mechanism. Adequate levels of opiate pain control are associated with the development of lower PTSD symptom levels among burn injury survivors who are children. Thus opiate analgesics may be important agents to consider for future acute care efficacy trials targeting PTSD prevention. The use of narcotic analgesics may be very problematic in most of the populations receiving emergency care after a traumatic event, thus compounds with combined analgesic and anxiolytic properties but without a narcotic profile may hold promise as early PTSD-preventive agents. Gabapentine and Pregabalin are two drugs that accomplish these properties but they have never been tested in auditory threat conditioning. Testing the effect of these drugs in the consolidation of threat memories should be the first step to in order to elucidate their potential effect in preventing and/or treating PTSD.

Methods: Rats are habituated to the conditioning context for 15 min, as well as to handling and weighing. Threat conditioning will occur the next day, during which rats will be trained in a single conditioning trial consisting of two pairings of a tone (5 kHz, 80 dB) with a footshock (0.5 sec, 0.7 mA). Immediately after conditioning animals will be intra-peritoneally injected with the different doses of Gabapentine and Pregabalin or its vehicle. Afterwards rats will be returned to their home cage. Long term memory test will be performed 24 hours after conditioning with 10 presentations of the 20-sec tone alone in a modified context.

Results: Rats treated with Gabapentine and Pregabaline showed significantly less freezing during long-term memory test than the rats treated with its vehicle, thus suggesting an impairment of threat memory consolidation.

Conclusions: These results may represent the first step to acutely treat patients after a trauma with drugs that prevent the development of subsequent trauma-associated psychiatric disorders.

Keywords: PTSD, Threat Conditioning, Trauma exposure, Gabapentin, Pregabalin

Disclosures: Nothing to disclose.

W54. Withdrawn

W55. CorrECTing ConnECTomes: Brain Network Correlates of ECT Treatment and Response

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Background: Previous work by our group and others using multimodal neuroimaging techniques have demonstrated that major depression is associated with network-level alterations such as reduced network efficiency, altered hub organization, and reduced network resilience. The goal of the present study was to determine whether electroconvulsive therapy (ECT) can correct or compensate for network-level alterations observed in major depressive disorder (MDD).

Methods: We examined 68 participants (42 diagnosed with MDD and 26 healthy comparison (HC) subjects) who underwent resting-state functional magnetic resonance imaging (rs-fMRI) scanning. MDD participants were scanned before and after the ECT series. HC subjects were scanned at a similar interval. Functional connectomes were generated from the rs-fMRI data. Global and nodal graph theory based metrics were calculated (efficiency, betweenness centrality, modularity). We examined the effect of

disease (MDD vs. HC), effect of ECT treatment (time 1 vs time 2), and correlations with ECT treatment response.

Results: Depressed subjects had significantly reduced global efficiency compared to HC (p = 0.025), however this difference remained significant after ECT (p = .003). Baseline nodal efficiency in the frontal pole (r = .42, p = .007) and right inferior gyrus (r = 0.4, p = .01) was significantly associated with treatment response. Hub strength (measured with betweenness centrality) in the left hippocampus (r = .44, p = .004) and right supracalcarine cortex (r = -.47, p = .002) were significantly associated with treatment response. Modularity analyses revealed altered community structure in the bilateral inferior temporal gyrus of depressed patients that was not present after ECT (p < .001).

Conclusions: These results demonstrate that while global brain network properties in depression remain state-independent, altered local network properties can be improved with ECT and correlate with treatment response. Thus, these local brain network alterations may serve as potential neuroimaging-based biomarkers for ECT response.

Keywords: neuroimaging, electroconvulsive therapy, connectome, major depression

Disclosures: Nothing to disclose.

W56. Lurasidone Adjunctive to Lithium or Divalproex for Prevention of Recurrence in Patients with Bipolar I Disorder: Results of a 28-Week, Randomized, Double-Blind, Placebo-Controlled Study

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Background: Bipolar disorder, with a lifetime prevalence of approximately 4.4%, is a chronic and often disabling condition with a highly recurrent course of illness. This study was designed to evaluate the relapse prevention efficacy and safety of lurasidone (in combination with lithium or divalproex) for the maintenance treatment of bipolar I disorder.

Methods: This study consisted of an open-label stabilization phase (12-20 weeks) followed by randomization to a 28-week, double-blind, placebo-controlled phase. Patients randomized to lurasidone were treated with flexible doses of 20-80 mg/d adjunctive with lithium or divalproex during both phases; patients randomized to placebo continued to receive adjunctive lithium or divalproex. Patients who met DSM-IV-TR criteria for bipolar I disorder were enrolled if they had ≥1 manic, mixed manic, or depressed episode in the past 2 years, and a current YMRS or MADRS total score ≥14 (if on lithium or divalproex), or ≥18 (if not on lithium or divalproex). Patients were randomized to the 28 week double-blind phase if they achieved consistent clinical stability, defined as MADRS and YMRS total scores ≤12 for ≥12 weeks; clinical worsening on one visit was permitted (YMRS ≤13; MADRS ≤14), except during the last 4 weeks prior to randomization. Time to recurrence of any mood event (primary efficacy endpoint) was evaluated from double-blind baseline; recurrence was defined as meeting any of the following 5 criteria: (1) meets DSM-IV-TR criteria for manic, mixed manic, hypomanic, or depressive episode; (2) requires treatment for a mood episode; (3) requires hospitalization for a mood episode; (4) YMRS or MADRS total score ≥18, or CGI-BP-S score ≥4 at 2 consecutive assessments; or (5) discontinuation from the study because of a mood event. Cox proportional hazards model was used to assess the hazard ratio of time to recurrence of any mood disorder (primary outcome), and a depressive episode, between the two treatment groups. Kaplan-Meier estimates of the probability of time to recurrence were also calculated.

Results: A total of 496 patients met stabilization criteria and were randomized to adjunctive lurasidone (N = 246) versus adjunctive placebo (N = 250). Patient characteristics at double-blind baseline were comparable for lurasidone and placebo for mean age (45.7 vs 43.2 years), male (44.7% vs 42.8%), index episode of depression (58.5% vs 47.6%), mean MADRS total score (4.0 vs 4.1), and mean YMRS score (2.2 vs 2.2). Patients in the adjunctive lurasidone group had a numerical longer time to recurrence of any mood event during 28 weeks of treatment compared with patients in the adjunctive placebo group, with a hazard ratio [95% CI] of 0.71 [0.49, 1.04] (P = 0.078), indicating a 29% reduction in recurrence risk (primary a priori Cox proportional hazards model analysis). Kaplan-Meier estimates of the probability of time to recurrence of any mood event at 28 weeks were 20.9% for the adjunctive lurasidone group and 51.5% for the adjunctive placebo group (log-rank test, P = 0.055). Time to recurrence of a depressive episode was significantly longer for lurasidone versus placebo, with a hazard ratio [95% CI] of 0.57 [0.34, 0.97] (P = 0.039). The mean daily dose of lurasidone during the double-blind phase was 54.4 mg. All-cause discontinuation rates were 32.5% for lurasidone and 40.0% for placebo. The most frequent adverse events (incidence ≥5% during the double-blind phase of the study) for the adjunctive lurasidone versus placebo groups were: weight increased (9.8% vs 5.2%), headache (8.5% vs 7.2%), nasopharyngitis (6.1% vs 4.8%), and tremor (6.1% vs 4.4%).

Conclusions: In this double-blind study of patients with a bipolar I diagnosis who had been stabilized on lurasidone plus lithium or divalproex, 28 weeks of continued treatment with adjunctive lurasidone was associated with a trend-significant risk reduction in time to recurrence of any mood event compared with placebo plus lithium or divalproex, and a significant reduction in time to recurrence of a depressive episode. Clinicaltrials.gov: NCT01358357

Keywords: Bipolar I Depression, Recurrence risk, Atypical antipsychotics

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Partners, Hoffman LaRoche, Lilly, Lundbeck, Otsuka, Scientia, Sunovion, Takeda, and Teva.
Drs. Pikalov, Cucchiaro, Xu, Loebel; and Ms. Streicher are employees of Sunovion Pharmaceuticals.

W57. A Novel Study Design to Evaluate the Rapid Reduction of the Symptoms of Major Depressive Disorder, Including Suicidal Ideation, in Subjects Assessed to Be at Imminent Risk for Suicide

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Background: Major depressive disorder (MDD) is associated with an elevated rate of mortality, primarily due to suicide. The risk of suicide in those with MDD is about 20 times that of the general population, with approximately half of all suicides occurring in depressed individuals. While conventional antidepressants are often effective in treating depressive symptoms including suicidal ideation (SI), their delayed onset of action significantly limits their utility in the treatment of patients with MDD who are at imminent risk of for suicide. Recently, several studies of ketamine and esketamine have demonstrated that these agents can improve symptoms of depression in individuals with MDD within hours of administration. Additionally, preliminary studies of ketamine suggest it may have a similarly rapid effect in significantly reducing SI in subjects with MDD. As such, Janssen R&D is developing intranasal esketamine for the rapid reduction of the symptoms of MDD, including SI, in patients who are assessed to be at imminent risk for suicide. The pursuit of this novel indication has required the development of an innovative study design that allows for the detection of rapid symptom change, and addresses the safety and ethical concerns of studying an acutely suicidal patient population. This poster will discuss the design and implementation of PeRSEVERe (ESKETINSUI2001), an ongoing proof-of-concept study to evaluate the efficacy and safety of intranasal esketamine in subjects with MDD who are assessed to be at imminent risk for suicide.

Methods: PeRSEVERe is an ongoing 12-week, randomized, double-blind, placebo-controlled, multicenter Phase 2 study of intranasal esketamine in 70 adult subjects with MDD who are assessed to be at imminent risk for suicide. Eligible subjects must have active suicidal ideation and intent, and be in need of psychiatric hospitalization. The primary objective is to evaluate the efficacy of intranasal esketamine 84 mg compared with intranasal placebo in reducing the symptoms of MDD, including SI, in subjects who are assessed to be at imminent risk for suicide, as measured by the change from baseline on the MADRS total score at 4 hours post-dose on Day 1. Secondary efficacy objectives include the assessment of single and repeated doses of intranasal esketamine compared with intranasal placebo on the clinician's assessment of suicide risk as measured by the Clinical Global Judgment of Suicide Risk from the Suicide Ideation and Behavior Assessment Tool, and the subject's report of the severity in suicidal ideation as measured by the Beck Scale for Suicide Ideation, through the end of the

double-blind treatment and follow-up phases. Additionally, the study evaluates response rates ($\geq$ 50% reduction in MADRS total score from baseline, with onset by Day 1 sustained through the end of the double-blind treatment phase) across treatment groups. Safety objectives include the assessment of dissociative and psychosis-like symptoms, sedation, nasal tolerability, vital signs and suicidal thinking and behavior.

The study consists of a 24-48 hour screening evaluation performed prior to the Day 1 intranasal dose, immediately followed by a 25-day double blind treatment phase, and a 56-day follow up phase. Given the vulnerability of the patient population, the study is being conducted in the context of standard clinical care, with all subjects receiving standard antidepressant medication and initial in-patient hospitalization.

Results: PeRSEVERe is the first large-scale, prospective, placebo-controlled trial of a rapidly acting antidepressant in subjects with MDD who are assessed to be at imminent risk for suicide. The study is being conducted in the United States and is currently ongoing, with over 70% of subjects enrolled. Details and rationale for the study design, outcome measures, and key efficacy times points will be discussed. Additionally, key operational insights and challenges unique to the study of a rapidly acting antidepressant in a suicidal patient population will be highlighted.

Conclusions: PeRSERVERe employs innovative clinical trial methodology in a Phase 2 study for a novel indication, in a vulnerable patient population – i.e. the rapid reduction of symptoms of MDD, including SI, in subjects assessed to be at imminent risk for suicide. Should this approach prove successful in Phase 2, similar methods will be considered in the design of subsequent pivotal trials of esketamine, and possibly other rapidly acting antidepressants. Results of PeRSERVERe are expected to be available in 2016.

Keywords: Rapid Antidepressant, Suicide Assessment, Clinical Trial Design, Esketamine

Disclosures: All authors are full-time employees of Johnson & Johnson (parent company of Janssen R&D and Janssen Pharmaceuticals).

W58. Ventral Tegmental Area Muscarinic Acetylcholine Receptor Mechanisms Mediating Responses to Stress and Anxiety in Preclinical Models

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Background: Recent clinical and preclinical evidence strongly suggests that dopaminergic and cholinergic mechanisms likely play an important role in the pathogenesis of depression. Muscarinic cholinergic antagonism has been shown to induce rapid antidepressant effects in depressed human subjects. Recent rodent studies have also suggested that phasic dopamine signaling in the ventral tegmental area (VTA) is an important substrate for antidepressant action. However, the role of VTA cholinergic mechanisms

in depression and in antidepressant efficacy is poorly understood - despite the evidence that cholinergic mechanisms in the VTA robustly regulate phasic dopaminergic activity in the mesolimbic system. In the current studies, we addressed the hypothesis that projections from the LDTg to VTA might mediate responses to stress and anxiety via VTA muscarinic receptor regulation of phasic dopamine signaling. To test these hypotheses, we used pharmacological, optogenetic, and electrochemical approaches in rats to identify the role of midbrain cholinergic mechanisms in depression-related behaviors.

Methods: All procedures were performed in male Sprague-Dawley rats. We examined whether VTA cholinergic mechanisms mediate depressive-like, anxiety-related and anhedonia-related behavior, as measured in the forced swim test (FST), elevated plus maze (EPM) and sucrose preference test (SPT). Rats first received stereotaxic surgery where guide cannula were chronically implanted above the VTA. After a 1 week recovery period, rats received VTA infusion of cholinergic drugs immediately prior to behavioral examination in either a 10 min FST (24 hours after a 15 min pretest), 5 min EPM test, or a 1 hr SPT (after 48 hr exposure to 1% sucrose). We also integrated in vivo optogenetic and voltammetric approaches to determine whether optical activation of LDTg terminals in the VTA was sufficient to evoke phasic DA release in the NAc. Here, an adeno-associated viral construct with channelrhodopsin and EYFP (ChR2-AAV-EYFP), was stereotaxically infused into the LDTg. After 6 weeks, an optical fiber was inserted above the VTA and a carbon fiber microelectrode was inserted into the NAc for voltammetric recordings. Phasic dopamine release in the NAc was then measured following optical stimulation of LDTg terminals in the VTA.

Results: VTA administration of the acetylcholinesterase inhibitor, physostigmine (0, 1, or 2 micro g/side), dose-dependently increased immobility time in the FST ($F(2,12) = 25.04$, $p < 0.001$), decreased open arm time in the EPM ($F(2,19) = 5.744$, $p < 0.01$) and decreased sucrose preference in the SPT ($F(2,43) = 3.228$, $p < 0.05$). Similar to physostigmine, selective activation of VTA muscarinic acetylcholine receptors (mAChRs) with pilocarpine (0, 3, or 30 micro g/side), increased immobility time in the FST ($F(2,20) = 9.654$, $p < 0.01$). In contrast, VTA administration of the mAChR antagonist, scopolamine (0, 2.4 or 24 micro g/side), decreased immobility in the FST ($F(2,14) = 14.08$, $p < 0.01$). We also examined the 24 micro g/side dose of scopolamine in the EPM and found that scopolamine increased open arm time (independent t-test, $p < 0.05$, 0 vs 24 micro g/side). Importantly, VTA administration of these cholinergic drugs did not alter general locomotor activity. In combined optogenetic and voltammetry neurocircuitry experiments, optical stimulation of LDTg terminals in the VTA was sufficient to evoke phasic DA release in NAc.

Conclusions: Our findings reveal that enhancing VTA acetylcholine tone or activating mAChRs, which is known to induce phasic DA activity, induces a pro-depressive, anxiogenic and anhedonic-like behavioral phenotype. In contrast, VTA mAChR blockade, which is known to decrease phasic DA activity in the VTA to NAc pathway, promotes antidepressant and anxiolytic-like effects. Together, the data suggest that AChR regulation of phasic DA activity in the VTA to NAc pathway may mediate responses

that may underlie depression. In ongoing studies, we predict that optical stimulation of LDTg terminals in the VTA, which was sufficient to induce phasic DA release in the NAc, will promote pro-depressive and anxiogenic behavioral responses via activation of VTA mAChRs.

Keywords: Scopolamine, Depression, Dopaminergic system, voltammetry, Fast-acting Antidepressant

Disclosures: Nothing to disclose.

W59. Susceptibility to Chronic Social Defeat Stress Is Associated with Profound Alterations of the Brain Pituitary Adenylate Cyclase-Activated Polypeptide (PACAP) System

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Background: Depressive disorders, including major depressive disorder (MDD), are often characterized not only by depressed mood but also by anhedonia, defined as diminished interest or pleasure in activities. Stressful life events are well-recognized triggers of psychiatric diseases, and many of these conditions are thought to stem from an individual's inability to cope with such events. A major goal of current research is to identify which factors determine vulnerability or resilience to chronic or traumatic stressors. The polypeptide pituitary adenylate cyclase-activating polypeptide (PACAP) and its receptor PAC1 (PAC1R) are key regulators of the stress response, and dysregulations of the PACAP/PAC1R system have been proposed to contribute to the etiology of anxiety, trauma-related and depressive disorders.

Methods: Here we used the chronic social defeat stress (CSDS) model in rats as a model of stress susceptibility and depression induction.

Results: CSDS produced in a subset of rats (named susceptible) an increase in anxiety-like behavior, a reduction in social interaction and in the consumption of a sweet solution, compared to control and resilient animals. Rats subject to chronic social defeat also showed significant reductions in body weight gain and increases in immobility time in the forced swim test. Using immunohistochemistry, we also found that exposure to CSDS caused significant alterations in PACAP expression levels in several brain regions, including the central nucleus of the amygdala, the paraventricular nucleus of the hypothalamus and the dorsal raphe nucleus. Interestingly, some – but not all - of these molecular changes were found to be dependent on stress susceptibility or resilience.

Conclusions: Our data suggest that specific neuroadaptations in the PACAP/PAC1 system may mediate the susceptibility to stress observed following exposure to CSDS, and therefore that this system may represent a novel therapeutic target.

Keywords: Social defeat stress, major depression, post-traumatic stress disorder, PACAP, neuropeptides, central nucleus of the amygdala

Disclosures: Nothing to disclose.

W60. Seasonal Variation in Serotonin Transporter Binding in Seasonal Affective Disorder and Health: A [11C]DASB Positron Emission Tomography Study

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Background: Seasonal affective disorder (SAD) affects 1 to 6% of individuals and prevalence rates are higher at more Northern latitudes. Serotonin transporter binding potential (5-HTT BPND), as measured with [11C]DASB positron emission tomography (PET), is an index of 5-HTT levels and 5-HTT BPND has been found to be higher across multiple brain regions in the fall-winter relative to the spring-summer; a finding replicated by four independent research groups. DASB binds preferentially to 5-HTT on outer cell membranes. As such, it is likely that changes in 5-HTT BPND observed in vivo using [11C]DASB PET reflect 5-HTT protein levels at the cell surface where serotonin re-uptake occurs. The purpose of this study was to determine if the magnitude of seasonal variation in 5-HTT BPND, an index of 5-HTT levels, was greater throughout the brain in individuals with SAD as compared to healthy volunteers. A secondary hypothesis was that seasonal change in 5-HTT BPND would positively correlate with severity in both the prefrontal and anterior cingulate cortices (PFC and ACC, respectively), which include brain regions involved in mood-regulation.

Methods: 12 SAD (7 women and 5 men, mean age: 31.8 [4.9] years) and 12 healthy participants (7 women and 5 men; mean age 28.4 [3.8] years) underwent [11C]DASB PET and MRI scans in both summer and winter, in randomized order, to measure seasonal change in 5-HTT BPND. All participants were non-smoking, had no history of major medical or additional psychiatric illness, nor history of substance abuse, and all were medication free. Severity of seasonality was measured with the Seasonal Pattern Assessment Questionnaire (SPAQ) to assess change in seasonal depressive symptoms applying a summed global seasonality score (GSS) to determine degree of seasonality (i.e. seasonal change in sleep, mood, energy, appetite, weight and social activity). Regions assayed included the PFC, ACC, thalamus, caudate, putamen, midbrain, striatum and hippocampus.

Results: In winter, as compared to summer, a global elevation in 5-HTT BPND was observed in SAD as compared to health (magnitude, 14.0% vs. -2.40%, MANOVA, $F(8,15) = 3.52$, $p = 0.03$). A similar trend was also detected in individual brain regions assayed (ANOVA, $F(1,22) = 4.35-6.01$, $p = 0.02-0.049$), excepting the midbrain and hippocampus. A positive correlation was observed between severity of SAD (measured with the SPAQ) and seasonal change in 5-HTT BPND which was significant in the PFC ($r = 0.76$, $p = 0.004$), and in the ACC ($r = 0.58$, $p = 0.046$).

Conclusions: Since greater seasonal fluctuation in 5-HTT BPND is associated with SAD, and the severity of SAD, this suggests that fluctuation in levels of 5-HTT, most likely in the PFC, represents a component of the phenotype of SAD. This has pathophysiological implications since overexpression of 5-HTT is associated with reduced extracellular serotonin and [11C]DASB has strong preferential binding for the 5-HTT on outer cell membranes. To our knowledge this is the first brain marker of SAD so future study should investigate markers of other phenotypes implicated in mood disorders.

Keywords: seasonal affective disorder, Positron emission tomography, Serotonin Transporter, prefrontal cortex, anterior cingulate cortex

Disclosures: Nothing to disclose.

W61. Exploration of the Interrelationship between Cytokines and Other Inflammatory Markers across MDD Cohorts

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Background: Increasing evidence suggests that immune dysregulation plays a role in the molecular pathophysiology of major depressive disorder (MDD). In post-mortem studies, elevated cytokines have been detected in brain tissues from MDD patients. In clinical studies, elevations in circulating pro-inflammatory cytokines have been noted in MDD over healthy control individuals and patients in remission, with plasma levels of CRP, TNF-alpha, IL6, and IL1-beta being most commonly studied and found to be elevated. Cytokine signaling plays several roles in the CNS, which include effects on neurogenesis, synaptic plasticity and stress-coping mechanisms that have been implicated in the molecular pathology and therapeutic mechanisms of MDD.

Preclinical studies indicate that peripheral cytokines can enter the CNS directly and also can impact CNS function indirectly through a variety of downstream targets. However, the peripheral source tissue and stimuli, and the interrelationship between the cytokines and other inflammatory markers in MDD remain largely unknown. In this study, we explore cytokines and other inflammatory marker measurements in various MDD cohorts and report on their correlation patterns across studies. To investigate the specific tissues in which each cytokine of interest is predominantly expressed, we also analyzed an RNAseq database for cytokine expression profiles.

Methods: Inflammatory marker measurements were collected from two phase 2 randomized controlled clinical trials investigating the efficacy of ketamine and esketamine in subjects with treatment resistant depression (N = 77) conducted by Janssen, the Netherlands Study of Depression and Anxiety (NESDA) database (N = 1090; Penninx et al., Int. J. Methods Psychiatr. Res. 2008), a clinical trial using the anti-TNF-alpha monoclonal antibody, Remicade (N = 52; Raison et al., JAMA Psychiatry, 2013), and the Lundbeck database (N = 374 MDD, N = 202 healthy

controls; Jones and Thomsen, Molecular and Cellular Neuroscience, 2012). CRP, TNF-alpha, IL6, IL1-beta, and SAA were the most commonly measured inflammatory variables in these datasets. Measurements were performed using individual ELISAs, except for the Lundbeck data set, in which a multiplex assay was used. In studies that included a therapeutic intervention, only the baseline (pre-treatment) inflammatory markers were assessed. Correlation analyses were performed within each study using a rank-based Spearman correlation metric to measure pair-wise co-expression of inflammatory markers. Significance was evaluated as $p < 0.05$. Results from each study were compared to identify correlations that were significant in multiple studies. Unsupervised hierarchical clustering was performed in order to assess co-expression patterns of multiple inflammatory markers within a study. The RNAseq database is comprised of expression data from 85 different tissues or cell types throughout the human body and was generated using the Illumina HiSeq platform internally or by the NIH GTEx project (version 4; Lonsdale et al., Nature Genetics, 2013).

Results: Although absolute values of the measurements varied within cohorts as well as across studies, IL6, CRP, SAA, and body mass index (BMI) were consistently and significantly correlated with one another ($r = 0.28$-0.54) in all studies. TNF-alpha was only weakly correlated with the corresponding IL6, CRP, SAA, or BMI measures ($r = 0.01$-0.18) in the three studies performed using uniplex assays. Stronger correlations among all inflammatory markers were observed in the Lundbeck data set in which a multiplex assay was run. However, the correlation between TNF□ and IL1□ was consistently stronger than between TNF-alpha and the IL6/CRP/SAA/BMI markers ($r = 0.25$ vs. 0.01-0.18, uniplex; $r = 0.87$ vs. 0.57, multiplex). The RNAseq expression data revealed that TNF-alpha is expressed primarily by lymphocytes, including B-cells and CD4 and CD9 positive cells. CRP and SAA are primarily expressed by liver, with sizable expression also measured in adipose tissue. IL6 is expressed in high levels in adipose tissue, heart and lung, and in extremely low levels in CD4 and CD8 positive cells. The tissue with highest IL1-beta expression is whole blood. The levels of expression of all three cytokines are comparatively low in CNS tissue.

Conclusions: This study revealed different interrelationships between IL6, TNF-alpha, IL1-beta, CRP, SAA, and BMI. The relationships could be explained in part by source, e.g. expressing tissue; however, further confirmation is needed. The stimuli leading to cytokine elevations in depression are still largely unknown. Given the lack of correlation between CRP and TNF-alpha, chronic "low grade" inflammation might not be the only explanation of the cytokine elevations in depression. One hypothesis arising from the current data is that subpopulations of MDD subjects could be defined by individual cytokine profiles.

Keywords: Major Depressive Disorder (MDD), cytokines, Inflammatory Markers, meta-analysis

Disclosures: LY, XY, GS, JP, JS, WD, GW, and GC are employees of Janssen Research & Development. FL, BP, and AM have received research support from Janssen Research & Development. CT is an employee of Lundbeck.

W62. Distinct Subpopulations of Nucleus Accumbens Dynorphin Neurons Drive Aversion and Reward

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Background: The nucleus accumbens (NAc) and the dynorphinergic system are widely implicated in motivated behaviors. Prior studies have shown that activation of the dynorphin-kappa opioid receptor (KOR) system leads to aversive, dysphoria-like behavior. KOR agonists induce place aversions, depression-like behavior, and dysphoria in both human and animal models. Notably, direct infusion of KOR agonists into the NAc mediates conditioned place aversion and local antagonism of KOR in this region prevents depression-like behaviors. It is thought that monoamine output within the NAc is tightly regulated via presynaptic KORs on serotonergic and dopaminergic cells in the region, acting to suppress dopamine and serotonin transmission. However, the mechanisms and role of endogenous dynorphin in the regulation of KOR-mediated negative affective behaviors remain unresolved.

Methods: We virally targeted channelrhodopsin-2 to striatal dynorphinergic neurons and photostimulated neuronal populations in both the dorsal and ventral NAc shell to measure aversion and reward behaviors using place preference, aversion, and operant conditioning. We also injected the KOR antagonist NorBNI to determine the mechanism. We implanted wireless μ-ILED devices which allowed directionally controlled light to specific NAc subregions. We used an opto-dialysis probe implanted in the NAc of mice injected with channelrhodopsin-2, to collect dialysate before, during and after stimulation to detect dynorphin. Samples were analysed using liquid chromatography-mass spectrometry (LC-MS) detection. We injected modified rabies virus and cholera toxin retrograde tracers in both the dorsal and ventral region NAc to trace the neuronal projections that may act to mediate the aversion and preference.

Results: We found that photostimulation of dynorphinergic cells in the ventral NAc shell elicits robust conditioned and real-time aversion. In contrast photostimulation of dorsal NAc shell dynorphin cells induced a place preference and was positively reinforcing. Both the aversion and preference were blocked by local injection of NorBNI. Furthermore, activation of ventral NAc shell induced conditioned and real-time aversive behavior, while dorsal NAc shell stimulation resulted in a place preference, also shown to be positively reinforcing in operant slef-stimulation. These results show previously unknown discrete subregions of dynorphin-containing cells in the NAc shell that selectively drive opposing behaviors. Here we show that photostimulation of ChR2 expressing neurons in the NAc shell of dyn-cre mice significantly increases the release of dynorphin compared to non-ChR2 expressing neurons in controls using an ELISA. We were also able to detect photostimulated dynorphin release in the ventral NAc. Taken together these data support the conclusion that we can reliably drive dynorphinergic tone in dyn-expressing cells in the NAc.

Conclusions: In the present study we identify that photo-stimulation of dyn-expressing cells in discrete subregions within the NAc shell drive opposing motivational behavioral states. We show that photostimulation of dyn-expressing cells in the ventral NAc shell drives aversion behavior whereas photostimulation in the dorsal NAc shell drives a preference/reward behavior, both of which are inhibited following local NorBNI injection. Here we demonstrate this functional anatomical segregation using three different behavioral paradigms; real-time place testing using bidirectional wireless μ-ILED technology, conditioned place testing and operant self-stimulation.

How KOR is able to mediate these opposing behaviors in two distinct regions of the NAc shell is unknown, but it is possibly modulated by functionally distinct neuronal populations projecting to either the dorsal or ventral shell, which we are currently investigating.

For the first time we are able to detect the release of dynorphin following photostimulation of dynorphin containing cells. Understanding the regional specificity by which NAc dynorphinerigic cells regulate preference and aversion provides insight into motivated behaviors that are dysregulated in stress, reward and psychiatric disease.

Keywords: Nucleus Accumbens, kappa opioid receptor, optogenetics

Disclosures: Nothing to disclose.

W63. Striatal Sub-Region Resting State Connectivity in Bipolar Depression and Mania

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Background: Bipolar disorder (BP) is characterized by periods of depression (BPD) and (hypo)mania (BPM), but the underlying state-related brain circuit abnormalities are not fully understood. Striatal functional activation and connectivity abnormalities have been noted in BP but consistent findings have not been reported. To further elucidate striatal abnormalities across different BP states, this study investigated differences in resting state functional connectivity of six striatal subregions in BPD, BPM and healthy control (HC) subjects.

Methods: Ninety medication-free subjects (30 BPD, 30 BPM and 30 HC), closely matched for age and gender, were scanned using 3T functional magnetic resonance imaging (fMRI) acquired at resting state. Correlations of low frequency BOLD signal fluctuations for six previously described striatal subregions were used to obtain connectivity maps of each sub-region. Using a factorial design, main effects for differences between groups were obtained and post-hoc pairwise group comparisons performed.

Results: BPD had increased connectivity of the dorsal caudate putamen with somatosensory areas such as the insula and cuneus. The BPM group showed unique increased connectivity between left dorsal caudate and midbrain regions as well as increased connectivity between ventral striatum inferior and thalamus. In addition, both BPD and BPM exhibited widespread functional connectivity

abnormalities between striatal subregions and frontal cortices, limbic regions and midbrain structures.

Methods: Ninety medication-free subjects (30 BPD, 30 BPM and 30 HC), closely matched for age and gender, were scanned using 3T functional magnetic resonance imaging (fMRI) acquired at resting state. Correlations of low frequency BOLD signal fluctuations for six previously described striatal subregions were used to obtain connectivity maps of each sub-region. Using a factorial design, main effects for differences between groups were obtained and post-hoc pairwise group comparisons performed.

Results: BPD had increased connectivity of the dorsal caudate putamen with somatosensory areas such as the insula and cuneus. The BPM group showed unique increased connectivity between left dorsal caudate and midbrain regions as well as increased connectivity between ventral striatum inferior and thalamus. In addition, both BPD and BPM exhibited widespread functional connectivity abnormalities between striatal subregions and frontal cortices, limbic regions and midbrain structures.

Conclusions: BPD exhibited unique connectivity abnormalities of associative and somatosensory subregions of the putamen, while BPM exhibited connectivity abnormalities of associative and limbic caudate. Most other striatal subregion connectivity abnormalities were common to both groups and may be trait-related.

Keywords: R-fmri, Bipolar Disorder, striatum, Depression, mania

Disclosures: Nothing to disclose.

W64. The Adrenergic-Cholinergic Hypothesis of Affective Disorders Revisited

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Background: The adrenergic-cholinergic balance hypothesis of affective disorders, proposing that mania is caused by a predominance of catecholaminergic central activity, and depression caused by a predominance of cholinergic (muscarinic) activity was proposed more than 40 years ago. Research exploring this hypothesis over the next three decades consisted almost entirely of observations of the depression relevant mood (antimanic, depression inducing), behavioral (anergy, decreased thoughts), neuroendocrine (increased cortisol, beta endorphin, epinephrine, ACTH), and REM sleep latency (shortening) effects of centrally acting cholinesterase inhibitors and muscarinic agonists as observed in studies of humans and rodents. In the past decade a substantial body of therapeutic, imaging, genetic, neurotransmitter receptor and neurotransmitter - interaction information has accumulated, in large part supporting the hypothesis that muscarnic mechanisms are closely related to the phenomenology of depression and integrating the cholinergic hypotheses with other neurotransmitter and neuromodulator hypotheses of mood disorders. Although generally supportive of a role for acetylcholine in the etiology and phenomenology of mood disorders, this information has not had the exposure to the medical community that other hypotheses, such as the monoamine hypotheses and the glutamate hypothesis and its relationship to ketamine have had.

Methods: An extensive review of the current and past decade's medical literature occurred via Pub Med scrutiny. Key words including adrenergic-cholinergic balance, acetylcholine-depression, cholinergic -depression, scopolamine-depression, nicotinic-depression, cholinergic-mania were utilized to determine what relevant information has been published over the past decade.

Results: The following are acetylcholine-depression relevant observations.

Anticholinergic Therapies: The antimuscarinic agent intravenous scopolamine has proven in a series of studies done exclusively by the NIMH group to have rapid antidepressant effects in treatment -resistant major depressive and bipolar depressed patients. Additionally, a study performed in Iran demonstrated that oral scopolamine exerts an adjunctive antidepressant effect in citalopram treated individuals. Scopolamine impairs/attenuates anger and disgust recognition accuracy, providing a possible mechanism for its antidepressant effects.

Receptor Studies: Muscarinic M1 and M2 receptors appear to regulate learned helplessness in the forced swim test, a model of human depression. CHRM2 receptors and B2 nicotinic receptors are decreased in bipolar and/or major depressive disorder patients, suggesting a response to an increase in central acetylcholine. Hippocampus Cholinergic Studies: Increasing acetylcholine in the hippocampus leads to increased anxiety and depression- like behaviors and decreased social stress resilience in mice. Furthermore, increased CRF due to stress leads to increased hippocampal acetylcholine levels in rats. Stress- acetylcholine interaction studies: Stress effects appear increased when central hyppocampus acetylcholine is increased. Stress leads to increased CRF activity which increases central acetylcholine activity. Seasonal Mood Variations: Low activity photoperiod lengths (Fall, Winter) can cause elevated CRF and acetylcholine levels in mice, and such periods are associated with depression in humans. Cholinergic- Neurotransmitter and neuromodulator interactions: Acetylcholine interacts with and is effected by a variety of depression relevant neurotransmitters/neuromodulators including Dopamine, Glutamate, Serotonin, CRF, GABA and Epinephrine being most prominent, leading to a cascade hypothesis of affective disorders.

Conclusions: In the past decade considerable information has been presented supporting a role for acetylcholine in the etiology and phenomenology of affective disorders. Such information suggests that a further research in this area is indicated.

Keywords: acetylcholine, cholinergic system, Depression, Scopolamine

Disclosures: Nothing to disclose.

W65. Neural Substrates of Social Exclusion in Recent Suicide Attempters

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Background: More than 41,000 Americans died of suicide in 2013. One of the barriers to implement suicide prevention measures is the lack of understanding of the biological mechanisms leading to it. Suicidal events are usually triggered by social or interpersonal crises and conflict associated with intense psychological pain. We hypothesized that the experience of social exclusion in patients with a recent suicide attempt will be associated with distinct activity in brain regions controlling pain and in regulatory prefrontal regions.

Methods: We assessed clinical characteristics including suicidality, depression, anxiety, hopelessness and physical pain threshold in three groups of individuals: (1) recently depressed patients within three days after a moderate-high lethality suicide attempt, (2) psychiatric controls, and (3) healthy controls (n = 8-14 per group). Subjects then underwent functional magnetic resonance imaging (fMRI) while participating in the Cyberball game, a paradigm that simulates the social interactive experience of being excluded by others in a virtual ball tossing game.

Results: The suicide attempt group had higher levels of depression, suicidal ideation severity and intensity, and physical pain threshold than both the psychiatric and healthy control groups. No differences between the studied groups were observed during the inclusion condition. During the exclusion condition, medial prefrontal cortex activation was higher in the suicide attempt group compared with both control groups. The exclusion-inclusion contrast showed hypoactivation in the parahippocampus and rostral anterior cingulate with k > 25 voxels, p < 0.005 before correction.

Conclusions: These preliminary results suggest a specific imbalance between frontostriatal cognitive control and limbic networks during social exclusion in acutely suicidal behavior independent from depression. This accrues on the evidence of cognitive control deficits associated with acute suicidal behavior. The study of social exclusion may provide a tool for the exploration of pain processing in acutely suicidal patients.

Keywords: suicide, prefrontal circuit, fMRI, Depression

Disclosures: Nothing to disclose.

W66. Ketamine Treatment Translocates Gsalpha from Lipid Rafts in Cultured Glial Cells: Similar Effects to Several Antidepressant Compounds

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Background: Previous studies have demonstrated that all classes of monoamine-centric antidepressants increase coupling between the G protein, Gsalpha and adenylyl cyclase, resulting in sustained cAMP production. This effect requires sustained drug treatment and is observed after 3 days in cultured neural or glial cells or 3 weeks in rats. This is apparently due to Gsalpha being released from constraints of a lipid raft environment to cholesterol-poor regions of the plasma membrane. Consistent with this, both peripheral tissue and postmortem brain from depressed human subjects show a greater proportion of Gsalpha in lipid rafts. In an effort to determine whether ketamine showed an antidepressant "biosignature" similar to other

antidepressants, studies in cultured cells were initiated. It was hypothesized that ketamine would have an effect similar to antidepressants but along a shorter time course. **Methods:** C6 glioma cells were treated with 1 μM ketamine for 15 minutes or 24 hours. Cells were harvested and lipid raft fractions prepared and the amount of Gsalpha was assessed in raft and non-raft membrane fractions by Western blot analysis. A population of C6 cells with stable expression of a fluorescent Gsalpha fusion protein (GFP-Gsalpha) was treated similarly with ketamine and the mobility of GFP-Gsa was determined by Fluorescence Recovery After Photobleaching (FRAP). Three-day treatment of these cells with compounds showing antidepressant activity retard recovery, as the GFP-Gsalpha is more extensively coupled with adenylyl cyclase, a 12 membrane span protein with slow lateral mobility.

Results: Both methods produced results consistent with translocation of Gsalpha (or GFP-Gsalpha) from lipid raft domains to association with adenylyl cyclase in non-raft domains. Ketamine induced translocation is also dose-dependent and occurs at the therapeutically relevant concentration of 1uM. FRAP studies also showed that 15 minutes of ketamine treatment was sufficient to achieve substantial translocaiotn of GFP-Gsalpha. These results suggest that brief treatment of cells with ketamine produces an antidepressant signature similar to that seen after prolonged treatment of well established classes of antidepressants.

Conclusions: Brief ketamine treatment evokes a biochemical hallmark (translocation of Gsalpha) seen after prolonged treatment with several species of drugs that have antidepressant activity. This effect occurs in cells lacking NMDA receptors and is not mimicked by memantine, suggesting a possible additional site for ketamine action. Furthermore, the translocation of GFP-Gsalpha produced by ketamine and all tested compounds with antidepressant activity (but not mood-stabilizers, antipsychotics or anxiolytics) might serve as a useful platform for identifying compounds with potential antidepressant activity and for predicting clinical response.

Keywords: Antidepressant, G-protein, lipid rafts, Biomarker, cyclic AMP

Disclosures: MMR has significant financial interest in Pax Neuroscience Inc., has received research support from Eli Lilly and honoraria from Pfizer.

W67. WITHDRAWN

W68. Connectivity Analysis of Eeg in Depressed Patients Receiving Electroconvulsive Therapy and Magnetic Seizure Therapy

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Background: Depression is associated with resting-state brain network hyperconnectivity, manifested as higher theta and alpha EEG coherence in long range connections between frontopolar and temporal or parieto-occipital areas. Electroconvulsive therapy (ECT), the most efficacious treatment of severe depression, has been reported to increase low frequency coherence, affect interhemispheric symmetry in EEG, and alter functional network architecture by down-regulating connectivity in frontal circuits. Magnetic seizure therapy (MST), which induces seizures with high dose repetitive transcranial magnetic stimulation, has been introduced to improve the benefit/risk ratio of seizure therapy. However, understanding the mechanisms of action of seizure therapy remains incomplete. The purpose of this study is to use graph theory analysis to examine the effects of seizure therapy on brain network connectivity.

Methods: Patients were participants in a two-center randomized controlled trial contrasting the efficacy and neurocognitive effects of ultrabrief pulse right unilateral ECT and circular coil MST. Patients were considered responders if they showed at minimum a 50% reduction in the Hamilton Rating Scale for Depression score. Resting-state EEG was acquired at baseline and after the treatment course in 9 patients (5 MST and 4 ECT) for 10 minutes each in eyes opened and closed conditions. Fifty-nine cephalic channels were placed according to the international 10/20 system. Data analysis was performed using FieldTrip. The signals were bandpassed 0.25–50 Hz, re-referenced to the average signal, and artifacted using independent component analysis. The 10-minute recording was partitioned into 300 two-second epochs, and spectral analysis was performed using multitaper Fourier transform. Phase synchronization was estimated using the debiased weighted phase lag index (DWPLI). The undirected, weighted graph was thresholded to construct an adjacency matrix. Network structure was assessed using betweenness centrality, clustering coefficient, density, and characteristic path length. Differences in network measures between responders and nonresponders to seizure therapy were tested using the independent sample t-test. Statistical significance was defined as a two-sided p-value of less than 0.05.

Results: Of the 9 patients with EEG recording, 3 were responders. At baseline, responders and nonresponders exhibited similar DWPLI-frequency profile in the eyes opened condition. There was a significant post-treatment elevation in DWPLI in the delta band for the responders but not for nonresponders (p < 0.01). The increase in low frequency phase synchronization is consistent with previous analysis of EEG coherence before and after ECT, and may serve as a state marker of response. The post-treatment change in brain network density was significantly higher in the responders relative to nonresponders (p < 0.05). For the eyes closed condition, the responders exhibited a higher DWPLI in the theta, alpha, and beta frequencies compared to the nonresponders both at baseline and post treatment, which can potentially serve as a trait marker of response.

Conclusions: Our findings support the use of network measures derived from resting-state EEG as trait and state markers of response to seizure therapy in severely depressed patients.

Keywords: Magnetic seizure therapy, Electroconvulsive therapy, Graph theory, EEG biomarker

Disclosures: Nothing to disclose.

W69. Dysregulation of Striatal Dopamine Receptor Binding in Suicide and by Early Life Adversity

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Background: Suicide can be a devastating endpoint of psychiatric disease and is a major cause of death worldwide. Dopamine signaling in the striatum critically modulates motivational and emotional processing as well as reward association, which may be disrupted in mood disorders and suicide and exacerbated by environmental factors such as early life adversity (ELA). Despite data indicating that ELA increases risk of suicide, the neurobiological effect of ELA on striatal dopaminergic signaling in humans is unclear. We sought to determine, therefore, whether there are alterations in striatal dopamine systems in suicides and in subjects exposed to ELA.

Methods: We used quantitative receptor autoradiography on postmortem brain tissue from suicides and controls to measure binding to the dopamine transporter (DAT; 3H-Mazindole), and dopamine D1 (3H-SCH23390) and D2 receptors (3H-Sulpiride). 17 Suicides and 17 controls were matched for age, sex, and postmortem interval. An Axis I diagnosis of major depressive disorder (MDD) was present in 11/17 suicides. All subjects were characterized diagnostically and in terms of ELA by psychological autopsy and underwent toxicological screens. ELA was present in 6 control subjects and 10 suicides.

Results: Mean DAT, D2 receptor, and D1 receptor binding did not differ between suicides and controls. However, there was a positive correlation between D1 and D2 receptor binding in the dorsal striatum of control subjects ($R2 = 0.31$, $p < 0.05$) but not in suicides ($R2 = 0.00$, $p = 0.97$). In both suicides and controls who reported ELA, there was no correlation between striatal DAT and D1 receptor binding ($R2 = 0.07$, $p = 0.33$ in ELA subjects), although DAT and D1 receptor binding were positively correlated in subjects with no report of ELA ($R2 = 0.33$, $p < 0.05$). There were no mean ELA-related group differences in binding after controlling for age, as D1 receptor and DAT binding throughout the striatum decreased with age across all subjects.

Conclusions: The loss of association between dorsal striatal dopamine D1 and D2 receptor binding in suicide could reflect a dysregulation of dopaminergic signaling pathways that could perhaps in part be attributed to an ELA-induced change in DAT binding. An imbalance in dopaminergic function could contribute to the diathesis for suicide, and mediate the effect of ELA on risk of suicide.

Keywords: suicide, Dopamine, Dorsal striatum, early life stress, postmortem human brain
Disclosures: Nothing to disclose.

W70. Novel Molecular Signatures of Antidepressant Treatment Response

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Background: With over 350 million sufferers worldwide major depression is a life-threatening psychiatric disorder and a leading cause of disability. Despite the rapidly increasing lifetime incidence for developing a depressive episode, currently available antidepressants have limited efficacy and only one-third of patients experience remission after up to four months of treatment. Therefore, there is an urgent need to develop improved antidepressants in order to alleviate the burden of depression in the significant number of patients that are resistant to currently available treatments. One of the greatest challenges in identifying advanced treatment strategies results from the lack of knowledge about the neurobiological mechanisms that determine individual differences in antidepressant responsiveness and treatment resistance. On a neuroanatomical level, the dentate gyrus of the hippocampus has been shown to be a crucial mediator of antidepressant effects on behavior. In order to identify novel molecular targets that mediate antidepressant treatment responses, we used two complementary mouse models of chronic stress and antidepressant treatment. We then segregated responders and non-responders to chronic fluoxetine treatment and conducted RNA sequencing in the dorsal and ventral dentate gyrus.

Methods: In the first model, we used wild-type C57/BL6 mice that were socially defeated for 10 days by a dominant CD1 aggressor mouse and subsequently divided into stress-susceptible and resilient mice based on their social avoidance behavior in the social interaction test. Susceptible mice were then treated with fluoxetine (18mg/kg) for 28 days and treatment response was determined based on improvements in social interaction behavior after fluoxetine. Brains were flash frozen 24h after behavioral testing and dorsal and ventral dentate gyrus tissue was obtained using microbiopsy punches. RNA was extracted and sequenced using Illumina Hiseq 2500 with 100bp single-end reads and 30M reads/sample. In the second model, we treated wild-type Sv129 mice chronically with corticosterone (35μg/ml) for 8 weeks and administered fluoxetine during the last 28 days of corticosterone treatment. Mice were divided into fluoxetine responders and non-responders based on their latency to feed in the novelty-suppressed feeding test. RNA from dorsal and ventral dentate gyri was processed on an Illumina microarray. Genes with $p < 0.05$ were considered significant.

Results: In the social defeat model, 47% of mice responded to fluoxetine while 53% did not respond. In the chronic corticosterone model, 73% of mice responded to fluoxetine while 27% did not respond. When comparing gene expression profiles between responders and non-responders in the social defeat model, we identified 33 RNA transcripts that were significantly regulated in the ventral dentate gyrus and 36 transcripts that were significantly regulated in the dorsal dentate gyrus. In the chronic corticosterone model, we found 102 probes in the ventral dentate gyrus and 165

probes in the dorsal dentate gyrus. In order to identify common gene expression profiles across the two different animal models, we compared the significantly regulated transcripts of responders and non-responders in both models. In the ventral dentate gyrus, we identified 3 common genes that were increased in fluoxetine responders compared to non-responders: neuronal PAS domain 4 (Npas4; 6 fold & 26 fold increase), heparan sulfate 6-O-sulfotransferase 2 (Hs6st2; 2.3 fold & 1.8 fold increase), and connective tissue growth factor (Ctgf; 1.6 fold and 2.1 fold increase). In the dorsal dentate gyrus, we did not find any genes that were commonly regulated in both models.

Conclusions: Here we used two different animal models of chronic stress and antidepressant treatment and identified a common set of genes in the ventral dentate gyrus that characterize the behavioral response to fluoxetine. Interestingly, previous studies have implicated these genes in the regulation of neuronal activity, neuronal survival and angiogenesis, which may be of particular importance for antidepressant-dependent regulation of dentate gyrus function.

Keywords: Antidepressants, Depression, gene expression, mouse behavior, novel therapeutics

Disclosures: Nothing to disclose.

W71. Lithium Decreases Markers of Inflammation in an Animal Model of Mania

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Background: Bipolar disorder (BD) affects 1-4% of the population, incurs great costs to the US healthcare system, and is associated with high morbidity and mortality. Despite an unknown mechanism of therapeutic action, lithium is the standard-of-care treatment for BD. Recent studies suggest that the physiopathology of BD is related to immune system alterations and inflammation. In particular, people with BD have alterations in peripheral markers of inflammation IL-2, IL-4, IL-6, IL-10, and tumor necrosis factor alpha (TNF-α). In this study we investigated the effects of lithium on cytokine levels in a rat model of mania.

Methods: To induce mania-like behavior we treated Wistar rats with d-amphetamine (d-AMPH), or saline control for 14 days. On day 8 of the mania induction treatment we initiated treatment with lithium or saline placebo for 7 days. On day 14 of mania induction (day 7 of lithium or placebo treatment) we assessed spontaneous locomotor and rearing behavior in an open arena. Rats were then humanely euthanized and we collected peripheral blood, cerebrospinal fluid (CSF), hippocampus, striatum, and frontal cortex to assess cytokine levels.

Results: Rats treated with d-AMPH had increased locomotor and rearing activity ($p < 0.001$) in the open field arena, which was attenuated by treatment with lithium ($p < 0.001$), thereby verifying the mania model and lithium effect on mania-like behavior. Rats treated with d-AMPH had

increased levels of IL-4, IL-6, IL-10, and TNF-α (all $p < 0.001$) in the frontal cortex, striatum, and serum. Cytokine level increases in d-AMPH rats were completely reversed by lithium treatment ($p < 0.001$).

Conclusions: Rats treated with d-AMPH express mania-like behavior and have abnormal cytokine levels in peripheral and cerebral tissues. Lithium treatment ameliorates these behavioral and biochemical abnormalities. These results demonstrate the validity of the d-AMPH model for immune phenotypes associated with BD and point to a possible clinical mechanism of action for lithium.

Keywords: Bipolar Disorder, mania, Lithium

Disclosures: Nothing to disclose.

W72. Alterations in Cortical and Striatal Oprk1, PDYN and Oprm1 mRNA Expression May Underlie the Behavioral Effects of Buprenorphine in Unpredictable Chronic Mild Stress

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Background: Emerging preclinical and clinical evidence suggest that buprenorphine (BPN) may be an effective therapeutic for treatment-resistant depressed patients. BPN is an FDA approved drug, which acts as a partial agonist at mu opioid receptors (μ-ORs) and antagonist at kappa opioid receptors (κ-ORs). It also has some activity at nociceptin and delta opioid receptors. Our laboratory has previously shown that acute and sub-chronic administration of BPN produces behavioral effects in rodent tests relevant to anxiety and depression. Here, we evaluate alterations in mRNA expression of the genes encoding μ-OR (Oprm1), κ-OR (Oprk1) and the endogenous κ-OR ligand prodynorphin (PDYN) following unpredictable chronic mild stress (UCMS) with and without BPN treatment.

Methods: C57BL/6J mice were exposed to three mild stressors per day for three weeks. Commencing at the start of week three, buprenorphine (0.25 mg/k) or saline was administered to non-stress (NS) and UCMS exposed mice for a total of 14 days. Mice were euthanized by cervical dislocation. Brains were rapidly dissected into frontal cortex (FC), striatum (Str), amygdala (Amy) and hippocampus (Hp). RNA was isolated using a Trizol/chloroform extraction method and Taqman Gene Expression Assays (Applied Biosystems) were used in the Applied Biosystems StepOne Plus RT-PCR system to quantify the target genes during amplification. Two-way ANOVA with Newman-Keuls multiple comparisons were used to analyze the data.

Results: Within the FC significant stress*treatment interactions for Oprk1 ($F1, 52 = 5.436$ $p = 0.03$), PDYN ($F1, 52 = 3.723$, $p = 0.05$) and Oprm1 ($F1, 50 = 9.086$, $p = 0.004$) expression were observed. This reflected increased Oprm1 and PDYN expression and decreased Oprk1 expression post stress. These changes were reversed following BPN treatment. Significant stress*treatment interactions were observed in the Str for Oprk1 ($F1, 71 = 6.354$, $p = 0.02$) and

PDYN expression (F1, 71 = 4.945 p = 0.03), where treatment significantly reduced UCMS-induced increases in mRNA expression of these genes. A significant effect of BPN treatment was also observed for Oprm1 expression in the Str (F1, 71 = 9.664 p = 0.003) in UCMS-exposed mice only. In the Amy, a significant effect of stress on Oprk1 expression was detected (F1, 58 = 4.769, p = 0.03). BPN treatment did not reverse the UCMS-induced decrease in Oprk1 expression in this region. Conversely, a significant effect of BPN treatment was observed for Oprm1 mRNA expression in the Amy (F1, 61 = 4.366, p = 0.04). No alterations in Oprk1 mRNA expression were observed in the Hp. However, a significant effect of stress alone was observed for PDYN in the Hp (F1, 73 = 6.690, p = 0.01). Furthermore, a significant stress*treatment interaction was observed for Oprm1 expression in the Hp (F1, 72 = 3.990, p = 0.04), where BPN significantly reduced the UCMS-induced increase in hippocampal Oprm1 mRNA expression. The alterations in Oprk1 and Oprm1 expression in the FC were correlated with a decrease in pro-depressive behavior of UCMS treated mice in the forced swim test (FST) following BPN treatment. Additionally, the effects of BPN treatment in the FST were correlated with reductions in mRNA expression of Oprm1 in the Amy and Hp. Elevated expression of Oprk1 and PDYN in the Str were highly correlated with anhedonia in the sucrose preference test in UCMS exposed mice. Interestingly, decreased expression in the PDYN expression across all regions in UCMS + BPN treated mice were correlated with increased sucrose preference.

Conclusions: This is the first set of studies to evaluate the effects of BPN treatment in a rodent model of depression. Taken together these results highlight the FC and Str as regions of interest for future investigations, as BPN treatment resulted in normalization of UCMS-induced changes in Oprk1, PDYN and Oprm1 expression in these regions. These changes were highly correlated with the ability of BPN to reverse UCMS induced anhedonia and pro-depressive behavior. Overall, these data support the continued investigation of BPN as a novel antidepressant for treatment-resistant depressed patients.

Keywords: Buprenorphine, Major depression, chronic mild stress

Disclosures: Nothing to disclose.

W73. HIV-1 Disrupts Motivational Processes via Dopamine Transporter Dysregulation

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Background: Apathy is a common motivational alteration in HIV-1 + individuals, affecting between 30-60% of the HIV-1 + population. Motivational alterations in HIV-1 + individuals are associated with decreased performance on tasks involving frontal-subcortical circuitry and alterations in the nucleus accumbens. In these studies, the HIV-1 transgenic (Tg) rat was used to assess the effects of long-term HIV-1 exposure on motivated behavior using volun-tary wheel running and fast-scan cyclic voltammetry to assess the dopamine system in the HIV-1 Tg rat.

Methods: Adult female HIV-1 Tg animals (n = 21) to F344 controls (n = 26) were pair-housed under a 12:12 light/dark cycle. Voluntary running was measured with 34cm running wheels for 60 minutes per day for 3 months during both light and dark phases. In addition, we investigated DA uptake in striatal brain slices from HIV-1 Tg and F344 control female rats (during diestrous) using fast-scan cyclic voltammetry.

Results: There were no significant differences during the light phase between HIV-1 Tg and F344 control rats in voluntary wheel running. However, during the nocturnal phase of the light/dark cycle we found that the HIV-1 Tg animals initially ran more than F344 controls and reached an earlier asymptotic plateau; however, F344 controls continued to escalate their overall running and surpassed the stabilized HIV-1 Tg group after ∼4 weeks of nocturnal running, until reaching their asymptotic plateau at week 11. Maximal running speed was not different between the HIV-1 Tg and F344 controls, and the responsiveness to circadian cues remained intact in the HIV-1 Tg animals. In the HIV-1 Tg striatal slices, DA reuptake was significantly prolonged relative to the F344 control striatum, suggesting functional alterations of DAT within the striatum of HIV-1 Tg rats.

Conclusions: Collectively, selective alterations in the rewarding efficacy of voluntary wheel running and dysfunctional DA reuptake indicates that HIV-1 exposure effects key motivational circuitry. These findings may help determine the mechanisms of common motivational alterations, such as apathy, in HIV-1 + individuals.

Keywords: HIV-1, dopamine transporter, wheel running, apathy

Disclosures: Nothing to disclose.

W74. Targeting FKBP51 Mechanisms to Treat PTSD and Related Disorders

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Background: FK506 binding protein 51 (FKBP51), encoded by the FKBP5 gene, is an Hsp90 co-chaperone implicated in multiple psychiatric diseases, including depression and post-traumatic stress disorder (PTSD). FKBP51 levels increase with age due to reduced FKBP5 methylation, leading to vulnerability to stress and altered glucocorticoid signaling. Furthermore, single nucleotide polymorphisms (SNPs) in FKBP5 are associated with higher FKBP5 expression leading to increased risk for developing depression and PTSD, suggesting targeted inhibition of FKBP51 may be beneficial in neuropsychiatric diseases.

Methods: We used animal modeling and cell based drug screening techniques as well as biochemical assays to identify new treatment strategies for disorders related to increased FKBP51 production.

Results: Transgenic over-expression or knockout of FKBP51 in mice demonstrates behavioral and neuronal changes that further support the connection of FKBP51 to PTSD. Using FKBP51 knockout mice, we confirmed that one primary

mechanism through which FKBP51 regulates these effects in the brain is by inhibiting glucocorticoid receptor (GR) activity. Therefore, we designed a screening platform to identify compounds that could attenuate FKBP51-mediated suppression of GR activity. Using cell-based luciferase assays, we screened more than 1000 pharmacologically active compounds and identified lead molecules that abrogated the suppressive effect of FKBP51 on GR activity. Follow-up screens highlighted one compound in particular that had limited impact on GR activity, independent of FKBP51.

Conclusions: The compound we identified through our screening efforts has been used to treat other psychological disorders, but not PTSD or depression. We discovered that this drug most likely works by directly disrupting GR/Hsp90/FKBP51 binding and analogs of this scaffold were also active, suggesting that medicinal chemistry efforts around this scaffold could overcome some of its known liabilities while also preserving its capacity to regulate the FKBP51/Hsp90 interaction. These findings reveal new insights and tools to understand the role of FKBP51 in GR-dependent depression and PTSD.

Keywords: PTSD, FKBP5, glucocorticoid receptor, Hsp90, Depression

Disclosures: Nothing to disclose.

W75. Kappa Opioid Receptors Modulate Mammalian Target of Rapamycin (mTOR) through p38 MAP Kinase

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Background: Exposure to stress can exacerbate depressive symptoms and trigger depressive episodes, and the mechanisms by which stress can influence depression are not completely defined. Both the mammalian target of rapamycin (mTOR) and Kappa-opioid receptor (KOR) systems have been independently associated with stress-induced depression, but no study has attempted to link these two systems. One potential kinase that could provide this bridge is p38 MAPK, which has been shown to underlie the pro-depressant effects of stress and KOR stimulation, and has been associated with mTOR in limited studies. Inhibition of p38 MAPK signaling blocks aversion induced by KOR agonism, reduces immobility in the forced swim test, and also decreases social avoidance induced by social defeat stress. Several studies have demonstrated that stress can reduce phosphorylated (active) mTOR in the hippocampus and basolateral amygdala. Moreover, mTOR signaling has been shown to be critical for the anti-depressant activity of ketamine in the prefrontal cortex (PFC). In these experiments, we test the relationship between KOR activation and phospho-mTOR (p-mTOR) expression in vitro and in the spinal cord, hippocampus, and prefrontal cortex.

Methods: In vitro studies- KOR transfected HEK 293 cells were incubated with the KOR agonist U50,488 and were harvested at various time points from 5-60 min post-treatment. A subset of cells was pretreated with either DMSO or the p38 MAPK inhibitor SB203580 prior to KOR

treatment. Cells were then prepared for western blot analysis using anti-phospho-mTOR primary (Cell Signaling). In vivo studies- Experiments were performed in male C57Bl/6 mice. A subset of the animals was G-protein receptor kinase 3 knockout (GRK3 -/-) with appropriate littermate controls. Animals were injected with U50,488 (10 mg/kg) and/or norBNI (10 mg/kg) and brains or spinal cords were collected at 30 min or 28 hr post-injection. A second cohort of animals received nine injections of U50,488 (10 mg/kg) over 5 days and brains were collected 4 hr after the last injection. Homogenate tissue was prepared for western blot analysis using phospho-mTOR antibody. A third group of animals were subjected to repeated forced-swim stress (5 swims over two days) and sacrificed 28 hr after the last swim.

Results: Surprisingly, in HEK 293 cells, incubation of U50,488 (10 μM) significantly increased p-mTOR expression that peaked at 15 min post-administration and lasted up to 60 min. In cells pretreated with the 1 μM SB203580, the increase in p-mTOR was completely blocked, suggesting the acute response was dependent on p38 MAPK. In spinal cord samples, we also observed an increase in p-mTOR 30 min after U50,488 injection. This effect was not evident in GRK3-/- animals, again suggesting that p38 MAPK was required for acute mTOR signaling. Looking at both 30 min and 28 hr later in brain, U50,488 produced a bi-phasic response, where p-mTOR is significantly increased at 30 min in hippocampus and prefrontal cortex but significantly decreased at 28 hr in hippocampus. This decrease in p-mTOR signaling was norBNI dependent, although norBNI itself produced a small decrease in p-mTOR expression. After chronic U50,488 injection, p-mTOR expression in the PFC was significantly reduced. Finally, repeated forced-swim stress also reduced p-mTOR in the hippocampus.

Conclusions: These results suggest that KOR activation produces a biphasic p-mTOR response. The increase seen after acute injection could represent new protein production associated with learning about a stressful environment, while the subsequent decreases seen at 28 hr and chronically over 5 days could represent synaptic pruning classically associated with depression. Ongoing studies will look at the necessity of p38 MAPK for the 28 hr and sustained decreases in p-mTOR expression, as well as looking at the role of KOR activation on spine density in the prefrontal cortex and hippocampus.

Keywords: kappa opioid receptor, mTOR, p38 MAPK, chronic stress

Disclosures: Nothing to disclose.

W76. Subgenual Cingulate Cortical Activity and Connectivity in the Antidepressant Efficacy of Electroconvulsive Therapy

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Background: Electroconvulsive therapy (ECT) is the most effective treatment for depression, yet its mechanism of

action is unknown. Identifying key neuronal mechanisms underlying clinical response to ECT could provide new targets for innovative therapies in depression.

Our goal was to investigate the neurobiological underpinnings of ECT response using longitudinally collected resting state fMRI (rs-fMRI) in patients with treatment-resistant depression.

Methods: Data was collected in sixteen patients with a major depressive episode and at least 2 failed antidepressant trials in the past. 10 healthy comparison subjects underwent the same neuroimaging procedures. Patients received bifrontal ECT (at 150% of seizure threshold energy) 3 times a week under general anesthesia. Patients received on average 6.4 ECT administrations before their final MRI session (range: 4-8). We acquired rs-fMRI at 3 time-points: at baseline, after the 1st ECT administration, and after the course of the ECT treatment; depression was assessed with the Hamilton Depression Rating Scale (HAM-D). The primary measure derived from rs-fMRI was fractional amplitude of low frequency fluctuation (fALFF), which provides an unbiased voxel-wise estimation of brain activity. We also conducted seed-based functional connectivity analysis based on our primary findings. We compared treatment-related changes in HAM-D scores to pre- and post-treatment fALFF and connectivity measures.

Results: Subcallosal cingulate cortex (SCC) demonstrated higher BOLD signal fluctuations (fALFF) at baseline in depressed patients, and SCC fALFF decreased over the course of treatment. Decrease in SCC fALFF over the course of treatment was strongly associated with HAM-D change. In addition, connectivity of SCC with bilateral hippocampus, bilateral temporal pole, and ventro-medial prefrontal cortex was significantly reduced over the course of treatment.

Conclusions: These results suggest that the antidepressant effect of ECT may be mediated by down-regulation of SCC activity and connectivity. SCC function may serve as an important biomarker of target engagement in the development of novel therapies for depression that is resistant to treatment with standard medications.

Keywords: Depression, Subcallosal Cingulate, Connectivity, fMRI resting state, ECT

Disclosures: Nothing to disclose.

W77. Neurocognitive Clustering in Bipolar Disorder Patients and Their Unaffected Siblings

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Background: Deficits in cognitive functioning are common in patients with bipolar disorder (BD); however, there is considerable heterogeneity among patients, with a proportion of patients remaining cognitively spared. Our prior work suggested that there are 3 discrete cognitive subgroups in a large cohort of affectively-stable BD patients: 1) a group with severe cognitive impairment across all the cognitive domains (globally impaired; 40% of subjects); 2) another

group with mild to moderate deficits only seen in specific domains (selectively impaired; 30% of subjects); and 3) a group with intact cognitive performance relative to healthy controls (HCs) (intact; 30% of subjects) (Burdick et al. 2014). In this study we aim to expand upon this approach by first attempting to replicate the subgrouping in a sample of BD patients and then to determine if these subgroups might be associated with genetic risk by exploring whether their unaffected siblings (UAS) demonstrate a cognitive profile similar to that seen in the proband.

Methods: Demographic, clinical and neurocognitive data were collected in 219 subjects; 81 BD, 73 UAS, and 65 HC subjects. Cognitive functioning was evaluated using the California Verbal Learning Test (CVLT) total learning and short-delay recall (verbal learning and memory), Trail Making Test (TMT)-A and WAIS-Digit symbol subtest (processing speed), TMT-B (executive functions), and COWAT (verbal fluency). Mood symptoms were rated with the Hamilton Rating Scale for Depression (HRSD) and the clinician administered rating scale for mania (CARS-M). An estimate of premorbid IQ was obtained with the WRAT-3 Reading. BD, UAS and HC were compared in terms of demographic, clinical and cognitive characteristics using Analysis of Variance and Chi Squared as appropriate; mood symptoms and premorbid IQ were included as covariates in subsequent models. Hierarchical Cluster Analysis was used to investigate the presence of distinct cognitive clusters in the BD sample and BD subgroups were compared with the UAS and the HC sample. UAS were then subdivided into cognitive subgroups according their BD proband's cognitive cluster and then compared to HCs.

Results: BD, UAS and HC did not differ in terms of demographic features or premorbid IQ however BD had more severe mood symptoms than both UAS and HC (though still in the euthymic range). BD patients performed significantly worse than their UAS and the HC sample in digit symbol, CVLT, and in the cognitive composite score. UAS performed worse than HC on verbal memory (trend-level p = 0.052). Consistent with our previous report, cluster analyses revealed three distinct cognitive clusters in the BD sample characterized by a globally-impaired (37.7%), a selectively-impaired (17.4%), and a cognitively-intact subgroup (44.9%). The global and intact clusters follow a qualitatively similar pattern to one another, while the third group show a unique profile with selective impairment in processing speed (as measured by the TMT-A and Digit Symbol task). When siblings were categorized based upon their BD siblings' cognitive clusters, we found that UAS profiles followed the same pattern as that seen in their affected sibling.

Moreover, UAS with a globally impaired BD proband performed significantly worse than HC on the TMT-B (executive functions; p = 0.049), on the CVLT total score (p = 0.003) and delayed recall (p = 0.006), and on the general composite score (p = 0.018) – providing evidence of association with genetic risk.

Conclusions: Our study confirms previous findings regarding the presence of discrete cognitive subgroups of BD patients with a global impairment, a selective (i.e. processing speed) impairment, and an intact group. We further show that qualitatively similar cognitive clusters can be found in unaffected siblings of BD and that by accounting for heterogeneity in the proband sample, we are able to

accentuate the degree to which neurocognitive functioning can represent a meaningful endophenotype in BD.

Keywords: Bipolar Disorder, sibling, neurocognition, heterogenity

Disclosures: Nothing to disclose.

W78. Exploratory Genome-Wide Association Study of Acute Antidepressant Effects of Ketamine

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Background: Multiple studies have shown that the non-competitive N-methyl-D-aspartate (NMDA) receptor antagonist ketamine shows rapid antidepressant efficacy.

Methods: To investigate the role of genetic variants underpinning acute antidepressant effects of ketamine, we genotyped ∼700,000 single nucleotide polymorphisms (SNPs) in unrelated individuals of European ancestry with a major depressive episode who were collected at four US research centers (N = 149). The clinical study randomized subjects in proof of concept trials with ketamine and evaluated the efficacy of ketamine using the Montgomery-Asberg Depression Rating Scale and the Hamilton Depression Rating Scale. Change in depression rating at Day 1 was considered as the primary outcome.

Results: Linear regression analysis with PLINK v1.9 detected genome-wide significant evidence of association with a SNP in an intron of MCF2L2 (rs12696500, $p = 8.5e-9$; information = 0.73). Three nearby SNPs also showed evidence of association at the $p < 0.0001$ level.

Conclusions: MCF2L2 encodes a rho guanine nucleotide exchange factor that is highly expressed in brain, and has been previously associated with type II diabetes and metabolic/cardiovascular dysfunction. If replicated in larger samples, this finding could point toward novel mechanisms of rapid antidepressant action.

Keywords: GWAS, ketamine, antidepressant, genetics, treatment

Disclosures: Dr. Zarate is listed as a coinventor on a patent application for the use of ketamine and its metabolites in major depression. Dr. Zarate has assigned his rights in the patent to the US Government but will share a percentage of any royalties that may be received by the Government. All other authors have no potential conflicts of interest to disclose.

W79. Reduced Dopamine Transporter Function Enhances Probabilistic Learning, an Effect Blocked by Lithium

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Background: Bipolar disorder (BD) is a life-long psychiatric disorder represented by a myriad of symptoms but no cure.

Therapeutics have been limited to compounds with serendipitous origins and unknown mechanisms. Such therapeutics were designed to treat the affective symptoms of the disorder, not cognitive deficits experienced by patients despite such deficits closely associating with a patients functional outcome. In fact, current treatments (e.g., lithium) can impair cognition alone. Altered dopaminergic homeostasis has been linked to the mechanistic underpinnings of BD via genetic, PET, and molecular studies. Such homeostasis is controlled primarily by the dopamine transporter (DAT). Since genetic or pharmacological reduction in DAT function recreates several mania-relevant behaviors, we investigated whether: 1) Such DAT reductions would affect probabilistic learning (PL); 2) Chronic lithium treatment alone would affect PL; and 3) whether these treatments would synergistically interact on this behavior.

Methods: Male DAT wildtype (WT; n = 30) and knockdown (KD; n = 25) mice, as well as C57BL/6J (C57; n = 45) mice treated with the DAT inhibitor GBR12909 (GBR) or vehicle, were tested in the PL task. This task measures initial learning (trials to criterion) and serial reversal learning (switches) in a single session, consistent with clinical testing. The effects of chronic (7-10 days in water) lithium (0.6 or 1.0 g/L) on PL in C57 mice were also investigated, as was the effects of chronic lithium (0.6 and 1.0 g/L) on PL performance of GBR-treated C57 and DAT WT and KD mice respectively. All testing procedures were approved by the UCSD Institutional Care and Use Committee and all facilities met federal and state requirements for animal care as approved by the American Association for Accreditation of Laboratory Animal Care.

Results: Chronic lithium (1.0 g/L) impaired PL as measured by increased trials to criterion [$F(2,36) = 5.0$, $p < 0.05$], compared to water-treated mice ($p < 0.05$). A trend GBR by lithium interaction [$(F(1,39) = 3.2$, $p < 0.1$] revealed that GBR-treated C57 mice exhibited better PL performance compared to vehicle-treated mice as measured by increased switching ($p < 0.05$), an effect that was blocked by chronic lithium pretreatment (0.6 g/L; $p > 0.1$). Again, DAT reduction (KD mice) interacted with lithium (1.0 g/L) pretreatment in terms of switches [$F(1,33) = 12.8$, $p < 0.01$], revealing better performance of DAT KD compared to WT mice treated with water- ($p < 0.05$) but not lithium pretreatment ($p > 0.1$). No effect of lithium on general health, appearance, or weight was observed.

Conclusions: Consistent with some BD mania clinical studies, mice with reduced DAT function exhibited better switching during learning task, although not overall faster complex learning. Chronic lithium treatment - at clinically therapeutic serum levels (1.0 mmol/L) impaired learning, and blocked the improvement seen with reduced DAT functioning. These findings add behavioral findings to evidence of an interaction between lithium treatment and DAT function, e.g., lithium-induced suppression of phasic dopamine driven by DAT leakage, ultimately leading to elevated DAT levels. These studies support the premise that while current BD treatments may aid in the affective state of patients with BD, they may not offer the best treatment for patient's cognitive deficits. Hence, better treatments are required for the long-term treatment of BD patients.

Keywords: Bipolar Disorder, mania, Reward-based decision-making, genetic, Animal Models
Disclosures: Nothing to disclose.

W80. Social Buffering: Circuit Mechanisms of a Stress Coping Strategy

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Background: Social buffering is a widely recognized protective factor that reduces the incidence of stress induced mental health disorders, including depression. This benefit has been demonstrated across multiple species. Specifically, exposure to an affiliative conspecific enhances recovery following experiences of distress. Primarily, investigations have demonstrated the overwhelming benefit of social support in reducing malfunction of the hypothalamic-pituitary-adrenocortical (HPA) system, the noradrenergic system, and central oxytocin pathways. Despite the vast clinical evidence of the positive effects of social support in the face of psychological stress, surprisingly few experimental physiological circuit- and cell-type specific studies have been performed within the mesolimbic circuits, neural pathways which have been causally linked to depressive phenotypes. To explore the mechanisms of social support in the mesolimbic system and other brain circuits, we successfully developed a model of social buffering of chronic social stress (SBCSS). In the SBCSS, we modified a model of chronic social stress to include a variable of an affiliative conspecfic following the social defeat stress. This behavioral model has provided insights into the neuronal circuits involved in the protective benefit of social support.
Methods: A powerful and well-validated model of stress induced neural changes is a ten day chronic social defeat stress (CSDS). This model induces a robust long-lasting behavioral syndrome that includes social avoidance, anhedonia, anxiety and impaired coping responses to other environmental stressors in the majority of subjects. Only a narrow subset of subjects exhibit an active resistance to the deleterious effects of chronic social defeat stress. Utilizing a modified model of CSDS, a social buffer in the form of a littermate is provided daily following the stressful social encounter. The SBCSS paradigm is also ten days long, allowing for long-term behavioral and molecular changes to occur to establish a robust behavioral phenotype. To perform cell-type and circuit specific analysis, we utilize transgenic mouse lines in combination with retrograding viral markers to provide identification of projection specific neurons during electrophysiological recordings.
Results: We first performed behavioral analysis of repeatedly socially stressed mice in comparison with socially stressed mice that were provided with social buffering (SBCSS). We found that, unlike the CSDS, the large majority of subjects provided with a littermate companion post stress demonstrated healthy social behaviors. Specifically, mice that undergo chronic social defeat stress with a social buffer do not develop social avoidance and have sucrose preference scores similar to stress naïve animals. In investigating the mesolimbic circuit, we found that social buffering prevents the dopaminergic hyperactivity observed in the ventral tegmental area (VTA) of mice that develop depressive symptoms following the chronic social defeat stress. Next, we explored the effect of social buffering on medial prefrontal cortex (mPFC) projections to the basolateral amygdale (BLA), a projection known to be involved in mediating stress responses. Utilizing a retrograding AAV2/5-eYFP virus injection into the BLA, combined with in vitro slice recording in the mPFC, we performed electrophysiological recordings of mPFC neurons projecting specifically to the BLA (mPFC-BLA). We found that mPFC-BLA neurons of the mice provided with a social buffer, compared to stress naïve mice, exhibited an increase in the excitability. Interestingly, social buffering also reduces the hyperpolarization-activated current (Ih) in mPFC-BLA neurons.
Conclusions: The attenuated VTA neuron activity and increased excitation of the neuronal projections from the mPFC to the BLA following social buffering indicates that unique alterations within the circuit occur when social support is provided post stress. These findings strengthen the validity of SBCSS as a model to advance our understanding of the mechanisms of social buffering. Importantly, it raises the exciting possibility that a deeper understanding of the cellular mechanisms and circuits underlying social buffering can be elucidated for the development of mechanistically distinct therapeutics for depressive and anxiety related disorders.
Keywords: Social defeat stress, Depression, neural circuitry, Social buffering, VTA
Disclosures: Nothing to disclose.

W81. Effects of Repeated Lipopolysaccharide (LPS) Injections on Anxiety-Like Behavior and Brain Serotonin in Female BALB/cJ Mice

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Background: Inflammatory processes may promote the development of anxiety and mood disorders. It has been shown that female mice respond to bacterial endotoxin lipopolysaccharide treatment (LPS) with anhedonia-like symptoms. Also, it has been suggested that depressive symptoms after administration of an inflammatory challenge mostly stem from alterations in monoamine function. The goal of the present study was to analyze the effects of repeated injections of LPS on anxiety-like behavior and brain serotonergic function in female TPH2-sensitive mice.
Methods: Adult female BALB/c mice from Jackson Laboratory were treated three times, four days apart with an intraperitoneal injection of LPS (0.83 mg/kg dose) or a vehicle (saline). Anxiety-like behavior was assessed 72-h after each LPS or saline injection with the light/dark test (L/D test; a total of three different sessions), when pro-inflammatory cytokine expression is reported to be back to normal levels. Percentages of time spent in light, the percentage of the distance traveled in light and pokes into the light were automatically recorded. 2.5 weeks after initial LPS or saline

injection, mice were killed by decapitation and brains were immediately dissected and kept at -80C until subsequent analysis of brain tryptophan, serotonin (5-HT) and 5-HIAA by HPLC-ECD. The effects of the repeated LPS injections on behavior and neurochemistry were analyzed by repeated measures ANOVA.

Results: There was a main effect of LPS on anxiety-like behaviors and brain 5-HT in female BALB/cJ mice. LPS-injected mice spent less time in the light and traveled less distances in the light when compared with saline-treated mice. The repeated test session showed an adaptation-like effect of the saline-treated mice, whereas the LPS-treated mice showed the same anxiogenic-like effect. Hippocampus and amygdala 5-HT were significantly reduced in LPS-treated mice versus saline-treated mice one week after the last injection. In contrast, neither tryptophan nor 5-HIAA were affected by LPS treatment. Moreover, brain 5-HT turnover (ratio 5-HIAA/5-HT) was found to be increased in LPS-treated mice.

Conclusions: The repeated inflammatory challenge by LPS administration seems to produce a short-term anxiogenic-like effect and a long-term reduction effect on serotonergic neurotransmission in female BALB/cJ mice. The L/D test has been usually used for assessing anxiety-like behaviors. Healthy animals habituate to closely-spaced repeated L/D testing, but this habituation did not occur in animals receiving a repeated inflammatory challenge. Repeated injections of LPS did not enhance the anxiogenic-like effect among the different sessions. Further research on these strategies as possible models to study anxiety and mood disorders in female 5-HT-related impaired mice in comparison with animals with a more robust 5-HT system might be valuable. Repeated inflammatory challenge administration lead to persevering anxiety-like responses in L/D tests and a change in serotonergic function. Future studies should focus on elucidating the particular relationship between this behavior and the used neurochemical challenge procedure.

Keywords: Serotonin, LPS, Anxiety, Neuroinflammation

W82. What is the Nature of the Verbal Memory Deficit in Bipolar Disorder? A Discordant Sibling Pair Study

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Background: Verbal memory impairment is one of the most consistent deficits reported in patients with bipolar disorder (BD) and it is strongly correlated with functional disability. The exact nature of this impairment has not been systematically studied in BD but may be important in optimizing cognitive remediation strategies. We investigated the different components of a commonly used verbal learning measure in an effort to determine whether the deficit is seen during attentional aspects, learning (encoding/ consolidation), or memory (retrieval). We tested this in a large sample of BD patients, their unaffected siblings (UAS), and healthy controls (HCs), allowing us to further establish whether these deficits were related to genetic risk in BD families.

Methods: The California Verbal Learning Test (CVLT) was administered to 75 BD patients, 75 UAS and 119 HCs. Patients and UAS were well-matched to HCs with regard to age and IQ. All of the CVLT indices were converted to Z-scores for standardization using the HC group as a reference. We further subdivided learning and memory processes using the CVLT indices as follows: Attention (Trial 1), Learning [Total Recall 1 to 5, Learning Slope and Learning strategy (Semantic and Serial Clustering)] and Memory (Short Delay Recall, Long Delay Recall Percent Retention, and Recognition Hit Rate). The three groups were compared using one-way ANOVA.

Results: When compared to HCs we found that BD patients were impaired in Learning (Total Recall 1 to 5, $p < 0.001$; Slope, $p = 0.048$) and Memory Retrieval (Short Delay Recall, $p < 0.001$; and Long Delay Recall, $p < 0.001$; Percent Retention, $p = 0.037$). There were no significant differences (BD vs. HC) with regard to basic attentional capacity (Trial 1; $p = 0.095$) nor did BD patients show significant evidence of inefficient learning strategies (semantic and serial clustering $p = 0.135$ and $p = 0.075$ respectively). When we extended these analyses to compare the UAS with the unrelated HCs, we found that UAS were significantly impaired on both learning (Trial 1 to 5, $p = 0.016$; Slope $p = 0.022$) and memory components of the CVLT (Short Delay, $p = 0.030$).

Conclusions: BD patients showed verbal memory deficits in both encoding and retrieval processes. Although recognition was not abnormal among BD patients, the discriminability index suggested the hit rate was likely inflated due to a liberal response style. UAS also presented with deficits in both encoding and retrieval, however UAS benefited when semantic cueing was used during recall trials as well as during recognition performance; whereas BD patients did not appear to benefit from cues. It is likely that UAS are able to use these types of compensatory strategies in the context

of more mild impairments in learning and memory such that performance is normalized on some aspects of CVLT performance. However, the consistent deficits in both BD patients and their UAS on learning and short delay recall suggest that these impairments are related to genetic risk for the illness and may serve as useful endophenotypes in BD.

Keywords: Bipolar Disorder, Memory and Learning, Memory Encoding and Retrieval, Verbal Memory

Disclosures: Nothing to disclose.

W83. Effect of Subacute and Sustained Administration of Vortioxetine on Catecholaminergic System*s*

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Background: Preclinical studies show that selective serotonin reuptake inhibitors (SSRIs) not only cause a decrease in the firing rate of norepinephrine (NE) neurons of the locus coeruleus (LC) [1] and dopamine (DA) neurons of the ventral tegmental area (VTA) [2] but also decrease the extracellular levels of NE in the amygdala [3].Thus far it is conceivable that the lack of clinical response, side effects or residual symptoms in some major depressive disorder patients might stem from the inhibitory role of SSRIs on DA and NE neurotransmission [4]. Vortioxetine is a multimodal antidepressant that combines inhibition of the serotonin transporter (5-HTT) with direct modulation of 5-HT1A and four other types of serotonin (5-HT) receptors [5]. Since a 5-HT1A agonist, unlike a 5-HTT inhibitor, increases the firing activity of NE and DA neurons [6,7] in this study we have investigated the effects of subacute (4 days) and prolonged (14 days) administration of vortioxetine on factors controlling DA neurotransmission (firing activity of DA neurons) and NE neurotransmission (firing activity of NE neurons, activity of norepinephrine transporter (NET) and sensitivity of α2-adrenergic receptors expressed on pyramidal neurons of the hippocampus CA3 region). The regimen of vortioxetine in our study results in a similar level of 5-HTT occupancy to that of therapeutic doses of SSRIs (>80%) and also engages 5-HT receptor targets [8]. But since the affinity of vortioxetine for rat 5-HT1A receptors is 10-fold lower than that of humans, the effect of vortioxetine 5-HT1A receptor agonism on NE and DA neurotransmission may be underestimated in our study.

Methods: Male Sprague-Dawley rats, under chloral hydrate anesthesia, were used for in vivo single-unit electrophysiological recordings. Treated rats had ad libitum access to vortioxetine-infused chow (1.8 g/kg) and control rats received regular chow. This drug concentration has been documented to produce the same level of 5-HTT occupancy as the therapeutic doses of SSRIs (>80%) and also engages vortioxetine's receptor targets.

Results: Although 4-day exposure to vortioxetine decreased the mean firing rate of NE neurons by 40% (control: n = 144; vortioxetine: n = 51; P < 0.001), the dampened firing rate of NE neurons recovered to the level of the control group after 14 days of vortioxetine administration

(Controls: 2.2 Hz, treated: 1.6 Hz). Both regimens of vortioxetine administration left the bursting activity of NE neurons unaltered. RT50 is the time needed for the dampened firing rate of a recorded neuron to recover to 50% the baseline firing rate. Iontophoretic ejection of NE to hippocampal CA3 pyramidal neurons of 14-day vortioxetine-treated rats, revealed that RT50 values (an index of NE transporter activity) and number of spikes suppressed per nanoampere (a measure of sensitivity of α2-drenergic receptors on pyramidal neurons) were not altered as a result of the vortioxetine treatment. In the VTA, 4 days of vortioxetine administration did not alter the mean firing rate of DA neurons (control: n = 173, vortioxetine: n = 64), whereas 14-day administration significantly decreased (26%) the firing rate of these neurons (control: n = 173, vortioxetine: n = 100; P = <0.001). However, vortioxetine administration for 4 and 14 days did not change the bursting activity, the percentage of neurons firing in bursts or the number of spontaneously active DA neurons.

Conclusions: The present results indicate that dampened firing rate of NE neuron after 4-day administration of vortioxetine recovered to the level of the control group after 14-day vortioxetine administration. This recovery of firing rate, which is absent in SSRI- treated rats, could stem from the excitatory effect of 5-HT1A receptors, stimulated by vortioxetine agonism, on the firing activity of NE neurons. Neither vortioxetine regimens altered the bursting activity of NE neurons. In addition, 14-day vortioxetine administration, did not affect the activity of NET or the sensitivity of α2-adrenoreceptors on hippocampal CA3 pyramidal neurons. Unlike 4-day administration of vortioxetine, 14-day administration decreased the firing rate of DA neurons. Neither vortioxetine regimens altered the number of spontaneously active or bursting activity of DA neurons.

Keywords: vortioxetine, monoamines, electrophysiology

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W84. Involvement of SSAT in Depression in Post-Mortem Brains

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Background: Depression and suicidal behaviors are often observed in the same patients. However, only a subset of mood disorder patients ever attempt or complete suicide. Spermidine/Spermine N1-Acetyltransferase 1 is the rate limiting enzyme in the catabolism of polyamines and has been shown to be dysregulated in depressed suicides and in bipolar subjects with suicidal behaviors. The purpose of this study was to investigate brain gene expression changes associated with suicide in mood disorder patients in the dorsolateral prefrontal cortex (DLPFC), anterior cingulate cortex (ACC) and the nucleus accumbens (NACC).

Methods: Gene expression levels were of major SSAT1 isoforms were investigated by QPCR in a sample of 46 MDDs (18 non-suicides/26 suicides) and 20 Controls. Affymetrix HG-U133 Plus2.0 microarray chips data was available for a subset on subjects and was used for correlation analyses with other polyamine genes. Statistical analysis was performed using Partek Genomics Suite and an ANCOVA model was used to identify the differentially expressed genes between non-suicides and suicide controlling for age, gender, pH, and RNA degradation. Confirmation of results was performed by Western Blot using a Protein Simple imaging system and a commercially available anti-SSAT1 antibody (Novus).

Results: No correlation between gene expression levels and age, gender, pH or RNA degradation measures was observed. We confirmed the previously reported decrease in SSAT expression in depressed suicides and major depression subjects. We also observed a similar decrease in expression of another SSAT variant (isSSAT1) in major depressed subjects across brain regions. However, suicide overall did not play a major role as a factor in SSAT1 expression levels. Globally, expression levels were highly correlated between the most common SSAT1 isoforms across brain regions.

Conclusions: Major depressive disorder is a complex, multifactorial disorder, characterized by a significantly higher risk of committing suicide than the general population. Few post-mortem gene expression studies have been able to compare suicide versus non-suicide gene expression levels in post-mortem brains, and none have actually combined gene expression with protein expression in the same cohort. The identification of depression versus suicide risk factors could also lead to potential drug targets and a fundamental understanding of the molecular basis of these complex behaviors. Our findings support the involvement of the polyamine system and particularly of SSAT1 in major depression. The role of SSAT1 in suicide specifically is less clear as no suicide specific effect was observed.

Keywords: suicide, gene expression, dorsolateral prefrontal cortex, anterior cingulate cortex, nucleus accumbens

Disclosures: Nothing to disclose.

W85. Resting-State Functional Connectivity of Anterior Insula Differentiates Bipolar and Unipolar Depression

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Background: Mood disorders are characterized by emotion dysregulation and cognitive dysfunction, with bipolar disorder displaying more severe patterns of affective lability and cognitive deficits. Recent evidence suggests the anterior insula plays a key role in the adaptive regulation of affective and cognitive information, serving as an important hub for the integration and modulation of salience and executive control networks. Thus, variations in the extent of anterior insula functional connectivity within broader networks subserving the regulation of affect and cognition may provide clues towards potential biomarkers of dysfunction across the spectrum of mood disorders. Here, we explored patterns of whole-brain functional connectivity of bilateral anterior insula across a sample of unipolar depressed, bipolar depressed, and healthy individuals. Further, to understand the relationship between potential variability of anterior insula functional connectivity and dysfunction across mood disorders, we examined the relationship between anterior insula connectivity and behavioral measures of affective control, anxiety sensitivity and neuroticism.

Methods: Resting state fMRI data were collected from 39 patients (15 unipolar, 24 bipolar) and 40 healthy controls. After standard data preprocessing steps, whole-brain connectivity analyses were conducted using independently-defined bilateral insula seeds (k = 3; Kelly et al., 2014). Pearson correlations coefficients were computed between individual insula seed regions and all remaining whole-brain voxels. After r-to-z transformation, one-way ANOVAs and follow-up independent t-tests were run in SPM8 to identify significant differences in connectivity between groups. To examine the relationship between functional connectivity strength and behavioral outcomes, a series of whole brain regressions were conducted in SPM8 using scores from the Affective Control Scale (ACS), Anxiety Sensitivity Index (ASI) and NEO-Five Factor Model of Personality-Neuroticism Subscale (NEO-N) as predictor variables.

Results: Bipolar patients were characterized by weaker bilateral anterior insula–inferior frontal gyrus (IFG) and anterior insula-inferior parietal lobule (IPL) functional connectivity, key regions within the fronto-parietal executive control and salience networks. Further, weaker anterior insula-IFG functional connectivity was associated with greater dysfunction in affective control, and weaker anterior insula-IPL connectivity was associated with higher ratings of anxiety sensitivity. By contrast, patients with unipolar depression did not differ from healthy controls in anterior insula–IFG or anterior insula–IPL functional connectivity, but showed stronger anterior insula-amygdala and insula-striatal functional connectivity than bipolar patients or healthy controls. Patients across both groups were distinguished from healthy controls by increased anterior insula-cerebellum functional connectivity (bilaterally in bipolar patients, left lateralized in unipolar patients). Stronger anterior insula-cerebellar functional connectivity was predictive of higher behavioral ratings of neuroticism and greater dysfunction in affective control.

Conclusions: In a whole-brain functional connectivity analysis, unipolar and bipolar depression differed from healthy controls through both unique and overlapping patterns of functional connectivity to the anterior insula. Bipolar depression was uniquely characterized by deficits in functional connectivity between anterior insula and key regions implicated in emotion regulation and cognitive control. By contrast, unipolar depression was uniquely characterized by increased functional connectivity between anterior insula and salience and reward processing regions. These data suggest deficits in adaptive insular functional connectivity may be an important factor underlying specific patterns of pathology in depression subtypes, a potential biomarker in need of further research.

Keywords: mood disorders, Insula Connectivity, Resting State Functional Connectivity
Disclosures: Nothing to disclose.

W86. Genome-Wide Association Analyses on Clinical Response to Duloxetine and Placebo in Major Depression Implicates a Gene Locus Involved in Nociception to be Associated with Placebo Response

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Background: Major depressive disorder is a prevalent psychiatric disorder commonly treated with antidepressant medications. Duloxetine is a frequently prescribed antidepressant which is particularly prescribed with co-morbid pain symptoms. However, as with most other antidepressant medications, duloxetine is only beneficial to a subgroup of depressed patients while others do not show improvement or develop side effects. Genetic factors are known to influence outcome to antidepressant drugs while the first commercially available gene test for drug metabolizing enzymes are now increasingly being implemented in psychiatric practice. However, more studies are required to identify novel gene variants and to validate previous findings. Identifying a 'genetic signature' for duloxetine response will therefore help to predict responders to duloxetine and allow to implement improved personalized treatment care.
Notably, a substantial alleviation of depressed and pain symptoms is commonly also observed with placebo medication. Placebo response is poorly understood and identifying biological causes will help identifying new drug targets and help optimizing clinical drug trials.
This study investigated duloxetine and placebo response in major depression and if treatment effects may rely on similar genetic components.
Methods: We performed the first genome-wide association study in patients treated with either duloxetine (n = 215) or placebo (n = 235) for up to 8 weeks. Genotyping was performed using the PsychChip, which is endorsed by the Psychiatric Genomics Consortium. The samples and clinical data were provided by H. Lundbeck A/S under Lu activity number 15761. Treatment response was operationalized as MADRS score changes (%) from baseline and was used as the main outcome variable in an ANCOVA model, including baseline depression severity, length of treatment and cohort as covariates. High standard quality controls were applied on generated SNPs and on study subjects, controlling for factors such as ethnicity, heterozigosity rates, and degree of relatedness. In addition, we conducted imputation analyses allowing us to include close to two million gene variants for each individual.
Results: As for response to duloxetine, top hits were observed in regions on chromosome 1, 7 and 19 implicating a previously unnoted intergenic variant, a variant resulting in a missense signal in a gene involved in cell cycle progression, and an intronic variant in a gene involved in

endocytosis. However, none of the findings reached significance after correction for genome-wide analyses and thus would represent suggestive findings (p < 10-6). In contrast, as for response to placebo, a significant, genome-wide corrected hit was found in a region on chromosome 3 (p = 1.87E-09). This particular locus is located at a relatively short distance (150kb) from the SH3 and cysteine rich domain (STAC) gene, implicated in neuron-specific signal transduction, and expressed in nociceptive (pain processing) neurons. Carriers of the CC genotype improved on average by 49.6% of MADRS score while non-carriers only improved by 23.9%; a clinically relevant and meaningful difference. A second, suggestive finding (p < 10-6), was found with a marker located in a gene involved in thyroid functioning.
Conclusions: Interestingly, our results do not appear to support the notion that similar pathways were involved in each of the two treatment groups. The genome-wide corrected significant finding for placebo response is particularly interesting given the proximity to a gene involved in pain signalling mechanisms. Considering that placebo commonly improves pain symptoms, which are frequent co-morbid conditions and reinforcing risk factors of depression, this result bears some biologically plausibility. The region associated with placebo response in our study may harbour gene variants that act as remote promoters to regulate expression of the STAC gene. The suggestive finding related to thyroid functioning is also of interest, since dysfunction of the thyroid gland (i.e. hypothyroidism) is a well known risk factor for depression.
In summary and to the best of our knowledge, this is the first study detecting a genome-wide significant association with response to placebo in depressed patients. We are currently evaluating our findings in independent patient samples also treated with placebo for major depression.
Keywords: Major Depressive Disorder (MDD), chronic pain, pharmacogenomics, Placebo Response, duloxetine
Disclosures: Nothing to disclose.

W87. Combinatorial Pharmacogenomics for Personalized Antidepressant Therapy: Clinical and Economic Validity and Utility

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Background: Pharmacogenomic guidance for psychiatric medications has been pursued to increase treatment response, reduce adverse events, and decrease healthcare utilizations and costs. These clinical approaches should mitigate the considerable health care burdens and disabilities for patients with depression [Mrazek et al. 2014], anxiety, and psychosis [Kennedy et al, 2013]. A pharmacogenomic approach requires that diverse genomic information is integrated and provided to clinical caregivers through readily interpretable results.
Methods: GeneSight Psychotropic is a combinatorial pharmacogenomic test (CPGxTM) that creates a composite

phenotype based on allelic variations in six genes that encode cytochrome P450 (CYP) enzymes (CYP2D6, CYP2C19, CYP2C9, CYP1A2, CYP2B6, and CYP3A4) and two pharmacodynamic genes, the serotonin (5HT) transporter (SLC6A4) and the 5-HT2A receptor (HTR2A) [Altar et al, 2013]. The test stratifies 38 psychotropic medications into one of three cautionary categories, based on the metabolic pathways and mechanisms of action for each medication, and recommends dose adjustments to healthcare providers.

Results: Studies have demonstrated the ability of the CPGx test to predict symptom improvement for major depressive disorder (MDD) patients and predict decreases in healthcare utilizations. For example, unguided subjects who at baseline were prescribed genetically discordant "red" category medications ("Use with caution and with more frequent monitoring") showed only a 12% improvement in their depressive symptomatology, less than the 33% or 29% improvements of subjects prescribed yellow ("Use with caution"; p = 0.002), or green category medications ("Use as directed"; p = 0.02). Phenotypes ascribed to single genes failed to make these predictions for the same patients. Compared to unguided patients, those whose healthcare providers received the test had a 2.3-fold greater odds of clinical response (p = 0.004), a 57% greater improvement in depressive symptoms (p = 0.0002), and a 1.7-fold relative improvement in response (p = 0.01) [Hall-Flavin et al, 2013; Altar et al, 2015]. For MDD patients, GeneSight has predicted far higher healthcare utilizations than those whose medications were congruent with recommendations of the report [Winner et al, 2013]. GeneSight-guided therapy in psychiatric disorders saved the healthcare system $1,036 a year, during which time patients better adhered to medications and reduced polypharmacy vs. the unguided, standard of care patients. [Winner et al, 2015].

Conclusions: The clinical and economic validity and utility of CPGx in pharmacotherapy of depression and anxiety disorders may broaden its adoption. CPGx may be of benefit because it lessens the variability of the empirical approach that has traditionally been used to prescribe medications for psychiatric indications.

Keywords: Pharmacogenomics, antidepressant, depression, anxiolytic, anxiety

Disclosures: All authors in this study are employees of Assurex Health Inc.

W88. Clinical Outcomes Associated with Comorbid Posttraumatic Stress Disorder among Patients with Bipolar Disorder

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Background: Lifetime prevalence of bipolar disorder is 2.1% worldwide, with subthreshold forms affecting another 2.4%(Merikangas et al., 2007). Posttraumatic stress disorder has an estimated lifetime prevalence of 7.6% in general population(Kessler et al., 2005). The National Comorbidity

Survey Replication has shown that the lifetime prevalence of posttraumatic stress disorder among patients with bipolar disorder is 24% (Merikangas et al., 2007). Prior cross-sectional studies have shown that comorbid posttraumatic stress disorder is associated with worse quality of life and higher rates of suicide attempts among patients with bipolar disorder (Dilsaver et al., 2007; Quarantini et al., 2010). However, no study have assessed the impact of posttraumatic stress disorder in the core features of bipolar disorder.

Methods: Cross-sectional study of 284 subjects with bipolar disorder assessing the association between lifetime comorbid posttraumatic stress disorder and clinical characteristics. Participants were included from January 2006 to June 2009. We assessed age of onset and number of mood episodes, presence of rapid cycling, first drug use, suicide attempts, hospitalizations, functional impairment, and quality of life. Diagnostic, clinical, and functional assessments were carried out using the Structured Clinical Interview for DSM Disorders (SCID), the Functioning Assessment Short Test (FAST), and the World Health Organization Quality of Life scale (WHOQOL).

Results: The prevalence of lifetime comorbid posttraumatic stress disorder was 19.7% (56 subjects). Subjects with bipolar disorder and posttraumatic stress disorder presented an accelerated course of illness, with a lower age of onset of manic/hypomanic episodes (p = 0.009), and earlier initiation on illicit drug use (p = 0.008). In addition, they were more likely to be younger at the age they received the diagnosis of bipolar disorder (p = 0.036), and presented a higher number of manic/hypomanic episodes (p = 0.01). Of note, the last analysis was corrected by age of onset of manic/hypomanic episodes. Quality of life was worse in all domains among subjects who presented the comorbidity, as well as higher rates of functional impairment.

Conclusions: Comorbid posttraumatic stress disorder was associated with increased morbidity among subjects with bipolar disorder. Since patients with both disorders are associated with higher number of manic episodes, future studies should assess the impact of the use of antidepressants in such population. Psychosocial interventions that have been shown to improve both posttraumatic stress disorder and bipolar disorder may offer an important alternative in this subset of patients.

Keywords: bipolar disorder, functional impairment, posttraumatic stress disorder

Disclosures: Dr Jair C Soares has received grants/research support from Forrest, BMS, Merck, and has been a consultant for Roche and Abbott. The other authors report no financial or other relationship relevant to the subject of this article.

W89. Inflammation and Neurocognition in Bipolar Disorder

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Background: Cognitive deficits are highly disabling core features of bipolar disorder (BD); however, there is

considerable heterogeneity among patients; approximately 60% of euthymic BD patients show clinically relevant cognitive deficits and the remaining proportion are cognitively-spared. Why some BD patients are cognitively impaired while others are not remains unknown, hampering treatment and prevention.

BD has been conceptualized as a neurodevelopmental as well as a neuroprogressive illness, with worsening of cognitive deficits with each mood episode. One proposed mechanism for neuroprogression is the presence of elevated inflammatory markers during acute affective episodes in BD that are thought to result in toxic effects to the brain. There is converging evidence of a disturbance in inflammatory/cytokine and neurotrophic markers in BD, with prior studies reporting a pro-inflammatory profile (increased levels of IL-1, sIL-2R, IL-6, TNF-α-2, CRP) during acute phases, a profile which is only partially ameliorated during affective remission. The only published study investigating the relationship between plasma biomarkers and cognition in BD reported a significant correlation between TNF-alpha and inhibitory control performance, indicating a relationship between higher cytokine levels and neurocognitive impairment. These results are intriguing; however, replication and extension of these results in a larger cohort will be critical in understanding the role for these and other biomarkers in neurocognitive functioning in BD.

Methods: We have analyzed a sample of 40 BD I patients who have undergone a comprehensive clinical and neuro-cognitive battery alongside a panel of 41 peripheral markers (HIMC Human 41-Plex Luminex Plate) including, pro- and anti-inflammatory markers as well as growth factors. This assay represents the broadest approach reported to date. Patients were sub-grouped based on the presence (at least -1 SD below healthy controls) or absence of neurocognitive impairment and cytokine levels were compared between groups.

Results: 65% of BD patients (n = 26) met criteria for significant cognitive impairment and were classified as such; 35% were classified as cognitively intact (n = 14). Cognitive subgroups were well-matched with regard to clinical and demographic features but differed significantly (p < 0.05) on several of the inflammatory markers. Group differences included pro-inflammatory (IFN-r; IL-17a; IL-6; IL-8; TNF-alpha); anti-inflammatory (IL-10; IL-13; IL-RA); and growth factors (VEGF; FGF), with elevated levels across all markers seen in those BD patients who were cognitively intact. While in some instances these results may seem counterintuitive (e.g. pro-inflammatory elevations in cognitively intact relative to cognitively impaired BD patients), the importance of profiles including the full cassette of markers is highlighted. Specifically, when accounting for ratios among biologically interacting molecules such as IL-6 and TNF-alpha are taken into account, results are directionally consistent with higher levels of inflammation in BD patients who demonstrate significant cognitive impairment.

Conclusions: Our findings emphasize the importance of assessing a wide range of cytokines/immune markers to allow for detailed inflammatory profiles to be analyzed in the context of a biologically complex system. Ongoing analyses will substantially increase our sample size and provide opportunities for more sophisticated analyses incorporating both pro- and anti-inflammatory molecules. Our early results point toward a relationship between inflammation and cognition in BD providing new pathways to target for cognitive enhancement strategies.

Keywords: inflammation, Cognition, Bipolar Disorder
Disclosures: Nothing to disclose.

W90. Electroconvulsive Therapy (ECT) Modulation of Reward Processing and Circuit Dynamics in Depression

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Background: Electroconvulsive Therapy (ECT) is the most effective treatment in psychiatry, and the oldest brain stimulation modality. Although it causes a generalized seizure, its therapeutic benefit is not global but limited to specific clinical syndromes, of which mood disorders and major depressive episodes in particular are the most common indication.

Deficits in reward processing are key to depression, presenting as anhedonia and amotivation. In this study we investigated the modulation of the reward circuitry of depressed patients treated with ECT using functional connectivity MRI, and how this physiological modulation explains the therapeutic change in anhedonia, motivation and depression severity.

Methods: We studied 13 patients presenting with a major depressive episode treated with ECT. Before and after the acute course of treatment, we obtained resting state fMRI data, behavioral measures of reward anticipation and consummation with the Temporal Experience of Pleasure Scale (TEPS), and syndromal measures of depression severity with the Quick Inventory of Depression Severity (QUIDS).

After standard data preprocessing steps, whole-brain connectivity analyses were conducted using bilateral seeds of the (1) ventral tegmental area (VTA), (2) the nucleus accumbens (NAc) and (3) the reward core (RC: VTA + NAc). Pearson correlations coefficients were computed between individual seed regions and all remaining whole-brain voxels. After r-to-z transformation, paired t-tests were run in SPM8 to identify significant differences in connectivity between groups. To examine the relationship between functional connectivity and behavioral outcomes, whole brain regressions were conducted in SPM8 using scores from the TEPS-consummation, TEPS-anticipation and QUIDS as predictor variables.

Results: After ECT, the RC and NAc increased their connectivity with the left frontal pole and the right precentral gyrus. The VTA showed increased in connectivity with the DLPFC bilaterally and decreases with the left anterior middle temporal gyrus.

Changes in reward anticipation correlated positively with changes in connectivity of the RC and NAc to the posterior cingulate, precuneus and right angular gyrus, and negatively

with changes between the bilateral NAc alone to the left inferior frontal gyrus. No significant changes were observed with the VTA alone.

Changes in reward consummation correlated positively with changes in connectivity from the RC and NAc to the vmPFC and rostral ACC, and only the NAc to the left middle frontal gyrus and precuneus. Positive correlations were observed with changes from the VTA to the dorsal ACC and left inferior frontal gyrus.

Changes in depression severity correlated positively with changes in connectivity of the VTA and the dorsal ACC, and negatively with the VTA to left cerebellum. No correlations were observed with changes of RC or NAc connectivity.

Conclusions: Identifying the mechanism of action of our most effective therapies is a priority for our field. In this study we describe changes in functional connectivity within key disease-relevant circuits in patients with depression undergoing ECT, and are able to dissect different patterns of VTA and NAc connectivity that explain changes in anhedonia, motivation and depression severity. These data highlight the value of this paradigm for therapeutic targets discovery within a human systems neuroscience framework.

Keywords: electroconvulsive therapy, reward neural circuitry, Depression, Resting State Functional Connectivity, new targets

Disclosures: Nothing to disclose.

W91. The Efficacy of a Comprehensive Yogic Intervention on Major Depression – A Randomized Pilot Study with Inflammatory Biomarkers

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Background: Psychopharmacology and psychotherapy are effective treatments for Major Depressive Disorder (MDD); however, each typically provides remission rates between 35%-50%. Moreover, approximately 50% of patients treated with two trials of antidepressant interventions do not achieve clinical remission. Thus, additional evidence-based treatment modalities that can further treat this devastating disorder are needed. Mind-Body practices constitute a large and diverse group of practices that can substantially affect neurophysiology in both healthy individuals and those with psychiatric disorders. Initial studies with different mind-body interventions have been shown to improve outcomes in MDD. Studies utilizing Sudarshan Kriya Yoga (SKY) have demonstrated promising clinical benefits in patients ranging from dysthymic disorder to severe forms of MDD. However, these studies had methodological limitations. This pilot study employs a randomized design to assess the clinical and biological impact of a multi-component yogic intervention featuring SKY as an adjunctive treatment for MDD.

Methods: Consenting outpatients (n = 25) were diagnosed utilizing the Structured Clinical Interview for DSM-IV. All patients were diagnosed with MDD, symptomatic (Hamilton Rating Scale for Depression (HAM-D) $\geq$ 14) and continued

pre-study psychotropic medications for the entire duration of the study. No changes to medications were allowed during the 8 week study duration. Using a blocked randomization procedure, enrolled subjects are randomized to either the yoga intervention or waitlist control group. Additional post-intervention assessments at 1 month and 2 months were conducted for both the yogic treatment and waitlist arms. All evaluators were blinded to the treatment assignment. Response was defined by absence of DSM MDD, at least 50% reduction in HAM-D and a 17-item HAM-D $\leq$ 10. Responders who ended treatment with a HAM-D $\leq$ 7 were considered to be in clinical remission. Additional assessments included the Hamilton Anxiety Rating Scale (HAM-A), Beck Depression Inventory (BDI-II), Beck Anxiety Inventory (BAI) and Columbia-Suicide Severity Rating Scale (C-SSRS) were conducted. Serum biomarkers including TNF-alpha, IL-10, CRP and cortisol were assessed at baseline, 1 month and 2 months. The study will be compete in November 2015.

Results: A direct comparison between the yoga intervention and waitlist control groups at baseline, 30 days and 60 days was conducted on the 17-item Hamilton Rating Scale for Depression (HAM-D). A comprehensive yogic intervention decreased depression severity in unipolar depression by 50% at 1 month and 2 months. The waitlist control group did not demonstrate any changes in depression severity. A direct comparison between the yoga intervention and waitlist control groups at baseline, 30 days and 60 days was also conducted on the Beck Anxiety Inventory (BAI). A comprehensive yogic intervention decreased anxiety severity in unipolar depression by 50% at 1 month and 2 months. The waitlist control group did not demonstrate any changes in anxiety severity. The improvement in clinical symptoms positively correlated with reductions in serum levels of TNF-alpha, IL-10 and CRP.

Conclusions: This pilot study establishes feasibility and efficacy in the evaluation of a multicomponent yoga intervention for MDD on clinical measures and serum biomarkers of stress and inflammation.

Keywords: Depression, Major Depressive Disorder, Clinical trial

Disclosures: Nothing to disclose.

W93. Systematic Review and Meta-Analysis: Dose-Response Relationship of Selective-Serotonin Reuptake Inhibitors in Major Depressive Disorder

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Background: Previous studies suggested that the treatment response to Selective-Serotonin Reuptake Inhibitors (SSRIs) in Major Depressive Disorder (MDD) follows a flat response curve within the therapeutic dose range. Our study was designed to clarify the relationship between dosage and treatment response in MDD.

Methods: We searched PubMed for randomized placebo-controlled trials examining the efficacy of SSRIs for treating adults with MDD. Trials were also required to assess

improvement in depression severity at multiple time points. Additional data was collected on treatment response and all cause and side effect-related discontinuation. All medication doses were transformed into imipramine equivalent doses. The longitudinal data was analyzed with a mixed regression model. Endpoint and tolerability analyses were analyzed using meta-regression and stratified subgroup analysis by predefined SSRI dose categories in order to assess the effect of SSRI dosing on the efficacy and tolerability of SSRIs for MDD.

Results: We included 40 studies involving 10,039 participants. Longitudinal modeling [dose x time interaction $= 0.0007 (95\%\text{CI}:0.0001\text{-}0.0013; p = 0.0196)$] and endpoint analysis (meta-regression $\beta = 0.00053, 95\%\text{CI}:0.00018\text{--}0.00088, z = 2.98, p = 0.0029$) demonstrated a small, but statistically significant positive association between SSRI dose and efficacy. Higher doses of SSRIs were associated with an increased likelihood of dropouts due to side-effects (meta-regression $\beta = 0.00207, 95\%\text{CI}:0.00071\text{--}0.00342, z = 2.98, p = 0.003$) and decreased likelihood of all-cause dropout (meta-regression $\beta = \text{-}0.00093, 95\% \text{ CI-}0.00165\text{--}(\text{-}0.00021), z = \text{-}2.54, p = 0.01$).

Conclusions: Higher doses of SSRIs appear slightly more effective in MDD. This benefit appears to plateau around 250mg of imipramine equivalents (50mg of fluoxetine). The slightly increased benefits of SSRIs at higher doses are somewhat offset by decreased tolerability at high doses.

Keywords: Major depression, meta-analysis, SSRI

Disclosures: Nothing to disclose.

W94. Habenular Endocannabinoid Signaling Participates in Behavioral and Neuroendocrine Responses to Stress

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Background: Major depressive disorder (MDD) has a pervasive impact on both individual and societal functioning. MDD is the current leading cause of disability both in the US and worldwide, yet effective pharmacotherapeutic strategies for treating MDD remain elusive. Exposure to severe or prolonged stress is historically known to contribute to the development of MDD. However, stress does not invariably lead to MDD; indeed, most people exhibit resilience to stress and are able to implement effective coping strategies to avoid such pathologies. As such, studying the neurobiological mechanisms that confer divergent stress coping strategies is paramount to understanding the pathophysiological origins and treatment of MDD. The lateral habenula (LHb) has recently emerged as an intriguing new target for MDD, although the precise mechanisms by which the LHb influences stress coping and emotionality are currently unknown. Converging evidence suggests that the endocannabinoid (ECB) system plays a crucial role in regulating the neuroendocrine and behavioral response to stress. Given the role of the ECB system in regulating stress and emotional behavior, and recent studies demonstrating the importance of the LHb in the development and treatment of MDD, it is conceivable that ECB signaling in the LHb may dictate stress coping strategies, the dysfunction of which could have implications for the development of MDD. However, the functional role of ECB signaling in the LHb has yet to be empirically evaluated.

Methods: In the present study, we used a battery of preclinical tests to examine whether site-specific pharmacological blockade of CB1 receptors in the LHb alters behavioral stress coping strategies, anxiety-like behavior, and basal or stress-induced neuroendocrine responses in male and female Sprague Dawley rats. All rats were implanted with bilateral cannula aimed at the LHb, given a week to recover, and then subjected to the elevated plus maze (EPM) test, novelty suppressed feeding (NSF) test, forced swim test (FST), or exposed to a 30 min acute restraint episode where corticosterone (CORT) was assayed at 0 min (basal), 30, 60, or 90 min post-stress onset. All rats received intracranial microinfusions of the CB1 receptor antagonist SR141716 (0.3 ug/side) or an equivalent volume of vehicle prior to testing. For the EPM, the amount of time and number of entries into the open (i.e., exposed) arms and closed (i.e., safe) arms of the EPM were assessed during a 5 min test session. For the NSF test, the latency to approach and consume a familiar peanut butter chip in the center quadrant of a novel open environment was examined. For the FST, rats were subjected to two inescapable forced swim sessions separated by 24 hours (15 min pre-exposure session on day 1 and 5 min test session on day 2) and the amount of time spent immobile, swimming, or struggling/climbing was quantified. After testing, cannula placements were verified histologically and any placements that were deemed outside the boundaries of the LHb were excluded from further analyses.

Results: Local blockade of CB1 receptors in the LHb significantly increased the time spent exploring the open arms of the EPM and reduced the latency to approach/consume the food in the NSF, both of which are indicative of reduced anxiety-like behavior. Local CB1 receptor blockade also significantly reduced the time spent immobile in the FST and significantly increased struggling/climbing behavior in both male and female rats, suggestive of increased reliance on escape-directed active coping strategies. Local CB1 receptor blockade also significantly augmented basal and stress-induced CORT secretion at 30 min post-stress onset compared to vehicle-treated rats. Ongoing studies are currently assessing the impact of local CB1 receptor activation in the LHb on these same behavioral and neuroendocrine endpoints.

Conclusions: These preliminary data suggest that CB1 receptor blockade in the LHb exerts robust anxiolytic effects, promotes the adoption of active, escape-directed stress coping strategies, and augments basal and stress-induced CORT secretion in response to acute restraint stress in male rats. These findings contribute to our understanding of how ECB signaling in the LHb influences stress and anxiety-like behaviors and also points to the LHb as a putative novel site of action for the controversial anxiolytic and antidepressant-like effects of systemic CB1 receptor blockade in prior preclinical reports.

Keywords: Endocannabinoids, stress, Lateral Habenula, corticosterone, Anxiety

Disclosures: Nothing to disclose.

W95. Ketamine-Induced Inhibition of Glycogen Synthase Kinase-3 Contributes to the Augmentation of AMPA Receptor Signaling

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Background: Sub-anesthetic doses of ketamine have been found to provide rapid antidepressant actions, indicating that the cellular signaling systems targeted by ketamine are potential sites for therapeutic intervention. Ketamine acts as an antagonist of N-methyl-D-aspartate (NMDA) receptors, and animal studies indicate that subsequent augmentation of signaling by α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptors is critical for the antidepressant outcome.

Methods: Wild-type and GSK3 knockin mice were treated with 10 mg/kg ketamine for 30 min and membrane and synaptosome fractions were extracted from hippocampus and immunoblotted for the AMPA receptors and PSD95.

Results: Here we tested if the inhibitory effect of ketamine on glycogen synthase kinase-3 (GSK3) affected hippocampal cell-surface AMPA receptors. Treatment with an antidepressant dose of ketamine increased the hippocampal membrane level of the AMPA receptor GluR1 subunit, but did not alter the localization of GluR2, GluR3, or GluR4. This effect of ketamine was abrogated in GSK3 knockin mice expressing mutant GSK3 that cannot be inhibited by ketamine, demonstrating that ketamine-induced inhibition of GSK3 is necessary for up-regulation of cell surface AMPA GluR1 subunits. AMPA receptor trafficking is regulated by PSD95, a substrate for GSK3. Ketamine treatment decreased the hippocampal membrane level of phosphorylated PSD-95 on Thr-19, the target of GSK3 that promotes AMPA receptor internalization.

Conclusions: These results demonstrate that ketamine-induced inhibition of GSK3 causes reduced phosphorylation of PSD-95, diminishing the internalization of AMPA GluR1 subunits to allow for augmented signaling through AMPA receptors following ketamine treatment.

Keywords: Ketamine, glycogen synthase kinase-3, AMPA

Disclosures: Nothing to disclose.

W96. Epigenetic Mechanisms of Hippocampal Plasticity: P300-Driven mGlu2 Up-Regulation Mediates Resilience and Antidepressant Responses

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Background: A same event may be more or less stressful or have pathophysiological consequences for one individual compared with another depending on prior experiences even when the genotype is similar or identical1. A past history of stress has an important role in opening windows of plasticity for positive as well as negative events2. Depression and anxiety, major public health problems, are examples of a loss of resilience where an intervention at the right time can have a good effect. Recently, we devised a rapid screening method to identify susceptible individuals among genetically similar animals. Mice that spontaneously show increased anxiety have elevated expression of hippocampal mineralocorticoid receptors (MR), which, after acute or chronic stress, mediates a suppression of mGlu2, a presynaptic regulator of glutamate release and increases levels of anxiety- and depressive-like behavior, respectively, because of an uncontrolled overflow of the neurotransmitter, glutamate1,3.

Methods: Male C57black and BDNF Val66Met (7wk old) mice were housed five per cage under controlled conditions with food and water ad libitum. Mice were stressed for 2h for 21 consecutive days; euthanasia occurred 24h after chronic stress. For the RNAseq, tissues from animals with the same vulnerability were pooled together to yield the proper amount of RNA per sample. We aimed at a design with balanced-blocks by multiplexing samples, in order to eliminate potential confounding caused by lane effects. Thus each sample was provided with a unique adapter and all samples were put in the same pool. The pool was sequenced in two lanes using Illumina Hiseq2500 to yield the desired sequencing depth. The Fastq files were evaluated for quality control using FastQC and trimmed with Trimmomatic. Alignment and statistical analysis were performed using TopHat and EdgeR. Gene validation was performed by qPCR. For IHC, all groups were represented on each slide to avoid any difference in the DAB reaction. Electrophysiological experiments have been assessed at the same time point of the behavioral and molecular analyses4.

Results: RNA deep sequencing (RNA-seq) analysis in the DG of either chronically-stressed wild-type mice or susceptible BDNVal66Met mice show similar profile of gene expression changes, revealing altered expression of many glutamate genes, such as mGlu2, and epigenetic regulators of gene transcription, such as the histone acetylatransferase P300. When an acute stress is applied to either mice with a history of stress (CRS) or to mice with an inherent susceptibility to stress (BDNFVal66Met mice), we observed a rapid and transient up-regulation of mGlu2 by a P300-driven acetylation of histone-H3-lysine-27 (H3K27ac) along with a transient improvement in the depressive- and anxiety-like behaviors. This "window of epigenetic plasticity" is reflected also in electrophysiological changes in the DG in vitro as we found that DG-long term potentiation (LTP) is decreased after CRS compared to non-stressed mice with habituation when the same acute restraint stressor is applied to CRS mice. This suggest that in the immediate aftermath of chronic stress in wild-type mice as well as in heterozygous BDNFVal66Met mice, there is a "window of plasticity" that may be useful for treatment of stress-related disorders to, more permanently, increase plasticity. Indeed the depressive-like behavior associated with chronic stress in susceptible individuals as well as the naïve mice bearing the BDNFVal66Met SNP is corrected by treatment with the novel antidepressant candidate acetyl-L-carnitine (LAC)4,5,6. LAC rapidly up-regulates mGlu2 expression in hippocampus by an epigenetic mechanism of hippocampal plasticity driven by P300 that increases acetylation of H3K27 bound to Grm2 promoter, which encodes for mGlu2 receptors.

Conclusions: In all, these findings indicate that the behavioral, molecular and structural changes seen either after prolonged chronic stress in susceptible wild-type mice or in naive genetically susceptible mice carrying a BDNFVal66Met polymorphism (SNP) that represents at least 33% of the human population, are modifiable when treated with appropriate therapies at the right time. The speed of action of LAC could also be beneficial for suicide prevention.

This findings show that there is a "window of epigenetic plasticity" that offers vast opportunities for behavioral and pharmacological interventions to increase resilience by correcting imbalances of excitatory transmission through regulation of gene transcription via histone modifications and related epigenetic alterations and thereby reestablishing balanced neural circuitry in the hippocampus, prefrontal cortex and amygdala that become unbalanced in anxiety and depressive disorders.

Keywords: Fast-acting Antidepressant, Epigenetics, neurobiology, stress, depression, anxiety, adolescence, glutamate, Histone

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W97. Neuroendocrine and Behavioral Effects of Cort 118335, a Novel Glucocorticoid and Mineralocorticoid Receptor Antagonist in Male Rats

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Background:: Dysfunction of the hypothalamic-pituitary-adrenal (HPA) axis due to aberrant glucocorticoid secretion is associated with psychopathology. Consistent with this fact, compounds targeting both Type I (mineralocorticoid MR) and Type II (glucocorticoid GR) corticosteroid receptors are being advanced as putative antidepressants or anxiolytics. Previous findings from our laboratory indicate that GR antagonists decrease neuroendocrine, cellular, and behavioral (depression-like) responses to stress in male rats.

Methods: The goal of the present study was to determine the impact of GR/MR antagonism on neuroendocrine and behavioral responses to acute stress in male rats. This was accomplished by using CORT 118335, a dual GR/MR antagonist. In two separate experiments, adult male rats were treated for 5 days with vehicle, CORT 118335 (10mg/kg or 30mg/kg), or imipramine (10mg/kg) (tricyclic antidepressant) and exposed to either restraint stress or the forced swim test (FST).

Results: Relative to vehicle, both doses of CORT 118335 potently suppressed neuroendocrine responses to restraint and FST stress exposure. The decreased neuroendocrine output in animals treated with CORT 118335 was not accompanied by an antidepressant-like effect of the compound in the FST, as there was no difference in immobility between animals treated with this compound versus vehicle. Consistent with our previous findings, imipramine (positive control) modestly decreased neuroendocrine responses to both restraint stress and FST relative to animals treated with vehicle and decreased immobility in the FST, consistent with antidepressant effects. Notably, inactivation of the infralimbic cortex (IL) decreases depression-like behavior in rats and in alignment with the behavioral observations, imipramine decreased c-Fos immunolabeling in the IL.

Conclusions: Taken together, the data suggest dissociation between corticosterone responses and immobility in the FST. The data further suggest that compounds targeting both MR/GR may be useful for mitigating glucocorticoid hyper-secretion to stress, but their ability to modulate mood-like responses to acute stress (at least under the context of the present study) may be limited. Studies are currently underway to investigate the efficacy of this compound in mitigating neuroendocrine and behavioral anomalies in response to chronic stress in both males and females.

Keywords: Acute Stress, glucocorticoid receptor, Depression, forced swim test, antidepressants

W98. Female Rats Exhibit Lower Baseline Dopamine Neuron Activity and Higher Depression Behavior that is Exacerbated in the UCMS Depression Model: Impact of Ketamine

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Background: Depression is a highly prevalent and debilitating psychiatric disorder that affects women at roughly twice the rates as men (Kessler, 2003). Underactivation of the dopamine (DA) system has been implicated in the pathophysiology of depression (Belujon & Grace, 2015). Indeed, two distinct animal models of depression [i.e. unpredictable chronic mild stress (UCMS), learned help-

lessness] converge in decreasing DA neuron activity in the ventral tegmental area (VTA) of exposed male rats (Belujon & Grace, 2014; Chang & Grace, 2014). However, despite clinical findings indicating that females are more susceptible to depression (Parker & Brotchie, 2010) and preclinical studies establishing a causal link between the DA system and depression (Chaudhury et al., 2013; Tye et al., 2013), a role for the DA system in female depression vulnerability has not been examined. Furthermore, whether baseline differences in DA system function in males and females are associated with increased depression vulnerability in females is currently unknown. Ketamine, a novel fast-acting antidepressant (Browne & Lucki, 2013), reverses the decrease in DA neuron activity in male rats resulting from learned helplessness (Belujon & Grace, 2014). Here we assess whether ketamine's effect on behavior and DA neuron activity patterns extend to male and female rats exposed to UCMS, and whether ketamine can obscure baseline sex differences in behavior and the DA system.

Methods: Stress-naïve male and female rats were tested for baseline differences in depressive-like behavior using the Forced Swim Test (FST). Extracellular recordings of DA neurons in the VTA were conducted under baseline conditions to identify sex differences in DA system activity. A separate cohort of male and female rats underwent 4-6 weeks of UCMS, after which they were tested in the FST. Extracellular recordings of the VTA were conducted within a week after behavioral testing to determine UCMS-induced alterations in VTA DA neuron activity patterns in both sexes. Neuronal activity parameters evaluated included number of active cells per track, firing rate and amount of burst firing. A separate cohort of stress-naïve and UCMS rats were administered ketamine (5mg/kg i.p.) or saline (1mg/kg i.p.) to determine whether ketamine can obscure baseline differences between male and females and restore stress-induced alterations in behavior and DA activity.

Results: Under baseline conditions, females exhibit greater depressive-like behavior and approximately 40% lower DA neuron activity compared with males. UCMS induced greater immobility in the FST and reduced VTA DA neuron activity by approximately 50%, and these effects were more pronounced in females. Ketamine produced sex-dependent effects on depressive-like behavior, and studies aimed at determining whether these effects are also present at DA neuron level are in progress.

Conclusions: The results from this study indicate that increased female susceptibility to depression may be associated with lower baseline DA system activity and a higher impact of stress on behavior and the DA system. Ketamine appears to be effective at restoring behavior. Understanding the neural underpinnings of sex differences in vulnerability to stress and the antidepressant response will provide insight into mechanisms of disease and antidepressant action and lead to improved diagnosis and therapeutic approaches in both sexes.

Keywords: stress, Depression, Dopamine, sex differences

Disclosures: Dr. Grace receives consulting fees from Johnson & Johnson, Lundbeck, Pfizer, GSK, Merck, Takeda, Dainippon Sumitomo, Otsuka, Lilly, Roche, Asubio, and Abbott and receives research funding from Lundbeck, Lilly, Autofony, and Johnson & Johnson. Dr. Rincón-Cortés has nothing to disclose.

W99. Factor Analysis of Temperament and Personality Traits in Bipolar Patients: Correlates with Comorbidity and Disorder Severity

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Background: Temperament and personality traits have been suggested as potential endophenotypes for bipolar disorder based on evidence of the reliability and stability of measurement in patients and the heritable transmission in bipolar families. Some of these traits have also been successfully used in genetic studies of bipolar disorder to identify regions of linkage and association. Previous work has further suggested the presence of an anxious-reactive factor identified across temperament and personality scales that produced significant group separation and discriminated unaffected relatives of bipolar patients from healthy controls. As such a factor could potentially be useful in genetic analyses of bipolar disorder, we have attempted to reproduce and further characterize this factor structure in a large sample of bipolar patients.

Methods: A total of 1195 subjects with bipolar disorder were selected from the Bipolar Genome Study (BiGS) sample. All subjects had complete data available for the Temperament Evaluation of Memphis, Pisa, Paris, and San Diego Auto-questionnaire (TEMPS-A) and the Temperament and Character Inventory (TCI). Principal component analysis was conducted on all 235 items derived from both scales. Parallel analysis with 2000 simulations and a p value threshold of 0.01 confirmed the presence of 18 factors explaining 39% of the variance. Subsequent varimax rotation and standardization was performed, and Cronbach's alpha estimates indicated good internal consistency. Cluster analytic methods were then applied to the factors to identify groups of subjects with more similar profiles.

Results: The largest factors encompassed mood and energy shifts (factor 1) and general anxiety/worry (factor 2) and explained 5% and 3.5% percent of the variance, respectively. Other factors related to cooperativeness, social anxiety, dysthymic mood, motivation, irritability/anger, persistence/drive, spirituality, openness, novelty seeking, humor, low energy, impulsivity, physical anxiety, sentimentality, acceptance seeking, and extravagance. Subsequent analyses of each factor with clinical features and comorbid states associated with bipolar disorder revealed specificity for nearly every factor in a predicted pattern. Factor 1, which seems to represent core features of the disorder, was strongly associated with rapid cycling, as well as an earlier age at onset and increased suicide risk, anxiety comorbidity, and childhood ADHD symptoms. Cluster analysis defined one group by a strong lack of general anxiety (i.e., very low on factor 2), and low mood and energy cycling (factor 1). Subjects in this group also scored low on other factors related to anxiety and depression. This group further displayed a complete lack of comorbid diagnoses, as may be expected, and formed a relatively stable, less clinically affected group of subjects. The remaining subjects could be

distinguished into two groups based on the presence of either positive characteristics, including persistence/drive, spirituality, openness, humor, and impulsivity or negative characteristics of depression and anxiety.

Conclusions: These results suggest that cross-analyses of temperament and personality scales may have utility for identifying subgroups of bipolar patients with specific clinical profiles. By applying cluster analytic methods to the identified factors, we may be able to group these patients by clinical similarity and potentially reduce the degree of genetic heterogeneity typically associated with bipolar disorder. This reduction of heterogeneity should facilitate the identification of genetic variants specific to subtypes of bipolar disorder.

Keywords: Bipolar Disorder, temperament, personality, factor analysis, heterogeneity

Disclosures: Nothing to disclose.

W100. Categorization and Tractographical Correlation of Acute Intraoperative Behavioral Responses to Subcallosal Cingulate Deep Brain Stimulation

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Background: The clinical utility of monitoring behavioral changes during intraoperative testing of acute subcallosal cingulate deep brain stimulation (SCC DBS) is unknown. The experience of patients during SCC DBS testing is notable for certain stereotypical features, such as descriptions of 'a sudden calmness or lightness', disappearance of a 'void,' a sense of 'connectedness,' increased interest, and even sudden brightening of the room. Due to the idiosyncratic nature of these self-reports, quantification has yet to be standardized. In addition, a biological correlate of these responses has not been recognized. We examined structural connectivity patterns of subcallosal cingulate stimulation mediating acute intraoperative behavior responses with the goal to identify an intraoperative biomarker of optimal SCC DBS lead placement.

Methods: Intra-operative testing during DBS surgery for depression at Emory University was conducted in 9 consecutive adult participants. The stimulation protocol consisted of 12 trials (one at each of the 8 available contacts on the DBS leads; 4 left, 4 right plus 4 sham trials) of 3 minutes of electrical stimulation followed by 3 minutes of no stimulation using standard parameters employed for chronic SCC DBS (monopolar stimulation, frequency $= 130$ Hz, pulse width $= 90$ μsec, current $= 6$mA). Blinded ratings (On/Off; contact location) and categorization of acute behavioral effects was conducted. In positive response trials, features of the response were further classified into two categorical 'types' based on the salience, quality and magnitude of the self-report. Response Type 1 was defined by presence solely of a perceived change in body state (i.e., interoceptive awareness), or specific physical sensations. Response Type 2 was characterized by a more complex set of evoked thoughts and feelings commonly indicated by a shift in attention from themselves to others (exteroceptive

awareness). The number of responses (either Type 1 or Type 2) was summed for each hemisphere and a 'best' contact was then selected reflecting the most robust combination of interoceptive (Type 1) and complex behavioral phenomena (Type 2) overall. Once rankings were completed, trials were unblinded and contacts (Left 1 - 4; Right 1 - 4) were matched to trial and response types. These classifications were subsequently used for structural connectivity analyses to define white matter tracts mediating the 'Best' versus any 'Salient' Responses (either Type 1 or Type 2 alone) as well as differences between right and left hemisphere stimulation effects.

Results: Among the nine subjects, a total of 108 individual stimulation trials were recorded (72 active - 36 per hemisphere- and 36 sham). Thirty contacts generated a type I 'salient' response (17 on the left hemisphere, 13 on the right hemisphere) while 42 contacts evoked no response. Of the 36 sham trials, only 4 generated some mild Type 1 responses; none of Type 2. As previously observed, behavioral changes were apparent to subjects within the first minute of the initiation of the stimulation and effects were sustained while stimulation remained on; patients generally noted a clear fading of any effects within the first minute following discontinuation returning to their pre-trial baseline after about 2 minutes. The 'Best' contact for each of the 9 subjects was always in the left hemisphere. Self-reports from these 'Best' contacts showed robust Type 1 and Type 2 responses: 'lightening of mood', 'feeling warm', 'lighter', 'feeling more connected', 'I can get outside of myself to pay attention to you', noticing objects, people and activities ongoing in the operating room, interest and perceived capacity to engage in various personally relevant activities if they were home (taking a shower, washing the dishes, walking the dog). 'Salient' (positive, but non-best) responses occurred with equal frequency with stimulation of contacts in either hemisphere. Three common white matter bundles were impacted by stimulation of the 9 left-sided contacts mediating the 'Best' response: fibers connecting both ventromedial frontal cortices (ipsilateral (left) via the Uncinate Fasciculus and Forceps Minor and contralaterally through the Forceps Minor), as well as to the anterior cingulate cortex via the Cingulum Bundle. White matter bundles mediating the 'Salient' responses were limited to the ipsilateral cingulate bundle with a mirror pattern for right and left sided contacts. In contrast to 'Best' and 'Salient', the 'No behavior' contacts shared no common pathways regardless of hemisphere.

Conclusions: This analysis of acute intraoperative behaviors in SCC DBS, and the subsequent identification of unique connectivity patterns may provide a potential biomarker to guide and optimize surgical implantation and to refine and optimize algorithms for selection of contacts in chronic stimulation.

Keywords: deep brain stimulation, Treatment Resistant Depression, Biomarker, tractography

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W101. Why 7-Tesla fMRI is Needed to Study Depression?

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Background: Although the DMN is regarded as a homogenous network, increasing evidence demonstrates dissociation between anterior and posterior cortical midline regions in MDD patients. Anterior portions of the DMN are more strongly affected in MDD, with increased low-frequency fluctuation (ALFF) band compared with healthy subjects. Posterior portions of the DMN generally show larger BOLD signal change and consistency within and between subjects, suggesting anterior portions might not have been probed with high sensitivity in healthy subjects. BOLD signal changes associated with brain function are higher by, at least, a factor of 2 at 7T compared to 3T, suggesting increased sensitivity for detection of lower intrinsic temporal correlations. Our objective was to test whether ultra-high field might offer a sensitive tool for characterization of the topological organization of the DMN in a more finely grained spatial scale, specially needed for anterior cortical midline regions.

Methods: BOLD data were acquired at 3 and 7T at different isotropic spatial resolutions (1mm, 1.5 mm and 2 mm) from five healthy subjects. The spatial structure of the DMN was assessed using a seed-based analysis method (ventral, rostral and dorsal MPFC seed-ROIs). Magnitude of both, temporal and spatial correlation coefficients, were assessed across field strengths and resolutions. For the analysis of the Default Network architecture, network graphs and betweenness-centrality measures were also compared across field strengths using graph-analyses techniques.

Results: 7T fMRI revealed intricate cortical structures representing a not henceforth seen architecture of the default network. Comparing results between multiple resolutions we showed that the smaller voxel volumes (1 and 1.5 mm isotropic) permitted separations of detailed spatial features within the anterior- and posterior-DMN patterns as well as a better function to anatomy correspondence. We also show that the spatial pattern of the anterior DMN was improved at 7T (more active voxels) and the magnitude of temporal correlations within the anterior DMN was on average 65% greater than those for 3T. For the dorsal ("dorsal medial prefrontal cortex subsystem") and ventral MPFC nodes ("medial temporal lobe subsystem"), network graphs showed at higher field strengths an increased pattern of correlations within subsystems and elevated betweetness-centrality, modifying the topological organization of these brain regions within the DMN.

Conclusions: Advantages of ultra-high field (7T) allowed us to measure brain connectivity in areas of lower intrinsic network correlation within the DMN in healthy subjects. As such, using ultra-high field will benefit fcMRI in a way that is crucial in characterizing DMN in depression and elucidate the presence of previously unknown nodes within the anterior DMN that are not observed at lower field strengths.

Disclosures: Nothing to disclose.

W102. Tuning Circuits with Interleaved TMS/fMRI: One Session of 10Hz Left Dorsolateral Prefrontal Cortex rTMS (4000 pulses) Increases the Cortical and Decreases the Striatal Bold Signal Response to a TMS 'Ping' of that Circuit

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Background: Repetitive transcranial magnetic stimulation (rTMS) of the left dorsolateral prefrontal cortex (LDLPFC) (3000 pulses) daily for 4-6 weeks is an FDA-approved therapy for treatment resistant depression. Although the stimuli delivered to the LDLPFC are focal, the neurophysiological effects of rTMS are likely relayed to connected structures in a target-specific manner. It is difficult to measure these network relays using standard electrophysiological techniques. In this study, we used interleaved TMS/fMRI to examine how LDLPFC rTMS alters mood-regulating structures by analyzing their BOLD signal responses to single TMS pulses as surrogate markers of cortical and subcortical excitability.

Methods: 14 healthy subjects were included in this sham-controlled, single blind, crossover study. The experimental paradigm consists of three stages: 1) Pre-rTMS MRI scan (3T Siemens) using single pulse interleaved TMS/fMRI over LDLPFC 2) a 20-minute (4,000 pulse) session of either active or sham 10Hz rTMS treatment immediately following the scan, and 3) Post-rTMS MRI scan using single pulse interleaved TMS/fMRI over LDLPFC. Thus, TMS was used as a probe inside the scanner and as an agent of change outside the scanner. Participants were randomized to receive 10 Hz active rTMS on one visit and 10 Hz sham rTMS on the other visit. The visits were separated by at least 5 days and counterbalanced to prevent an order confound. Interleaved TMS/fMRI scanning sequence fMRI data were analyzed using SPM8 with the first 8 functional volumes omitted for consistency and reduction of movement artifact.

Results: fMRI whole-brain analysis ($n = 14$, cluster size 25, $p < .05$) suggests that a 20-minute session of 10 Hz rTMS over the LDLPFC changes activity in the connected circuit, with BOLD signal increases and decreases in cortical and subcortical brain structures, respectively. Increases in the BOLD signal response to a TMS pulse were seen in bilateral frontal lobes as well as the rostral and posterior cingulate cortices. Decreases in subcortical BOLD response to a TMS pulse were found in the ipsilateral striatum.

Conclusions: These findings suggest that a single session of 10 Hz LDLPFC rTMS increases cortical excitability and decreases striatal excitability as measured by subsequent BOLD signal response to single pulse LDLPFC TMS pings. These circuit level effects may be associated with prefrontal TMS's antidepressant and anti-nociceptive effects. Scanning

studies like this one may help improve efficacy or predict response to rTMS therapy.

Keywords: TMS, fMRI, interleaved TMS/fMRI, brain stimulation, Excitabilty

Disclosures: Nothing to disclose.

W103. Seizure Initiation with Focal Electrically Administered Seizure Therapy (FEAST)

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Background: Electroconvulsive therapy (ECT) efficacy and cognitive side effects remain influenced by several parameters including electrodes position and configuration, the applied current intensity, duration, and polarity. We applied a nonlinear interaction model on multichannel scalp EEG recordings paired with ECT and determined the functional interaction across cortical areas.

Methods: Patients were treated with unidirectional current FEAST using a modified MECTA spECTrum 5000Q device (MECTA Corp, Tualatin, Oregon) and following the same anesthesia protocol. 64 channel EEG recording (Neuroscan, Compumedics) were acquired during 2 cross-randomized treatment sessions at 6 times seizure threshold (6*ST) with opposite current polarity involved a simultaneous. We derived the non-linear dynamic interaction models from modified neuronal population activity models whose dynamics can reproduce basic features of ECT-induced seizures within local areas and across distant cortical areas. We applied the Square-Root Cubature Kalman filter in three EEG states: baseline under general anesthesia, ictal and post-ictal. This yielded the functional connectivity between right and left frontal and parietal regions.

Results: To date, we acquired 24 recordings from 12 patients with major depressive disorder (5 females, age = 44.5 ± 10 years). These 12 6*ST direct polarity and 8 6*ST reversed polarity with conserved electrodes configurations and 4 6*ST reversed polarity and electrodes configurations (172.8 ± 59.48 mC). Right Frontal-to-Parietal and Right Parietal-to-Frontal ictal parameters showed significant differences in functional connectivity values (4.07 ± 2.98, -0.46 ± 0.34, $p < 0.001$) between direct and reversed polarity. This difference was most accentuated between direct and reversed polarity with reversed electrodes configurations.

Conclusions: This innovative research highlights the regional relationships of ictal activity with FEAST. Differences between direct and reversed polarity treatment administration indicated that FEAST is clearly initiating seizure activity in the frontal region (right > left). Ongoing analyses are focusing on dynamic regional interactions over time. Future work will focus on comparing FEAST with more classic ECT modalities and relationship to clinical outcomes.

Keywords: ECT, brain stimulation, FEAST, EEG

Disclosures: FEAST machine on loan from MECTA Inc.

W105. Use of Mobile Technologies to Monitor Activity, Sleep, and Mood States to Identify Targets of Prevention of Mood Disorders

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Background: There is considerable evidence from clinical studies that variations in 24-hour patterns of motor activity and sleep schedules are strongly linked to the onset, course and patterns of treatment response of major mood disorders. Additionally, data from family and twin studies suggest that these phenomena may underpin the key pathophysiological aspects of bipolar and some unipolar depressive disorders. The ability to use mobile tracking devices has created the capacity to monitor patterns of motor activity and sleep over prolonged periods in the same individual, and to study the patterns of association with other neurobiological markers, changes in other key external factors (e.g. seasonality, light exposure), and the onset and course of these disorders. The aims of this presentation are to present findings from mobile monitoring of activity, mood states and sleep to examine the specificity of patterns associated with different subgroups of mood and anxiety disorders in diverse samples and the preliminary results of pilot studies of interventions in these systems in young adults.

Methods: We present data from two samples from a collaborative initiative on activity patterns and mood disorders including a community-based family study of mood disorders and a clinical sample of youth with emerging mood disorders. The NIMH Family Study of Affective Spectrum Disorder enrolled 242 adults in the U.S. and the Transitions Study of a clinical sample of young adults (n > 1000 young persons ages 12-30 in the early stages of mood disorders) in Sydney, Australia. These studies have integrated mobile tracking devices to obtain objective assessments of minute-to-minute activity and its association with mood, sleep, light, biologic measures, and temperature over time periods of two weeks. Common measures from each site are supplemented by a range of ancillary measures that can inform the biologic and environmental factors associated with homeostatic regulation of activity, sleep and emotional states.

Results: The results of these studies converge in demonstrating specificity of patterns of sleep and activity in subtypes of mood disorders. The NIMH Family Study data revealed that Bipolar-I disorder (BP-I) is characterized by lower average daytime activity, and greater variability in daytime activity patterns than other mood disorder subtypes or controls. In addition, there was a unidirectional association between energy, activity, and mood, net of sleep and other potential correlates of these associations, and greater reactivity to perturbations in these systems. The Transitions Study demonstrated the extent to which perturbations in 24-hour activity cycles are linked to bipolar-type disorders, more severe disability, changes in other key brain (as evidenced by MR Spectroscopy, dim-light onset melatonin rhythms) and neuropsychological variables, as well as more unstable longitudinal course. Sleep-wake disturbances, particularly delayed and disorga-

nized sleep patterns, characterize all disorders in youth and whereas those with primary anxiety or mood disorders showed marked sleep initiation difficulties with poor and unstable sleep consolidation, while those with primary psychotic and, to some degree, bipolar disorders were more prone to extended sleep duration seemingly linked to unstable sleep onset and oversleeping in the morning.

Findings from these studies have been used to design specific interventions in sleep and activity via a developed mobile phone application, RECHARGE, which also can be linked to activity monitoring systems. Intervention studies designed to evaluate the extent to which modification of sleep-wake and activity cycles may reduce the onset and consequences of major mood disorders across developmental stages are now underway.

Conclusions: Personal tracking and related mobile monitoring and intervention strategies provide a unique capacity to study the relationships between perturbations in 24 hour activity and sleep-wake cycles and the onset and course of major mood disorders – most notably in the key developmental period of adolescence and young adulthood. The use of a broad range of samples on the links between activity and bipolar disorder provides a powerful approach to establish procedures and define effective targets for prevention and intervention.

Keywords: Mobile technology, accelerometry, mood disorders, big data, Actigraphy, mood and anxiety disorders

Disclosures: Nothing to disclose.

W106. Inflammation-Related Decreases in Dopamine and Effects on Corticostriatal Reward Circuitry: Evidence from Humans and Non-Human Primates

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Background: Neuroimaging studies indicate that inflammatory cytokines decrease activation of the ventral striatum in association with increased symptoms of anhedonia. Our in vivo microdialysis studies in non-human primates suggest that these effects may be mediated by decreased striatal dopamine synthesis and release, which can be reversed by administration of the dopamine precursor, levodopa (L-DOPA).

Methods: Herein, we examined inflammation-related alterations in functional connectivity with the ventral striatum in relation to symptoms of anhedonia in medically healthy, medication free patients with major depression (n = 48). Pilot data was also collected from a subset of patients with high inflammation (as defined by plasma C-reactive protein [CRP]) who were administered L-DOPA before and after resting state fMRI.

Results: Increased inflammation (as determined by plasma CRP) was associated with decreased functional connectivity between the ventral striatum and ventromedial prefrontal cortex (vmPFC) in patients with major depression. These changes in functional connectivity were also associated with increased anhedonia (r = -0.48, df = 46, p = 0.001). Furthermore, preliminary data indicate that L-DOPA can reverse inflammation-associated decreases in corticostriatal connectivity.

Conclusions: These data support the hypothesis that inflammation effects on dopamine synthesis and release may play a role in corticostriatal dysfunction that underlies symptoms of anhedonia in major depression. Ongoing work with L-DOPA will provide a foundation for future studies investigating therapeutic strategies that facilitate availability of dopamine precursors to improve symptoms of anhedonia in patients with major depression and increased inflammation.

Keywords: Dopamine, inflammation, anhedonia, functional neuroimaging

Disclosures: Nothing to disclose.

W107. Sex Differences in Cytokine Networks in the Hippocampus after Systemic Immune Challenge

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Background: Systemic inflammation is associated with dysregulation of emotional states and cognitive function. Notably, increased circulating cytokine signaling, particularly interleukins -1β and - 6 (IL-1β, IL-6) and tumor necrosis factor (TNFα) may contribute to depression-like symptoms, cognitive impairments, and anxiety disorders including PTSD. In human studies, acute injection of lipopolysaccharide (LPS) to triggers inflammatory signaling and leads to changes in socio-emotional responses, and feelings of depression. Women are more vulnerable than men to the affective regulation of immune challenges, and this effect is often attributed to greater systemic immune responses compared with men. More recent work, however, suggests that sex-specific activation of types of immune cells and patterns of cytokines, rather than magnitude of response per se, are more important determinants of outcome.

Rodent models mirror many of the effects of immune challenge and have demonstrated a clear role for IL-1β, IL-6, and TNFα in modulation of behavioral tasks. However less is known about the role of the broader network of cytokines in cognitive and affective regulation. In addition, despite growing evidence for sex-specific roles of immune cells in the brain, few studies have directly studied sex differences in central cytokine regulation and their modulation of affective and memory processes.

Here we aimed to determine (1) sex differences in broad networked activation of cytokines in the hippocampus, and (2) and differentia vulnerability of males and females to depression-like versus memory modulatory effects of systemic immune challenge.

Methods: 9-11 Week old male and female C57Bl7/N mice (Harlan Laboratories) were housed individually in standard mouse caging, with ad lib access to food and water, a 12:12h light:dark cycle (lights on 7am-7pm) and maintained at 70F. All procedures were approved by University of Michigan Animal Care and Use Committee. Acute intraperitoneal (i.p) injections of lipopolysaccharide (LPS; E choli) at doses ranging from 62.5 - 250μg/kg were administered prior to

behavioral training (context fear conditioning, passive avoidance, forced swim test) or tissue dissection for cytokine analysis. 32-plex cytokine assays (EMD Millipore) were used according to the protocols provided, with minor adaptations for brain tissue. Plates were read on a MagPix (Luminex Corp) machine. The cytokines assayed were: CSF1, CSF2, CSF3, IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-9, IL-10, IL-12p40, IL-12p70, IL-13, IL-15, IL-17, IFN□, CXCL1, CXCL2, CXCL5, CXCL9, CXCL10, CCL2, CCL3, CCL4, CCL5, CCL11, TNFα, VEGF, LIF, and LIX. Statistical analysis used Multivariate ANOVA and post-hoc tests with Scheffe correction for multiple comparisons.

Results: We demonstrated (a) broad network of activation of cytokines in the hippocampus, with strong, persistent activation of chemokines downstream of the rapidly activated IL-1β, IL-6, IFN□ and TNFα; (b) qualitative and quantitative differences in cytokine profiles of males and females, as well as differential kinetics of activity; and (3) task- and sex-dependent modulation of memory.

Systemic LPS triggered broad activation of cytokines in hippocampus, with 28 of 32 cytokines showing significant changes. Surprisingly, the strongest elevations in cytokine protein were not IL-1β, IL-6, or TNFα, but CXCL2, CXCL9 and 10, and the colony stimulating factor (CSF) family. The sex-specific patterns of cytokine activity in the hippocampus were striking, with the IL-2 family (including IL-2, IL-15, and IL-4) upregulated in females, but not males. In contrast, IFN□ and IL-10 were only elevated in males. The CSF family also showed strong sex selectivity, with CSF1 and 2 only elevated in males, and CSF3 more strongly activated in females. Finally, we observed that males were more vulnerable than females to enhancements in fear-related memory after acute systemic LPS injection.

Conclusions: Together, these findings suggest that networked activity of cytokines in the brain, rather than individual cytokines, are central to vulnerability to cognitive and affective modulation. This is consistent with previous findings demonstrating that in peripheral immune responses, the function of individual cytokines depends on the context of the inflammatory (and neuroendocrine) milieu. The differential vulnerabilities of males and females to mnemonic and affective modulation by immune stimulation are there likely to be due to which cytokines are present, and their kinetics of activation and resolution. In these data, several sex differences stand out. The regulatory cytokines, IL-10, IL-13, and IL-4 are all activated in sex-biased manner. IL-13 and IL-4 are rapidly upregulated in females, whereas IL-13 and IL-10 are more slowly activated in males, providing one mechanism by which cytokine signaling may be shut off faster in females. Second, males show a bias towards IFN□-dependent signaling, whereas females show a shift towards IL-2, suggesting differential activation of astrocytes, microglia and neurons in the brain, and a role for the distinct downstream signaling pathways. Understanding how these sex-specific patterns of cytokine signaling contribute to vulnerability to cognitive and affective modulation will be critical for targeting dysregulation of neuroimmune signaling in disorders including depression and PTSD.

Keywords: cytokine, neuroinflammation, Hippocampus, learning and memory, Depression

Disclosures: Nothing to disclose.

W108. Effects of Ketamine on Tests of Antidepressant Efficacy and Stress-Induced Anhedonia in Rats

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Background: Major depressive disorder is a chronic mood disorder that can be triggered by stress exposure and is characterized by loss of pleasure (i.e., anhedonia) and disturbances in the brain's reward circuitries. Ketamine has been shown to have rapid antidepressant effects in some patients with treatment-resistant depression, as well as antidepressant-like effects in non-human animals. However, little is known about whether ketamine would be effective in reversing stress-induced anhedonia. The aim of the present studies was to determine whether treatment with ketamine would decrease immobility in the forced swim test (FST), a screen for antidepressant efficacy, and/or reverse stress-induced deficits in brain reward function.

Methods: In the FST, male Wistar rats were exposed to a 15 min habituation swim followed by a 5 min test swim 24 hr later. Ketamine (10 mg/kg, IP) was administered 30 min before the test swim. Swimming, climbing, and immobility behaviors during the test swim were scored. Brain reward function was assessed in a separate group of rats using the intracranial self-stimulation procedure (ICSS). Bipolar stimulating electrodes were surgically implanted in the posterior lateral hypothalamus and rats were trained on a discrete-trial current-intensity ICSS procedure. Baseline reward thresholds were determined for each rat, and rats were subsequently exposed to 21 days of chronic social defeat. Reward thresholds were assessed immediately after social defeat and for 15 days after termination of social defeat. Susceptibility and resilience to social defeat were determined based on elevations or no change in reward thresholds, respectively, in response to the stressor. Susceptible, resilient, and control rats were then treated once with ketamine after termination of social defeat (10 mg/kg; 23 hr pretreatment).

Results: Ketamine decreased immobility and increased swimming behavior without affecting climbing behavior in the FST. In the stress-induced anhedonia test, ketamine did not reverse stress-induced reward threshold elevations in susceptible rats, reflecting anhedonia. In addition, ketamine did not affect reward thresholds in resilient and control rats.

Conclusions: The decrease in immobility in the FST in response to ketamine, which is consistent with results from other laboratories, confirms the antidepressant efficacy of ketamine in patients with treatment-resistant depression. However, the lack of reversal of stress-induced reward threshold elevations by ketamine suggests that this medication may not effectively treat anhedonia, a core symptom of depression. Thus, while some aspects of depression may be treated with novel glutamate-based medications, further research is required to determine whether ketamine and other glutamate-based medications may specifically alleviate the reward-related symptoms of depression and other psychiatric disorders.

Keywords: Ketamine, anhedonia, Social defeat stress, intracranial self-stimulation, Reward

W109. Associations between Increased C-Reactive Protein, Emotional Dysregulation, and Depression in a Sample of Trauma-Exposed African-American Women with Type 2 Diabetes Mellitus

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Background: C-reactive protein (CRP), a marker of systemic inflammation, has been associated with both depression and post-traumatic stress disorder (PTSD). Emotion dysregulation is a trans-diagnostic risk factor for a number of psychiatric disorders, including depression and PTSD. A growing body of research suggests that emotion dysregulation may be an important mechanism by which early life adversity confers lifetime risk for psychiatric illness. Emotion dysregulation may also be a risk factor for chronic systemic inflammation which has emerged as a factor complicating stress-sensitive psychiatric and medical disorders. However, no studies to date have examined the association between emotion dysregulation and CRP among traumatized and chronically ill adults with high rates of depression and PTSD. One population at particular risk for chronically elevated systemic inflammation is individuals with Type 2 diabetes mellitus (T2DM), and so in the present study, we examined the differential associations between trauma exposure, current depression and PTSD, and severity of emotion dysregulation on levels of CRP among (N = 32) African American females with T2DM recruited from an urban hospital.

Methods: Subjects were recruited from the primary care and diabetes specialty clinic of a large county hospital. Emotion dysregulation was measured using the Difficulties in Emotion Regulation Scale, PTSD was measured using the Clinician-administered PTSD Scale, depression was measured using the MINI International Neuropsychiatric Interview, child abuse was measured using the Childhood Trauma Questionnaire, and trauma load was measured using the Traumatic Events Inventory. High sensitivity CRP (hsCRP) was measured using standard immunoturbidometric methods.

Results: Using bivariate correlation analysis, we found that emotion dysregulation and current depression were both significantly associated with higher levels of CRP (p < .01). Child abuse, overall trauma load, and current PTSD were not significantly related to CRP levels. In a stepwise regression model, emotion dysregulation was significantly associated with higher CRP (p < .001) above and beyond demographic variables, body mass index, trauma exposure, and depression and PTSD diagnoses, accounting for 33% of unique variance in CRP. Examining the six dimensions of emotion dysregulation in relation to CRP, we found that difficulty with emotion regulation strategies in particular was predictive of higher levels of CRP independent of other emotion dysregulation dimensions and variables of interest.

Conclusions: These findings suggest that emotion dysregulation may be an important risk factor for chronic inflammation beyond already known risk factors among women with T2DM. It may be particularly beneficial to target emotion dysregulation in treatment of women with T2DM as reduction of systemic inflammation may have favorable effects on the course of illness.

Keywords: type 2 diabetes mellitus, Women's Mental Health, systemic inflammation, Trauma exposure, African American

W110. Variability in Sleep Duration Mediates the Relationship between Chronic Stress and Symptoms of Depression and Anxiety in Midlife Women: The SWAN Sleep Study

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Background: A large body of research links stress to poor sleep and impaired mental health. There is also preliminary evidence that day-to-day variability in sleep duration has a stronger association with mental health than does average sleep duration. However, no studies have examined the longitudinal relationships between stress, variability in sleep duration, and mental health outcomes. This study tested the hypothesis that high chronic stress would be prospectively associated with more variability in sleep duration and greater symptoms of depression and anxiety.

Methods: Participants were 262 women (42-52 years of age) enrolled in the Study of Women's Health Across the Nation (SWAN) Sleep Study. Upsetting life events were assessed annually for up to 9 years, and then trajectory analyses were used to quantitatively identify three distinct chronic stress groups: low stress, moderate stress, and high stress. Sleep duration was assessed by actigraphy for a minimum of 7 days (maximum of 35 days) during the ninth year of the study, and symptoms of depression and anxiety were assessed 3 years later. Multivariate analyses tested the prospective associations between chronic stress group, variability in sleep duration, and mental health. Analyses adjusted for sociodemographics, health characteristics, baseline sleep problems, depression and anxiety at the time of the Sleep Study, and acute life events at the time of the Sleep Study.

Results: Women characterized by high chronic stress had more variability in sleep duration and worse depression and anxiety symptoms compared to women with low or moderate chronic stress. Furthermore, variability in sleep duration partially mediated the relationship between chronic stress group and later symptoms.

Conclusions: Chronic stress in midlife women is prospectively associated with increased variability in sleep duration and increased symptoms of depression and anxiety, even after adjusting for a number of relevant health indicators. The results suggest that variability in sleep duration may be a mechanism linking chronic stress to impaired depression and anxiety. The findings are also consistent with models that emphasize the cumulative and long-term impact that stressful life events can have on important health outcomes.
Keywords: chronic stress, sleep, Depression
Disclosures: Nothing to disclose.

W111. The Challenge: Estimating the Onset of Drug Effect in the STAR*D Data

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Background: A longitudinal study of the onset of an individual's response to therapeutic medication generally involves two periods connected by onset. In designing studies to probe the time of onset, investigators typically follow a number of subjects, measuring the phenotype of interest, beginning immediately after initiation of drug treatment and continuing at a sequence of later time points. Thus the longitudinal phenotype data for each subject consists of a sequence of measurements regarding the key symptom. For different drugs, the longitudinal measurements behave in either of two ways: 1) a latent period with no change in symptoms followed by a period when the drug effect is manifest through changes in symptoms, or 2) the period when the drug effect is manifest followed by a period when the drug effect is stable. Mathematically, one of these scenarios is the reverse of the other.
Methods: We recently developed a method that can be used to estimate the onset of drug effects and assess the association between onset times and possible contributing factors. The proposed model identified the associations of those variables with time to onset of drug effects. We tested this model in the well-known Sequenced Treatment Alternatives to Relieve Depression (STAR*D) study (NCT00021528), in which different treatment strategies were sequentially tested in patients with major depressive disorder, beginning with a standard first-line drug treatment.
Results: Baseline depressive symptoms are measured using the 17-item Hamilton Rating Scale for Depression (HAM-D17) and the Quick Inventory of Depressive Symptomatology (QIDS; Rush et al. 1996). Subsequent depressive symptoms were measured with clinician and self-rated versions of the QIDS (QIDS-C; QIDS-SR). The study followed participants across four sequenced levels of treatment. In level 1, participants received citalopram for 12 to 14 weeks.
We fit our model to the STAR*D dataset, estimate the effects of the covariates, and make predictions about onset of response. In general, it takes an average of 6 weeks of treatment to detect antidepressant response. Accordingly, we choose early or late drug onset points at weeks 4 and 6. For individuals classified as having anxiety at baseline, drug onset tended to move from week 4 to week 6. In addition, anxiety at baseline experienced later onset of response (at week 6), compared to 2% of those without baseline anxiety (p-value = 0.034). This observation supports the reports that anxious depression is linked with poorer treatment outcomes (Fava et al. 2008) and slower response to treatment (Keller et al. 1991).
Conclusions: This type of research may result in better-targeted and more effective treatment protocols. However, potential problems arising from missing data and large variance in individual response may make it difficult to generalize conclusions from this study. Further research on drug onset data is needed.
Keywords: Antidepressants, genetics, statistical methods
Disclosures: Nothing to disclose.

W112. Cortical Inhibition in the Pathophysiology and Ect Treatment of Major Depressive Disorder

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Background: Dysfunctional cortical inhibition has been postulated as a mechanism through which the symptoms of Major Depressive Disorder are mediated. Treatment resistant depression comprises the subset of Major Depressive Disorder defined as lack of response to at least 2 adequate antidepressant trials. Treatment with electroconvuslive therapy, the treatment of choice for treatment resistant depression, has previously been associated with enhanced cortical inhibition. Our objective is to evaluate if the therapeutic mechanisms of electroconvulsive therapy response in treatment resistant depression are related to enhanced cortical inhibition, as evaluated by the cortical silent period and short interval cortical inhibition.
Methods: Twenty five patients with treatment resistant depression were enrolled in an acute course of electroconvulsive therapy. Cortical inhibition was measured in the motor cortex with two transcranial magnetic stimulation investigational paradigms: the cortical silent period, which has previously been shown to index GABA-B receptor-mediated inhibitory neurotransmission, and short interval cortical inhibition, which has been shown to index GABA-A receptor mediated inhibitory neurotransmission. Cortical inhibition was measured at two time points: just prior to beginning the acute electroconvulsive therapy course and within one week of its termination.
Results: Analysis of pre-treatment and post-treatment motor cortical inhibitory measures showed no significant increase in either measure of GABA mediated inhibitory neurotransmission after administration of the electroconvulsive therapy course. However, there an association was found between baseline measures of the cortical silent period (ie. GABA-B receptor-mediated cortical inhibition) and response to electroconvulsive therapy. A shorter baseline cortical silent period was associated with response to electroconvulsive therapy (F = 0.928, p = 0.44), however

this did not survive the bonferroni correction for multiple comparisons.

Conclusions: In contrast to previous smaller scale studies, analyses of this larger sample did not show increases in neurophysiological measures representing GABAergic cortical inhibition in response to an acute course of electroconvulsive therapy. However, subjects with a shorter baseline cortical silent period were more likely to respond to electroconvulsive therapy. That is, they were more likely to have a decrease in their depressive symptoms. Our unusual results may, in part, be attributed to the assessment of cortical inhibitory measures from the motor cortex, while stimulating the frontal cortices with electroconvulsive therapy. Future directions include investigating a baseline sample of brain stimulation patients with treatment resistant depression, by assessing cortical inhibitory measures from the dorsolateral prefrontal cortex, in comparison to healthy controls.

Keywords: Treatment Resistant Depression, Cortical inhibition, electroconvulsive therapy, brain stimulation, transcranial magnetic stimulation

Disclosures: Nothing to disclose.

W113. In Vivo Proton Magnetic Resonance Spectroscopy Study of the Relationships Between Lactate, Depression Severity, and Ketamine Treatment in Major Depressive Disorder

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Background: Inflammation, oxidative stress (e.g., dysregulation of glutathione, GSH) and related mitochondrial dysfunction have been implicated in the pathophysiology of neuropsychiatric conditions including major depressive disorder (MDD). These changes, including lactate (Lac) levels, may provide a potential illness- and treatment-associated biomarker that may offer insight into MDD pathophysiology. Using proton magnetic resonance spectroscopy (1H MRS) we recently found elevated ventricular cerebrospinal fluid (CSF) lactate in MDD. Though pharmacotherapies may affect these potential biomarkers, few prospective studies have characterized their intracranial levels in the context of a clinical trial for MDD. A single subanesthetic dose of the NMDA receptor antagonist ketamine is associated with rapid, though transient, antidepressant effects. We recently demonstrated the feasibility, tolerability, and potential acute efficacy of intranasal ketamine in MDD. In this study, we examined relationships between metabolites, including GSH and lactate, and depression severity before and after treatment with intranasal ketamine or placebo. We hypothesized that depression severity would correlate negatively with GSH and positively with lactate levels while ketamine treatment would increase GSH while decreasing lactate.

Methods: Nineteen adults with MDD in a current major depressive episode and enrolled in a randomized controlled clinical trial of intranasal ketamine (50 mg) vs. placebo (0.9% saline), underwent 1H-MRS scans at baseline and at 24 hours after each treatment in a crossover study. Treatments were separated by 1-2 weeks and other psychotropic medications were not withdrawn. All the imaging studies were conducted on a 3 Tesla MR system using an eight-channel phased-array head coil. The standard J-edited spin-echo difference technique was used to record spectra showing a clear and unobstructed GSH peak. Levels of GABA, glutamate (Glu), glutamate + glutamine (Glx), choline (Cho), creatine (Cr), and N-acetyl Aspartate (NAA), in addition to GSH and Lac, were measured in the dorsal anterior cingulate cortex and then expressed semi-quantitatively as ratios relative to the area of the synchronously acquired unsuppressed water resonance (W). Depression severity was assessed at baseline, before treatment, and on the day following each treatment, which coincided with the day-of-scan. The primary efficacy outcome for the clinical trial was absolute change from baseline in the Montgomery-Asberg Depression Rating Scale (MADRS) score at 24 hours following treatment and response (MADRS decrease of $\geq$ 50%) and remission represented secondary clinical outcomes.

Data were analyzed using linear mixed-effects models with metabolite levels (or change in metabolite level) as outcomes. Subject was the only random effect, and the fixed effects were change in MADRS and treatment (ketamine or placebo). An alpha level of .05 was used to determine significance.

Results: We found a significant effect of treatment on change (from baseline) in Lac/W ($t = 2.41$, $df = 10$, $p = 0.034$), when controlling for change in MADRS. Under placebo Lac/W levels decreased from baseline by $0.00134 +/- 0.00241$, while under ketamine, Lac/W decreased from baseline by $0.00085 +/- 0.00270$. There was no statistically significant association between change in MADRS and change in Lac/W level ($t = 2.15$, $df = 10$, $p = 0.055$) when controlling for treatment. Levels of GSH and other metabolites were not associated with changes in depression severity or response to ketamine treatment. There was no evidence of carryover effect for any of the metabolites. Because of the crossover design with data collected at baseline, demographic characteristics, including age, sex, current medications, and number of previous failed antidepressant trials did not confound our results. Among subjects with data from baseline and at least one post-treatment scan, 6 of 15 (40%) participants were responders 24 hours after receiving ketamine while following placebo, 2 of 17 (12%) were responders at 24 hours.

Conclusions: In this study, we found that when separating the impact of ketamine's antidepressant effects on lactate, ketamine was associated with higher lactate levels 24 hours after treatment in this sample. These findings suggest that MRS biomarkers may have utility in predicting treatment response and can provide further insight into pathophysiology and mechanisms of treatment efficacy. Further studies are indicated to replicate our findings and to determine whether MRS data can provide clinical guidance for ketamine treatment.

Keywords: Ketamine, Lactate, MDD, Antidepressant, 1H MRS

Disclosures: Kyle A. B. Lapidus has received research support from the National Institute of Mental Health (K23

W114. Concurrent Benzodiazepine Treatment Delays Antidepressant Response to Repeat Dose of Intravenous Subanesthetic Ketamine in Treatment Resistant Depression

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Background: Preclinical studies suggest that ketamine's mechanism of action is associated with a rapid and transient increase in glutamate release, possibly mediated by inhibition of tonic firing of gamma-aminobutyric acid (GABA) interneurons. The efficacy of ketamine through its antagonistic effects over the N-Methyl-d-aspartate receptor and secondary GABA/glutamate signal transduction could be affected by concurrent psychotropic agents, especially GABAergic (ie, benzodiazepines, gabapentin) and antiglu-

tamatergic (viz., divalproex, carbamazepine) treatments. A brief report (Frye et al., 2015) of variable-number serial ketamine infusions in treatment-resistant depression (TRD) showed significantly lower daily doses of benzodiazepines (BZD) among 4 ketamine responders compared to 2 non-responders. Interestingly, patients with anxious depression responded better than those with non-anxious depression (Ionescu et al., 2014).

As most ketamine studies have required patients to taper and discontinue previous psychiatric medications, the effect of concurrent psychiatric medications over ketamine response remains a poorly characterized area of inquiry. Here, we present a post-hoc analysis of an original study (Shiroma et al., 2014) of six consecutive ketamine infusions in TRD with continuation of their pre-ketamine psychiatric medication regimen throughout the course of the study.

Methods: TRD subjects, defined as having three or more adequate trials of antidepressants from at least 2 different antidepressant classes as determined by Antidepressant Treatment History Form criteria (Sackheim, 2001), were required to have a minimum 2-month period of stable doses of psychiatric and other medications before entering the study. Subjects received six IV infusions of 0.5 mg/kg ketamine over 40 minutes on a Monday-Wednesday-Friday schedule during a 12-day period. Those who met response criterion after the last dose of ketamine were followed for 4 consecutive weeks or until relapse towards their baseline level of depression symptomatology. Response was defined as $\geq 50\%$ improvement in pre-infusion/baseline depressive symptoms as measured by the Montgomery–Åsberg Depression Rating Scale (MADRS). Remission was established by a MADRS score ≤ 9. Relapse was defined as $<50\%$ improvement in pre-infusion/baseline MADRS score. For each infusion, MADRS scores were ascertained at baseline (t0), at the end of infusion (t+40 min), at t+100mins and again at t+160mins. Other outcome measures, all administered at the same time points as the MADRS, included self-rated Visual Analog Scales (VAS) for happiness, sadness, energy, tiredness, calmness, worry, worthlessness; four-item positive symptom subscale of the Brief Psychiatric Rating Scale (BPRS) and the Clinician-Administered Dissociative States Scale. Hemodynamic parameters were monitored throughout the infusion and post-infusion recovery period.

Results: Out of fifteen subjects enrolled, thirteen completed all six infusions. Twelve (92%) achieved response criterion while eight (61.5%) remitted. Five of twelve subjects remained in response status throughout the four weeks of follow-up. The mean time to relapse after the last ketamine infusion was 16 days (range 7-28 days). Of the 13 individuals who completed all 6 infusions, 4 were taking GABAergic benzodiazepine medications (mean daily dose 2.75 mg lorazepam equivalents). Post-hoc analysis showed no difference in MADRS score after 6 infusions (BZD users = 11 vs BZP non-users = 9; t = 0.35, df = 11, two-tailed p = 0.73). While both BZD users and BZD non-users eventually responded, the mean time to response differed significantly between groups: 11 days for BZD users as compared to 6.2 days for BZP non-users (t = 2.52, df = 11, two-tailed p = 0.029). After 3 consecutive infusions, no BZP users achieved response while 7 of 9 BZD non-users were already in response.

Conclusions: This post-hoc analysis provides further support for the hypothesis that GABAergic neurotransmission is relevant for achieving optimal ketamine response. Though limited by small sample size, this report corroborates previous reports of attenuated response to multiple ketamine infusions via GABAergic mechanisms. Controlling for concurrent BZD treatment with ketamine would be relevant for future clinical trials aimed at maximizing drug-placebo differences.

Keywords: Ketamine, benzodiazepine, treatment resistant depression, antidepressant

Disclosures: Nothing to disclose.

W115. The Actions of Corticotropin Releasing Hormone in the Nucleus Accumbens

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Background: There are behavioral studies that indicate that corticotropin releasing hormone (CRH) acts in the nucleus accumbens (NAc) to invigorate behavioral outputs and promote appetitive behavior. However, it is unclear whether the source NAc CRH is from neurons within the NAc or rather from projections to the NAc. One goal of this study is to investigate the source of endogenous CRH to the NAc and its effect on striatal neuronal excitability, dopamine neurotransmission and behavioral output.

Methods: We used transgenic CRH-ires-cre mouse line crossed to a fluorescent cre-reporter mouse line (Ai14;td-Tomato) to assess the localization of CRH positive neurons in the CNS. To investigate the behavioral ramifications of local CRH release in the NAc we used adult male CRHloxP/loxP mice to knock-out endogenous CRH peptide selectively in the NAc by expressing Cre recombinase tagged with eGFP. We used voltammetry and electrophysiology to assess the actions of CRF on dopamine and the firing of cholinergy interneurons, respectively.

Results: We found a small percentage of cre positive cells within the NAc (1% of all accumbal neurons). Preliminary anatomical and electrophysiogical data showed that the majority of cre positive neurons were small, spontaneously active low-threshold-spiking interneurons ($\sim$60%). In addition, approximately 15-20% of cre positive neurons were parvalbumin positive, fast spiking interneurons and 15-20% of cre positive cells appear to be medium spiny projection neurons based on their electrophysiological signatures. Collectively, these data indicate that the majority of CRH-cre + neurons in the NAc are interneurons and suggests that CRH can be locally released within the NAc. Targeted deletion of CRF peptide from neurons within the NAc produced a 40% reduction in CRH mRNA within the NAc, but did not affect basal corticosterone levels in blood, suggesting that CRH effects in the NAC are independent of HPA axis activity. Deletion of CRH peptide in NAc caused a reduction in novelty-induced locomotion and also reduced exploration of the center of an open field compared to littermates injected with eGFP viral particles. Importantly, once animals habituated to open field chamber, the locomotor activity was similar in mice with CRH-NAC deletion compared to littermate controls. we replicated our previous findings that CRH (100 nM) enhances dopamine transients evoked by electrical stimulation (20% above baseline) and measured with fast scan cyclic voltammetry in ex vivo slice containing the NAc. Novelty-induced locomotion and exploratory behavior has been linked to elevations in phasic dopamine as well as changes in the activity of accumbal projection and interneurons. However, how CRH modulates projection neurons and interneurons within the NAc is poorly understood. Exogenous bath application of CRH also enhanced dopamine transients evoked by optogenetic stimulation of cholinergic interneurons (22.7%) and caused a robust increase in the firing rate of cholinergic interneurons (275% above baseline). We found that blockade of nicotinic acetycholine receptors (nACh-R) potentiated CRF's effect on dopamine release, while blockade of muscarinic acetycholine receptors (mACh-R) reduced CRF's effect on dopamine release.

Conclusions: In summary, these data show that CRH can be locally released from striatal interneurons to enhance both dopamine and acetylcholine in order to potentiate motor output and exploratory behavior.

Keywords: CRH, Dopamine, genetic

Disclosures: Nothing to disclose.

W116. A Single Dose of SSRI Alters the Neural Circuit Underlying the Management of Attentional Resources to Emotional Distraction within 3 Hours

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Background: Accumulating evidence supports Selective Serotonin Reuptake Inhibitor (SSRI) drug-action to reduce the attentional bias to negative information, which has been proposed to underlie depressive symptomatology (Browning, Holmes, & Harmer, 2010). This change in emotional processing is mediated by a prefrontal-limbic neural system that SSRIs modulate on a time-scale that is much shorter than the 10-14 days typically viewed as required for an adequate antidepressant response (Harmer & Cowen, 2013). However, existing work in this field is limited by the paucity of paradigms that can reflect on the ability to manage attention resources in the face of emotional distraction. An emotional modification of a perceptual load task (emo-PLT) has recently been applied to investigate neural changes in emotional processing induced by a dietary supplement (Terburg et al., 2013). This paradigm allows for variation of cognitive difficulty in the midst of emotional distraction. Although the management of processing emotional information during varying cognitive challenges is a skill often substantially impaired in depression, little is known about the response in the underlying neural network to the most commonly prescribed antidepressants. To address this, we study the acute neuropharmacological impact of a single dose of escitalopram on the neural circuit underlying the management of attentional resources to emotional distraction.

Methods: To investigate this, 21 healthy subjects performed an emotional modification of a perceptual load task (Okon-Singer et al., 2014) during fMRI scanning following a single oral dose of escitalopram (20 mg) or placebo in a randomized, cross-over design. fMRI data was acquired on a 3-Tesla MR scanner at tmax for escitalopram and analyzed in SPM8 using standardized preprocessing. We applied a flexible factorial design with emotion (negative/neutral) and load (low/high) as within-subjects factors for the SSRI and the placebo conditions. We conducted a whole-brain voxel-wise general linear model (GLM) analysis on the first level and tested the resulting contrast maps for effects of load, emotion and their interactions (whole-brain family wise error (FWE) corrected $p < 0.05$, and extend-threshold 10 voxels) to validate the task. To identify the functional localization of the task-specific cortico-limbic regions implicated in navigating attention to varying cognitive load during negative emotional distraction, we performed a paired t-test comparing load (whole-brain FWE corrected $p < 0.05$, and extend-threshold 10 voxels) only during negative distraction for the respective single-subject contrasts for the placebo condition. We then performed a secondary region-of-interest (ROI) analysis in these regions to assess the extent of acute SSRI-specific neural signal change in the emotion-cognition circuit.

Results: A 2 x 2 x 2 repeated-measures ANOVA with drug, load, and emotion as within-subject factors revealed main effects of load (F $(1,20) = 304$, $p < 0.00001$) and emotion (F $(1,20) = 8,197$, $p < 0.01$) for reaction times, and a main effect for load (F$(1,20) = ,5,567$ $p = 0.029$) for accuracy. In the placebo condition, these behavioral effects were paralleled by positive main effects of load (high – low) in frontal, visual and cerebellar regions (whole brain, FWE-corrected $p < 0.05$). The contrast of negative minus neutral pictures showed that during distraction by negative emotion, there was significant BOLD activation in brain regions known to be involved in the management and processing of emotional information, namely the insula, temporal cortex, amygdala/hippocampus and orbitofrontal cortex (OFC) (whole brain, FWE-corrected $p < 0.05$). A single oral dose of escitalopram, however, substantially attenuated the BOLD responses in these brain regions: ROI analyses revealed a 146 ± 24 (mean $\pm$ SD) percent signal change in amygdala, insula and OFC ($p < 0.0000001$, Bonferroni corrected).

Conclusions: We demonstrate feasibility to apply a paradigm that detects the management of attentional resources for a cognitively challenging task in the midst of emotional distraction in a single-SSRI-dose psychopharmacological fMRI-design. Our data are consistent with the emerging theory that changes in the neural circuit underlying emotion-processing following SSRI-intake are detectable on an acute time-scale as we find changes as early as 3 hours post-administration. Furthermore, we provide strong evidence that a single dose of escitalopram can significantly reduce the BOLD response in the neural circuit underlying the behavioral interference of irrelevant emotional distractors contrasting different levels of cognitive load. These results emphasize that the management of attentional resources to negative stimuli during varying stages of cognitive challenge could represent a key mechanism of action during SSRI treatment. Our findings have important implications for the early evaluation of antidepressant efficacy in clinical populations and individuals at risk.

Keywords: Serotonin, fMRI, emotion processing, cognitive/emotional task performance

Disclosures: Society in Science Branco Weiss Fellowship & Research Funds from the Max Planck Society.

W117. Phasic Locus Coeruleus Activity Regulates Cortical Processing of Salience

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Background: The noradrenergic nucleus locus coeruleus (LC) projects broadly throughout the central nervous system and is the near exclusive source of norepinephrine (NE) to cortex. Among its targets, LC sends strong projections to primary sensory regions and has long been posited to have an important role in modulating sensory processing. LC neurons fire in two distinct modes, tonically, as characterized by irregular baseline activity (1-6Hz, physiological range) and phasically during short bursts of stimulus evoked activity (10-15Hz). Under normal conditions tonic LC activity changes with arousal and/or stress whereas phasic LC activity is associated with focused attention and behavioural responses to salient stimuli.

Methods: To causally probe LC influence on cortical sensory processing, we selectively expressed ChR2 in LC-NE neurons and drove LC activity during non-salient somatosensory stimulation while recording activity from neurons in deep layers of primary somatosensory cortex (S1). This paradigm allowed us to dissociate cortical representations of somatosensation from those that have been modulated through sensory evoked LC activity. All procedures strictly complied with Medical University of South Carolina IACUC protocols and were in accordance with the guidelines described in the US National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Results: We demonstrate that selective optogenetic stimulation of LC-NE in association with non-salient sensory signals can change the population dynamics within sensory cortex and differentially alter the sensory response (n = 135 single units). Phasic, but not tonic LC activity could generate a specific ERP in both cortical EEG and LFP. This Phasic LC ERP was not associated with temporal specific changes in cortical neuron activity. However, during non-salient sensory stimulation, phasic LC activity interacted with sensory processing to generate a late burst of activity in NE gated S1 neurons during the phasic LC ERP window. Similar evoked late responses from NE gated S1 neurons could be generated by increasing stimulus intensity/salience that endogenously engages phasic LC.

Conclusions: Collectively these results indicate that a major role of LC activity is to regulate sensory processing by providing discrete saliency signals to cortical targets. We propose that phasic LC salience ERP coordinates cortical targets and subsequent behavioral responses. The LC-salience signal we identify may provide a translational

measure of phasic LC activity in SUA, LFP and EEG to investigate attentional processing across species.
Keywords: Locus coeruelus, ERPs, Salience, Attention, optogenetics
Disclosures: Nothing to disclose.

W118. Sex Differences in Fear Conditioning and Extinction in Trauma-Exposed Individuals With and Without Post-Traumatic Stress Disorder

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Background: Fear conditioning and extinction paradigms have proven extremely valuable for studying fear learning and unlearning processes, particularly in populations who exhibit exaggerated fear responses, such as posttraumatic stress disorder (PTSD). Individuals suffering from PTSD show both behavioral and neural activation deficits during fear extinction paradigms, exemplified by elevated fear responses and lower ventromedial prefrontal cortex (vmPFC) activation during recall of the extinction memory. While this past literature is very informative, limited studies have used fear conditioning and extinction paradigms to study sex differences in PTSD. This is surprising due to the fact that women face significantly higher risk of developing PTSD following trauma exposure. Using a fear conditioning and extinction paradigm, we investigate how PTSD and trauma-exposed non-PTSD (TENP) individuals differ from each other within men and women.
Methods: We recruited 33 men (18 TENP, 15 PTSD) and 39 women (18 TENP, 21 PTSD). During their initial visit, all participants had a diagnostic interview. They then underwent a well-validated two-day fear conditioning and extinction paradigm while inside the fMRI scanner. Day 1 consisted of the fear conditioning and extinction learning phases, followed by the extinction memory recall phase the next day. During conditioning, participants saw three different colored lamps, two of which were partially reinforced by a shock (CS+) and one of which was never paired with a shock (CS-). During extinction learning, one of the two CS+s was presented along with the CS-, without the pairing of a shock. The next day, the participants returned for the extinction recall phase in which they were presented with all three cues (the extinguished CS+, the non-extinguished CS+ and the CS-). Throughout the three phases, we recorded skin conductance responses (SCR) as the index of conditioned responses and Blood Oxygen Level-Dependent (BOLD) signal was used to quantify functional neural activation.
Results: When looking at SCR during the early phase of conditioning, TENP women showed better discrimination between the CS+ and the CS- relative to PTSD women. The reverse pattern was found in men, with the PTSD group showing better discrimination relative to the TENP group. For the late phase of conditioning, these differences were still present but attenuated, and did not reach significance. Regarding fMRI findings during early conditioning, PTSD women showed greater activation in the dorsal anterior

cingulate cortex (dACC) relative to TENP women, whereas TENP men had greater activation in both the amygdala and insular cortex relative to PTSD men. During the late phase of conditioning, TENP women showed greater activation in the rostral anterior cingulate cortex (rACC) compared to PTSD women. During the early phase of extinction, SCR data revealed greater discrimination in TENP women compared to PTSD women. Regarding fMRI findings during early extinction, TENP women had greater activation in the hippocampus compared to PTSD women. During the late extinction phase, TENP women also showed greater activation in the insular cortex compared to PTSD women. During extinction memory recall, PTSD men exhibited greater SCR to the extinguished cue when compared to TENP men. This higher fear response was accompanied by significantly lower activation in the vmPFC.
Conclusions: Although complex, these data highlight distinct patterns between trauma-exposed individuals with and without PTSD in men and in women. During conditioning, women with PTSD seem to show worse discrimination between fear and safety cues, and this seems to be connected to greater activation in the dACC. During recall, men with PTSD show higher fear along with lower vmPFC activation compared to TENP men. Taken together, these results seem to suggest that PTSD women show dysfunctional fear acquisition whereas PTSD men show dysfunctional extinction memory recall. Our findings highlight the importance of further investigating the role of sex and sex hormones in the etiology of PTSD. This could allow enhanced treatment to target sex-specific neurobiological deficits.
Keywords: Extinction memory recall, Trauma exposure, Sex differences, fMRI, Skin conductance response
Disclosures: Nothing to disclose.

W119. Associations of White Matter Integrity with Discrepancies between Verbal and Performance IQ

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Background: Verbal-Performance IQ (VIQ-PIQ) discrepancies are common across childhood developmental disorders. For example, PIQ is typically greater than VIQ (PIQ>VIQ) in Autism, Dyslexia, and Language Disorder and VIQ>PIQ in children with Non-Verbal Learning Disability. In healthy individuals, this discrepancy is associated with significant cortical thinning (VIQ>PIQ) or thickening (PIQ>VIQ) in posterior cortices, frontal portions of frontostriatal circuits (inferior frontal gyrus and anterior cingulate cortex) and with reduced activation of frontal cortices during the engagement of cognitive control. Herein, we assessed the association of white matter integrity with the VIQ-PIQ discrepancy.
Methods: Using diffusion tensor imaging to analyze white matter connectivity, we examined the white matter integrity of fiber tracts associated with the VIQ-PIQ discrepancy in 166 healthy control participants. We correlated the VIQ-PIQ discrepancy with fractional anisotropy (FA) at each voxel on the cerebral surface.

VIQ-regressed-on-PIQ Residual Score. The VIQ-regressed-on-PIQ residual score was calculated with each participant's VIQ and PIQ score obtained from the WASI (Wechsler, 1999). To create the VIQ-regressed-on-PIQ score, we regressed the VIQ score onto the PIQ score, setting the intercept to zero, and saved the residual. These VIQ-regressed-on-PIQ residual scores were normally distributed. DTI Pulse Sequence. MRI scans were performed on a 3.0 Tesla MR Scanner (GE Health Care, Milwakee, WI) using an 8–channel head coil. DTI slices were acquired in an axial oblique orientation parallel to the AC-PC line using single-shot echo-planar DTI imaging sequence, with $TR = 13925$ ms, $TE \sim 74$ ms, $FOV = 19x19$ cm2, $Flip = 90°$, acquisition matrix $= 132x128$ (acceleration factor $= 2$) zero-padded to 256x256, for 60 oblique–axial slices positioned parallel to the AC–PC line, slices thickness $= 2.0$ mm. We acquired 3 baseline images with $b = 0$ s/mm2, and 11 diffusion weighted images at $b = 600$ s/mm2 with diffusion gradients applied in 25 directions sampling 3D space uniformly. Image processing and Data Analysis. We corrected magnetic field inhomogeneities, eddy-current distortions, and head motion using the FSL toolbox. We then computed the diffusion tensor at each voxel by fitting an ellipsoid to the DWI data acquired along 25 gradient directions and 3 baseline images using a Levenburg-Marquardt algorithm to achieve a robust non-linear least-squares fit, while constraining the diffusion tensor to be positive definite. FA maps generated from the diffusion tensor model were then spatially normalized to the template brain using a rigid body similarity transformation, followed by a nonlinear warping using a method based on fluid dynamics. Statistical Analyses. Multivariate linear regression at each point on the reference surface examined associations of the VIQ-regressed-on-PIQ residual score with FA. False Discovery Rate was used to account for the multiple correlations computed across the cortical surface. The p-value of the correlation between FA and VIQ-regressed-on-PIQ residual score was evaluated using a Student's t-test.

Results: The VIQ-regressed-on-PIQ residual score associated positively with FA in structures composing the corticobulbar and corticospinal tract (bilateral posterior corona radiata, posterior internal capsule), the superior occipitofrontal fasiculus (bilateral parietal lobe white matter), and in the cingulum. The VIQ-regressed-on-PIQ residual score associated inversely with FA in the right external capsule. Exploratory fiber tracking indicated regions with significant correlations of FA with VIQ-regressed-on-PIQ residual score were interconnected through the fiber tracts identified in the voxelwise analyses.

Conclusions: Voxelwise analysis showed inverse associations of the VIQ-regressed-on-PIQ residual score and FA in the corticocortical association fibers of the right external capsule, and positive associations of the VIQ-regressed-on-PIQ residual score and FA in structures that compose the superior occipitofrontal fasiculus and cingulum, fiber tracts that support spatial attention, visuospatial ability, and executive function. The cognitive capacities subserved by the white matter tracts associated with the VIQ-regressed-on-PIQ residual score are important for successful completion of tasks included in the performance portion of IQ tests. In our previous work we identified variation in cortical anatomy associated with the VIQ-regressed-on-PIQ residual score in regions that appear to underlie dysfunction in information processing that would specifically affect performance on IQ tasks. Taken together these findings suggest a distributed network that explains one aspect of variability in normal intelligence and that is likely relevant to the genesis of many childhood developmental disorders.

Keywords: DTI, Intelligence Quotient, Cognition

Disclosures: Nothing to disclose.

W120. Skin Conductance Responses and Neuroimaging Correlates of Fear Conditioning and Fear Renewal in Healthy Controls and Traumatized Individuals With and Without Post-Traumatic Stress Disorder

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Background: Neuroimaging investigations, along with animal data, have allowed the identification of key brain regions involved in both fear and extinction expression, notably the amygdala, the hippocampus, the insular cortex, the dorsal anterior cingulate cortex (dACC) and the ventromedial prefrontal cortex (vmPFC). Fear conditioning and extinction paradigms have been used as laboratory models to study the processes underlying fear learning and unlearning. These paradigms have shown to be of special relevance for fear-related psychopathology, such as post-traumatic stress disorder (PTSD). So far, most studies have focused on extinction learning and extinction recall and the majority of them have compared PTSD individuals to trauma-exposed controls. A key finding, which has been replicated in various studies, is that PTSD individuals fail to activate the vmPFC during extinction training and recall. The pattern of activation linked to fear learning and renewal has not been studied as extensively and has for the most part, yielded inconsistent or negative findings. Moreover, the inclusion of a non-traumatized healthy control sample has most of the time been omitted. Here, we study the psychophysiological and neural correlates observed during fear acquisition and fear renewal in three distinct cohorts differing on trauma exposure and diagnosis status.

Methods: Twenty-one healthy individuals never exposed to trauma (Healthy Controls; HC), sixteen trauma-exposed non-PTSD individuals (TENP) and eighteen trauma-exposed individuals with a PTSD diagnosis (PTSD) were recruited. All participants underwent a two-day fear conditioning and extinction protocol in the fMRI scanner. The fear-conditioning phase occurred on day 1 where three colored lamps were presented in one context (i.e. library or office), two of which were partially reinforced with the delivery of a finger shock. This was then followed by an extinction learning phase in a second context. On Day 2, extinction memory recall was first tested in the extinction context and fear renewal was then assessed in the fear conditioning context. The analyses presented here focus on both conditioned and unconditioned responses during conditioning and conditioned responses during fear renewal. Skin conductance responses (SCR) and blood-oxygen-level-dependent (BOLD) signal were measured throughout

all phases of the paradigm. Statistical analyses performed on demographic data revealed a main effect of age and years of education, with the HC group being slightly younger and more educated than the two other groups. Therefore, these two variables were used as covariates for all analyses.

Results: For SCR, no between-group differences were observed during conditioning for the conditioned and unconditioned responses. In terms of fMRI data during the early phase of conditioning, both trauma-exposed groups (TENP and PTSD) exhibited significantly higher vmPFC activation than the HC group. During shock delivery, PTSD individuals showed significantly more vmPFC activation than the TENP group. When comparing the trials where a shock was delivered to the trials where the shock was expected but not delivered, there was significantly less dACC and insular cortex activations in the TENP group relative to both HC and PTSD cohorts. In terms of SCR findings during fear renewal, HC showed greater return of fear to both CS + s when compared to the PTSD and TENP groups. The BOLD data revealed significantly greater deactivation of the vmPFC to the non-extinguished cue relative to both PTSD and HC cohorts.

Conclusions: Our data suggest that PTSD individuals activate more the vmPFC during fear learning and shock delivery. These data suggest that the vmPFC in PTSD is not necessarily hypoactive, but rather dysfunctional. We also showed that the TENP group had lower insular cortex and dACC activation during the shock vs. omitted shock contrast compared to both PTSD and HC cohorts. This result seems to suggest that a blunted response in these areas following trauma exposure could be a marker of resilience. When tested for renewal, the HC cohort showed a more robust return of fear in terms of SCR. The pattern was slightly different when looking at the neural correlates, with the TENP group showing the most robust deactivation of the vmPFC. Our data highlight the importance of looking at both conditioned and unconditioned responses and also illustrate how the inclusion of 3 cohorts (non-traumatized healthy controls, traumatized healthy controls and traumatized with PTSD individuals) reveals pertinent information about trauma exposure and diagnosis separately.

Keywords: Trauma exposure, PTSD, fMRI, Conditioned responses, Unconditioned responses

Disclosures: Nothing to disclose.

W121. Development, Safety, and Tolerability of Transcutaneous Auricular Vagus Nerve Stimulation (taVNS), a Novel Form of Noninvasive Vagus Nerve Stimulation

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Background: Cervically implanted vagus nerve stimulation (VNS) is FDA-approved for treating epilepsy or major depressive disorder. Additionally, VNS has shown promise in animal studies, with applications ranging from attenuation of inflammatory response, increased survival rate in heart failure, reduction of infarct size in cerebral ischemia, motor rehabilitation post-stroke, and tinnitus. VNS is thus a reemerging area of interest and has significant translatable applications. Although invasive cervical VNS is relatively safe and reasonably effective, there are risks involved in surgical implantation and it is expensive (about $30,000 to implant). Moreover, only 30% of epilepsy patients have a robust anti-epileptic response, and there is currently no way to predict who will ultimately respond. Thus many patients are implanted without benefit. Interest is growing in whether VNS can be administered non-invasively through the auricular branch of the vagus nerve that innervates the ear. We have developed a device that can electrically stimulate the auricular branch of the vagus (called transcutaneous auricular vagus nerve stimulation (taVNS)). This study aimed to determine feasibility, safety and tolerability of taVNS. We tested 9 different stimulation parameter combinations in order to determine the optimal stimulation parameters that modulate the vagus system. We hypothesized that taVNS would be safe, tolerable, and increase parasympathetic nervous system activity as measured by heart rate, skin temperature, and skin conductance.

Methods: This single-blind, sham controlled, crossover study consisted of 2 separate visits during which custom built ear clip electrodes (10mm diameter stimulation surface) were used to deliver direct electrical stimulation via a constant current stimulator (Digitimer Ds7a) to either the left tragus (active) or left earlobe (sham) in 15 healthy participants (Mean age = 26.5, 7 female). Each visit consisted of the participant lying supine with their torso and head slightly elevated. They then received nine different randomized stimulation parameters of varying pulse width and frequency (pulse widths: 100μs, 200μs, 500μs; frequencies: 1Hz, 10HZ, 25Hz). Current strength was delivered at 200% perceptual threshold (tragus perceptual threshold ranged from 1.5 to 4.64mA, earlobe threshold ranged from 0.99 to 3.3mA, dependent on pulse width). Each stimulation period lasted 60 seconds, flanked by a 90 second baseline period and a 180 second recovery period. Participants were asked to rate painfulness from 0 (no pain) to 10 (extreme pain) on a visual analog scale (VAS) after each stimulation period. Heart rate, skin temperature, and skin conductance were recorded during the entire visit and subjects were monitored for both minor (skin discomfort, irritation, headache, dizziness, facial pain) and major (dramatic drops in heart rate) adverse events.

Results: In terms of safety, no minor or major adverse events were reported or observed in any subjects. Active taVNS was non-significantly more painful than was sham taVNS. Mean pain VAS scores, dependent on stimulation parameters, ranged from 0.2 to 0.83 (Sham) and 0.57 to 1.94 (Active). With respect to heart rate changes, there was no significant decrease in heart rate between all active and all sham stimulations in an overall group analysis (n = 135 sessions, active mean HR decrease = 1.86 beats per minute (4.47 SD); Sham 1.47, (5.05 SD); p = 0.521). Of the 9 different parameters tested, stimulation pulse width of 500μs at 25Hz had the largest effect on heart rate compared to sham (active: 4.3 beat per minute decrease (8.05 SD); sham: 0.05 beat per minute decrease (6.33 SD)). Complete heart rate, skin conductance, and skin temperature data

time series analyses are being conducted and will be presented at the poster.

Conclusions: Short doses of taVNS at these parameters in a small sample of healthy young adults is feasible, tolerable, and reasonably safe. Like all forms of brain stimulation, the parameters of stimulation are important in the ultimate biological effects (electrical current strength (mA), pulse width (μs), and frequency (Hz)). With these safety data in hand, and indications of which parameters are important for changing heart rate, we will now explore the effects of taVNS on brain activity within the fMRI scanner and ultimately clinical trials.

Keywords: Brain Stimulation, Neuromodulation, Vagus Nerve Stimulation, Central autonomic network, Peripheral Nerve Stimulation

Disclosures: The Medical University of South Carolina holds a provisional patent on taVNS and the first author and presenter Bashar W. Badran is listed as the sole inventor.

W122. Lower Dorsolateral Prefrontal Cortex Activation is Associated with Higher Self-Transcendence in Healthy Controls Taking Clomipramine

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Background: Previous functional magnetic resonance imaging (fMRI) studies examined neural activity responses to emotive stimuli in healthy individuals after acute/subacute administration of antidepressants. We previously reported decreased neural activation in emotion processing related areas (i.e. amygdala, insula, dorsolateral pre-frontal cortex and anterior cingulate gyrus) during emotional processing in healthy individuals taking clomipramine, a tricyclic antidepressant with serotonin–norepinephrine reuptake inhibition properties. Previous studies in depressed individuals exploring the effects of anti-depressive treatment on personality measures typically reported a decrease in harm avoidance factor (theoretically related to serotonin system). No study previously investigated the relationship between personality measures and brain functioning after antidepressant use in healthy controls.

Methods: Eighteen subjects with no personal or family history of psychiatric disorders were selected from a 4-week open label trial of small doses of clomipramine (range 10-40mg). Personality was measured using the temperament and character inventory (TCI) before and at the end of the open trial. The TCI describes personality in seven dimensions: four temperament (harm avoidance, reward dependence, novelty seeking, and persistence) and three character factors (self-directedness, self-transcendence, and cooperativeness). The delta difference between each TCI factor was calculated and considered significant if different from zero using one-sample t-test at $p < 0.05$. Neural response to fear-, happiness-, anger-provoking and neutral pictures from the IAPS were investigated using functional magnetic resonance imaging (fMRI). All subjects were scanned in a 1.5T scanner at the end of the open label trial (i.e. under the effect of clomipramine). Images was analyzed using the Statistical Parametric Mapping (SPM). Correlation analysis was performed comparing significant TCI factors and a-priori regions of interest that we previously showed to be decreased under the effect of clomipramine and that are implicated in emotion processing and regulation.

Results: Harm avoidance factor was not significantly different before and after clomipramine use in healthy controls. All other factors were also not significant except self-transcendence (ST, $p = 0.008$). Using correlation analysis between the ST factor and neural activation under the effect of clomipramine, we found a significant negative correlation between ST and left dorsolateral prefrontal cortex ($p = 0.007$) and left insula ($p = 0.04$) during fear processing. Here, the lower the neural activation, the higher the self-transcendence score. Furthermore, we found a positive correlation between ST and left anterior cingulate gyrus ($p = 0.04$) during anger processing. Here, the higher the neural activation, the higher the self-transcendence score.

Conclusions: The prolonged use of low doses of clomipramine increased self-transcendence character factor in healthy controls. Self-transcendence is characterized as identification with everything conceived as essential and consequential parts of a unified whole, and high scores are associated with intuition, patience, imagination, and spirituality. During fear processing higher ST scores were associated with lower activation in the dorsolateral prefrontal cortex which is an area associated with voluntary emotion regulation. This finding suggests that in the context of clomipramine use there may be a more efficient emotion control, thus requiring decreased brain areas activity. During anger processing higher ST scores were associated with higher activation in the anterior cingulate gyrus, which could be related to the fact that an anger provoking stimuli requires a more complex processing, compared to a fear provoking one. Taken together, these findings suggest that in healthy individuals clomipramine may enhance personality characteristics which are linked to protective factors for depressive disorder (e.g. spirituality), and lead to a more efficient emotion regulation.

Keywords: clomipramine, healthy individuals, personality, dorsolateral prefrontal cortex, anterior cingulate cortex

Disclosures: Nothing to disclose.

W123. A Novel Strategy for Delivering Candidate Therapeutics to Dopamine Neurons

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Background: Monoamine neuron dysfunction is implicated in a range of neuropsychiatric disorders, including schizophrenia, depression, ADHD, addiction, and Parkinson's disease. The majority of current therapeutic modulators of monoamine neurons target specific extracellular mem-

brane-bound receptors or transporters, some acting acutely, others requiring a period of days or longer to produce a cascade of neurobiological changes necessary for symptom relief or remission. Notwithstanding the moderate safety and efficacy of conventional drug therapies, there remains a compelling need to broaden therapeutic strategies for these diseases, i.e. to address lengthy remission times, or to reverse disease processes and progression. New developments in the treatment of neuropsychiatric disorders will increasingly focus on small molecules or biological therapies that target intracellular processes. These will require innovative methods for targeted delivery. This research focuses on developing strategies for targeted delivery to dopamine neurons in brain, of small molecules implicated as neuroprotective or neuroregenerative agents. Our approach was to covalently tether a candidate therapeutic to a high affinity selective dopamine transporter drug, with this bond potentially degradable in brain via esterases released along with dopamine in the Substantia nigra and striatum. Conceivably, an ester link or other hydrolysable functionality on the tether can permit dissociation of the active molecule at high concentrations at dopamine neurons, or alternatively, the entire complex may be sequestered intracellularly, as has been shown recently Ferrés-Coy et al 2015). The dopamine transporter (DAT) is an excellent model for exploring the feasibility of specifically targeting a monoamine neuron, as it is expressed exclusively on dopamine neurons. To develop a prototype, we designed a high affinity DAT compound and tethered it, with a hydrolysable bond, to the large polar compound inosine. The purine nucleoside inosine was selected because of its high polarity together with early research implicating it as a neurotrophic growth factor.

Methods: Starting with two high affinity dopamine transport inhibitors, CFT (WIN 35,428) and its dichloro analog (dichloropane), we designed eight compounds to test the premise: with four different linker arms, to optimize retention of transporter affinity. In vitro potencies at the dopamine transporter were conducted with conventional transporter assays, using {3H]CFT to label the DAT. Affinities were measured with various concentrations of the novel compounds. To measure brain penetration and occupancy of the dopamine transporter in vivo, macaque monkeys were injected with 11C]CFT to measure the DAT availability. One hour later, one of the novel compounds (1 mg/kg) was injected iv, and occupancy determined by the extent to which [11C]CFT bound to the DAT was displaced by each compound.

Results: Inosine alone displayed no affinity for the DAT or serotonin transporter or SERT (100,000 nM, 60,000 nM, respectively), whereas CFT (12 nM) and dichloropane (1 nM) both bound with high affinity to the dopamine transporter Adding a 7-carbon spacer to CFT reduced affinity to a greater extent (72 nM) than the corresponding addition to dichloropane (4.2 nM). The latter retained relatively high affinity for the SERT (26 nM), whereas CFT affinity for the SERT was considerably lower (725 nM). The addition of inosine to dichloropane-spacer retained relative high affinity for both DAT (34 nM) and SERT (117 nM). Similarly, CFT-linker-inosine also bound with relatively high affinity for both DAT (30 nM) and SERT (69 nM). Both

CFT and dichloro-inosine complexes occupied 15-26% of [11C]CFT binding sites on the DAT, in living brain. Conceivably occupancy rates would be higher at higher doses of the novel compounds.

Conclusions: New developments in the treatment of neuropsychiatric disorders are increasingly focused on small molecules or biological therapies (e.g. siRNA) that modify intracellular metabolism or regulate transcription of proteins produced at pathological levels. These strategies will require innovative methods for targeted delivery. The present study shows the feasibility of designing small molecules that target neuron-specific membrane proteins which are conjugated to highly polar putative therapeutic compounds. This preliminary study requires a number of additional experiments for proof-of-concept. These include determining whether the ester link of the tether was hydrolyzed, whether inosine accumulated intracellularly and was functional, and whether imaging of the DAT revealed occupancy of CFT tethered to inosine, or whether the detected occupancy was due to a CFT hydrolysis product after release of inosine. Although these results are preliminary, they provide a promising strategy for pursuing delivery of polar and bulky candidate therapeutics to dopamine and possibly other monoamine neurons in brain. High affinity selective probes that target the dopamine (and/or serotonin) transporters,were developed in our laboratory.

Keywords: dopamine transporter, Regeneration, PET Imaging, serotonin transporter, depression

Disclosures: BK Madras is a U. S. and foreign patent holder on patents for dopamine transporter ligands and for dopamine transporter imaging agents. The patents are licensed by Navidea and Alseres. She serves on the Scientific Advisory Board of Rivermend Health; She has served on an Advisory Panel for the National Football League and as a consultant for Prexa Pharmaceuticals.

W124. Cortical Structure and Religious Use of Ayahuasca

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Background: The hallucinogenic tea, Ayahuasca, is used within religious contexts. The brew contains N,N-Dimethyltryptamine and several β-carboline alkaloids. In some animal models, high concentrations of β-carbolines have the potential to induce cerebellar damage via over-excitation of the Purkinje cells. However, the persisting effect of Ayahuasca on cortical and cerebellar structure in humans has not been established. In the present analysis, cortical volumes were examined in individuals from an Ayahuasca-using church (AUC) to assess the impact of Ayahuasca on the brain.

Methods: As part of a larger investigation on religious participation and neuropsychological function, 20 individuals from an AUC and 16 individuals from a matched control group (MC) were recruited from the community. Cortical structure was assessed using two neuroimaging

analysis techniques: (1) Freesurfer, a semi-automated analysis of volumetric measures from specific brain regions, and (2) Voxel-Based Morphometry, an unbiased, voxel-wise assessment of gray matter volumes.

Results: Results from both volumetric analysis techniques revealed no significant differences between the AUC and MC groups (p < 0.001). Additionally, within the AUC sample, cortical structure was assessed relative to months of participation and results showed a significant positive correlation between duration of Ayahuasca use and gray matter volume in a single cluster that included regions of the right corpus callosum, parahippocampal gyrus and precuneus (P < 0.001).

Conclusions: Overall, results suggest when used in a religious context, Ayahauasca is not associated with smaller cortical or cerebellar volumes.

Keywords: Human Neuroimaging, hallucinogens, brain structure

Disclosures: Nothing to disclose.

W125. Pharmacological Characterization of the Novel M1 Positive Allosteric Modulator Compound 25

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Background: M1 muscarinic acetylcholine receptor (mAChR) activators are of interest as potential novel therapeutics for the treatment of cognitive impairments associated with Alzheimer's disease and schizophrenia. Compound 25 (C-25; Davoren et al., submitted) is a potent M1 positive allosteric modulator (PAM) containing an azoindole core, that is > 180-fold selective against M2-5 mAChRs.

Methods: Experiments were performed using human M1 receptors stably expressed in CHO cells. Functional activity was measured using five different techniques: (1) a calcium mobilization fluorometric imaging plate reader (FLIPR) assay, (2) a PathHunter® β-Arrestin assay, (3) an inositol phosphatase (IP1) homogenous time resolved fluorescence (HTRF) assay, (4) a [3H]N-methylscopolamine ([3H]NMS) binding assay, and (5) electrophysiologically, using multi-electrode arrays to record spontaneous action potentials in the CA1 stratum radiatum of rat hippocampal slices. C-25 was tested either in the presence (PAM mode) or absence (agonist mode) of an EC20 concentration of either acetylcholine (ACh) in the FLIPR and IP1 assays, or carbachol in the hippocampal slice assay. Acetylcholine concentration response curve shift experiments were performed using FLIPR, β-Arrestin and [3H]NMS binding assays, in the presence of increasing concentrations of C-25. Animal studies were conducted in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals, and were approved by the Pfizer Animal Care and Use Committee. The effect of subcutaneous (SC) administration of C-25 (1, 3.2 or 10 mg/kg) on in vivo IP1 activity was measured in mouse striatum, hippocampus and prefrontal cortex tissue in the presence of

LiCl to enhance brain IP1 accumulation. LiCl (100 mg/kg, SC) was administered an hour before administration of C-25 or vehicle. Mice were microwaved three hours after LiCl and two hours after C-25 administration, respectively, and their respective brain regions were dissected out and processed with the CisBio IP1 kit. Groups of 5 mice were used per dose. The effect of C-25 (1, 3.2 or 10 mg/kg) on mouse locomotor activity (LMA) was determined in the absence and presence of amphetamine stimulation. Mice were administered C-25 or vehicle, placed in testing chambers, and spontaneous LMA was recorded for 90 minutes. For the amphetamine-stimulated LMA, mice were allowed to habituate to the testing apparatus for 60 minutes and then were administered C-25, haloperidol (0.1 mg/kg; SC) or vehicle, and then returned to the testing chamber. Data were recorded for 30 minutes and then the mice were removed and dosed with d-amphetamine (1.78 mg/kg; IP) or vehicle, before being placed back into the testing apparatus for the next 90 minutes.

Results: Using the FLIPR assay, C-25 activated M1 mAChRs with EC50 values of 55 ± 8 nM (mean ± SEM, n = 15; PAM mode) and > 1.4 ± 2 .7 μM (n = 13; agonist mode), demonstrating a PAM-agonist profile. This profile was also observed using the M1 IP1 assay, for which EC50 values were 181 ± 61.0 nM (mean ± SEM, n = 3) and 2.5 ± 0.5 μM (n = 3), in PAM and agonist mode, respectively, and for the native rat receptor hippocampal slice assay, in which C-25 increased a carbachol EC20 concentration CA1 neuron firing response with an EC50 of 225 ± 49 nM (mean ± SEM, n = 6). In the absence of carbachol this effect was 15-fold weaker, with an EC50 of 3.33 ± 0.59 μM (n = 7). At a concentration of 10 μM, C-25 was shown to increase the affinity of ACh for M1 mAChRs, inducing a concentration-dependent leftward shift of the ACh concentration response curve in the FLIPR assay (167-fold shift) and β-Arrestin assay (212-fold shift), as well as increasing the ability of ACh to displace [3H]NMS binding assay (339-fold shift). Having demonstrated robust in vitro activity with C-25, in vivo activation of the M1 receptor was then measured using mouse IP1 and LMA assays. In line with M1 mAChR activators functioning through a Gαq-coupled intracellular signaling cascade, C-25 (10 mg/kg, SC) was shown to increase IP1 levels in mouse striatum, hippocampus and prefrontal cortex tissue by 8.5 ± 1.9, 4.1 ± 1.0 and 4.3 ± 0.9-fold (mean ± SEM; n = 5), respectively, relative to vehicle. C-25 was also shown to be effective in an amphetamine-stimulated locomotor activity study in mice, with amphetamine-induced hyperactivity being significantly reduced in mice dosed with 3.2 and 10 mg/kg C-25.

Conclusions: These data characterize C-25 as a selective M1 PAM-agonist in both human cell line assays and a native rat brain slice assay, with in vivo activity demonstrated using both biochemical and behavioral read-outs. Further work will be required to determine whether C-25 is effective at improving cognition read-outs in vivo and whether a PAM-agonist profile offers any advantage over an M1 agonist profile.

Keywords: muscarinic acetylcholine receptor, positive allosteric modulators, Cognitive Enhancement

Disclosures: All authors are employed by Pfizer Inc.

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W126. Examining the Effects of Microbiome Depletion on the Behavioral Phenotypes of Selectively Bred High-and Low-Responder Rats

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Background: One of the most important events early in life is the establishment of the gut microbiota, which affect various aspects of host physiology, including brain development and function. Emerging evidence suggests that the gut microbiota is able to influence behavior through modulation of the gut-brain axis. A number of recent studies have demonstrated a relationship between gut microbiota, stress-reactivity and stress-related behaviors—implicating a role for the gut-brain axis in vulnerability to psychiatric disorders. In addition, the gut microbiota has been associated with memory performance and locomotor activity in animal models. Here we utilized a unique genetic rat model to examine the effects of gut microbial depletion on their known behavioral phenotypes. Specifically, we used selectively bred high-responder (bHR) and low-responder (bLR) rats. These rats are bred based on locomotor response to a novel environment, but have been shown to differ on a number of other traits. Compared to bLRs, bHRs exhibit elevated exploratory behavior, are more impulsive, more aggressive, seek stimuli associated with rewards, and show a greater tendency for drug-seeking behavior or relapse. In the current study, we examined the effects of gut microbiota depletion on addiction- and anxiety-related traits in these two rat lines.

Methods: bLR and bHR rats from our in-house selective breeding colony were used and divided into two groups per phenotype: control and antibiotic treatment (ABX). ABX-treated rats received a combination of 1 g/L ampicillin sodium, neomycin sulfate, and metronidazole; 0.5 g/L vancomycin hydrochloride in tap water for four weeks. The antibiotic mix was changed biweekly. Behavioral testing occurred following depletion of the gut commensal microbiota. We examined the effects of gut commensal microbiota depletion on the known behavioral phenotype of bHR and bLR rats using four behavioral assays: 1) Pavlovian conditioning (or autoshaping), to assess individual differences in food-associated cue reactivity; 2) conditioned reinforcement, to assess the motivational properties acquired by a food-associated cue; 3) locomotor response to a novel environment, as an index of novelty-seeking behavior; and 4) elevated plus maze as an index of anxiety-like and risk-taking behaviors. The Pavlovian conditioning paradigm consisted of 5 sessions, each comprised of 25 trials of cue-reward pairings, which in this case was a lever and food reward. Conditioned responses directed towards either the lever (i.e. sign-tracking) or the food cup (i.e. goal-tracking) were examined. The conditioned reinforcement test assessed the rats' motivation for the food-associated cue, in the absence of food itself. Thus, responding in an active port that resulted in cue (lever) presentation was examined. Locomotor response to novelty was examined by assessing locomotor activity for 1 hour after rats were placed in a novel environment. Behavior in the elevated plus maze was examined during a 5-min test, for which the number entries into the open vs. closed arms and the latency with which the rats moved into the open arms was examined.

Results: 10% of bHR and 70% of bLR rats refused to drink the water containing antibiotics and were eliminated from the study. Thus, these experiments were conducted with 10 control bHR rats, 9 ABX-treated bHR rats, 10 control bLR rats, and 3 ABX-treated bLR rats. Our results indicate that depleting the gut microbiota of rats selectively bred for their locomotor response to novelty alters their behavioral phenotype. Specifically, in bHR rats, ABX treatment appears to increase the propensity to attribute incentive motivational value or incentive salience to food-associated cues. That is, ABX treatment enhanced sign-tracking behavior in bHR rats and increased their motivation to respond for a food-associated cue. Further, although the percentage of time spent in the open arms did not differ between treatment groups, ABX-treated bHR rats entered the open arm of the elevated plus maze faster than bHR controls. ABX treatment did not alter locomotor response to novelty in bHR rats, but ABX-treated bLRs showed a tendency towards enhanced activity immediately after being placed in the novel environment when compared to control bLRs. However, for bLRs, there were no significant differences observed on any of the behavioral tests, but this is likely due to the small sample size.

Conclusions: Here we show that depletion of the gut microbiota via antibiotic treatment in drinking water alters the behavioral phenotype of selectively bred rats. These findings suggest that gut microbiota depletion enhances addiction-related and risk-taking behaviors in rats with an inherent predisposition for these traits. Ongoing studies are exploiting these selectively bred rats lines to examine the microbiome and metabolome under basal conditions (i.e. prior to any manipulation), and following some of the testing and manipulations described above. In addition, we will utilize this model to further explore the relationship between the gut, brain and behavior.

Keywords: Gut Microbiome, individual differences, Anxiety, addiction, sign-tracking

Disclosures: Nothing to disclose.

W127. In Vivo Serotonin Chemistry and Local Cytoarchitecture: A Combined Voltammetric, Mathematical and Microscopy Study

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Background: Fast scan cyclic voltammetry (FSCV) can provide sub second in vivo measurements of evoked serotonin release and reuptake. We previously used FSCV to describe serotonin dynamics in the mouse substantia nigra, pars reticulate (SNr) and developed a mathematical model to fit experimental data corresponding to serotonin's two discrete reuptake mechanisms, Uptake 1 and Uptake 2. Here, we expand this work to two important regions, the

CA2 region of the hippocampus and the prefrontal cortex (PFC). We describe two novel voltammetric circuitries for evoking and measuring serotonin in the CA2 region and the PFC. We compare evoked serotonin release and reuptake with FSCV and ambient serotonin levels (with a novel technique, fast scan adsorption controlled voltammetry (FSCAV)) between the SNr, CA2 region and PFC. Our models allow us to determine the average contribution of Uptake 1 and 2 in each region and correlate these contributions to local cytoarchitecture, as determined with 2-photon microscopy.

Methods: A single carbon fiber was aspirated into a glass capillary, pulled apart under heat and cut to 150 μM. The resulting CFM was electroplated with Nafion. Adult, male C57BL/6 mice weighing 20-25g were anesthetized with urethane. Mouse procedures were in compliance with WSU's Guide for the Care and Use of Laboratory Animals, approved by the Institutional Animal Care and Use Committee (IACUC). Stereotaxic surgery was performed to implant the CFM into the CA2 region of the hippocampus, a stimulating electrode into the medial forebrain bundle (MFB) and a reference electrode into the contralateral brain hemisphere. Electrical pulses were delivered via a linear constant current stimulus isolator.

Results: We found that serotonin is evocable in the SNr, CA2 region and the PFC with a geographically common electrical stimulation of the medial forebrain bundle (MFB). Evoked serotonin and ambient serotonin differ in all three regions. Ambient serotonin levels are lowest in the CA2 and mathematical models suggest the least contribution from Uptake 1 in this region. This chemical finding is backed by immunohistochemical imaging of all three regions with 2-photon microscopy, revealing the least axonal innervation and SERT density in the CA2.

Conclusions: The novel combination of our methods provides important insight into the chemistry of different brain regions. This is of particular interest for studies that probe the functional neurobiology and involvement of different brain areas in disease.

Keywords: voltammetry, Serotonin, computational modeling

Disclosures: Nothing to disclose.

W128. Clathrin Nanoparticles Efficiently Deliver Antibodies to Targeted Dopamine Brain Regions

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Background: Antibodies (Abs) have great promise for detection and treatment of central nervous system (CNS) disorders. However, the blood-brain barrier (BBB) is a major impediment to effective delivery. Only 0.1% of plasma Abs enter the CNS. Abs may take days to diffuse only a few millimeters and CNS concentrations may still be insufficient for therapeutic efficacy. Our goal was to develop a new nanotechnology method for efficient noninvasive intranasal delivery of antibodies to targeted dopamine brain regions.

Methods: Dopamine-3 receptors (D3R) in rats have a restricted CNS distribution and D3R antagonists may be of value in treatment of drug dependence and psychosis. Thus, D3RAbs were selected, PEGylated and conjugated to clathrin triskelia through cysteine residues. Transmission electron microscopy and dynamic light scattering determined nanoparticle size and shape. Nanoparticle immunoreactivity was tasted by Western Blot. Low doses (64 μg/kg) of nanoparticles were delivered intranasally in rats. Control animals received D3RAbs or saline. Animals were perfused and sacrificed three hours after intranasal administration and immunohistochemistry analyses were performed. ELISA was used to quantify D3RAbs in different brain regions. Rhodamine-PEGs were then attached to D3RAb-nanoprobes and their in vivo stability was tested with confocal microscopy.

Results: D3RAb-nanoprobes (42.3 nm) remained immunoreactive after the modifications. Three hours after intranasal administration D3RAb-nanoprobes were found only in D3R brain regions in rats. Fluorescent and light microscopic examination confirmed specific targeting of CNS D3-receptors with D3RAb-nanoprobes. The highest nanoprobe concentration (2,753 ng/g) was detected in basal forebrain (islands of Calleja and ventral pallidum) and nucleus accumbens (1,028 ng/g). Low concentrations were detected in the cerebellum. D3RAbs delivered intranasally but without clathrin did not enter the brain. Confocal laser microscopy confirmed integrity of the nanoparticles in rat brain. Clathrin and D3RAb fluorescence co-localized in the D3R brain regions.

Conclusions: Clathrin-nanoprobes successfully bypassed an intact BBB after intranasal administration. Nanoprobes were able to target D3 receptors and deliver adequate concentrations of Abs inside neurons (17.2% ID/g) by using doses 300 times smaller than previously reported in BBB technologies studies. This nanotechnology holds promise for delivering antibodies to treat neurodegenerative disorders, to suppress neuroinflammation, infection or cancer growth, to regulate GPCR receptors, and serve as nanoprobes for diagnosis and monitoring of cellular events.

Keywords: Neurotechnology, Clathrin nanoparticles, CNS antibody delivery, Dopamine 3 receptor imaging

Disclosures: GV and FV own equity in ExQor Technologies, Inc., the company that owns patent rights to the bioengineered clathrin protein nanotechnology (patents #7216038, #7219017, #7219018, #7393924).

W129. Results of a Survey of Clinician Rated Outcome Measures in International Cns Clinical Trials

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Background: Most trials in neuropsychopharmacology utilize clinician administered rating scales. This has implications in international clinical trials with regards to translation, validity and cultural adaptibility. In addition unlike patient rated outcome measures, there are no

guidance documents on the administration of these clinician administered rating scales.

Methods: A survey was sent to drug development scientists who are engaged in conducting international clinical trials in neuropsychopharmacology. Of 442 recipients of the survey 78 responded. The average experience of responders in being involved in clinical trials was 16 years, with over 55% having had formal training in psychometrics.

Results The results indicate a lack of clear guidance on the administration of these clinician rated scales and issues with translations, and cultural adaptability.

Conclusions: This survey supports the need for clear guidance on the administration of clinician rated outcome measures, especially in the context of international clinical trials.

The authors are developing such a guidance.

Keywords: Rating scales, international, clinical trials

Disclosures: Amir Kalali is full time employee of Quintiles, Richard Keefe is affiliated with Neurocog Inc., Elizabeth Pappadopulos is an employee of Pfizer, Monika Vance is affiliated with Santium.

W130. Generation of a Functional Human Cortex from Pluripotent Stem Cells

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Background: The development of the human cortex is one of the most intriguing biological processes, and disruptions in this elaborate succession of cellular events have been associated with neuropsychiatric disease. The ability to reprogram human somatic cells into pluripotent stem cells (embryonic stem cells or induced pluripotent stem cells) and differentiate these cells in vitro opens a unique opportunity to study normal and abnormal corticogenesis.

Methods: We present a simple and reproducible 3D culture approach for generating a laminated cerebral cortex-like structure, named human cortical spheroid (hCS), from human pluripotent cells.

Results: These hCS contain both deep and superficial layer cortical neurons and map transcriptionally to in vivo fetal development. The majority of neurons are electrophysiologically mature, display spontaneous activity, are surrounded by non-reactive astrocytes, and form functional synapses. Importantly, physiology experiments in acute slices of hCS demonstrate that cortical neurons participate in network activity and are capable of producing complex synaptic events associated with postsynaptic neuronal spike firing.

Conclusions: These 3D cultures allow the interrogation of human cortical development and disease in unprecedented detail and represent a versatile platform for generating other neuronal and glial subtypes in vitro.

Keywords: Induced pluripotent stem cells (iPSCs), cortex, neurodevelopment

Disclosures: Nothing to disclose.

W131. Neural Architecture of a Pair Bond: Calcium Imaging of the Nucleus Accumbens in Awake-Behaving Prairie Voles

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Background: Despite the strong evidence that social bonds impact numerous aspects of human health, the neural basis for bonding remains poorly understood. This is in part due to the relative shortage of appropriate animal models; biomedical research relies heavily on rats and mice, but these animals do not form strong, selective bonds with other adult conspecifics. However, laboratory-amenable prairie voles offer further opportunities to understand how social bonds are formed and maintained because, as a monogamous species, they form intense bonds between mated partners. Multiple lines of converging evidence suggest that the nucleus accumbens (NAcc) plays a critical role in pair bonding. Monogamous prairie voles exhibit high levels of oxytocin receptors in the NAcc. Local antagonists or reduction of oxytocin receptors in the NAcc inhibits bond formation, while increasing levels of these receptors can enhance bond formation. In addition, both dopamine D2 and μ-opioid receptor signaling within the shell of the NAcc are required for bonding. Further, plasticity in gene expression within this brain region has been observed following pair bond formation. This suggests that coordinated action of multiple neuromodulatory systems converge in the NAcc during pair bond formation and that neuroplastic changes within this region may help to maintain social bonds. However, despite the clear importance of the NAcc in pair bonding, very little is known about the local circuit within the NAcc that mediates this behavior, and how this circuit changes upon bond formation and over time.

Methods: In order to understand the neural circuits that underlie the formation of social bonds, we have used calcium imaging in vivo to monitor activity of large populations of NAcc neurons in prairie voles during epochs of social interaction. This allowed us to observe population activity in response to different social and non-social stimuli before and after bond formation.

Results: Analysis of NAcc neuron firing rate revealed that a subset of these neurons code for both the type of stimulus (social versus non-social) and the type of the social interaction. Our ongoing efforts are aimed at identifying how this population code within the NAcc is related to interactions with a bonded partner versus a novel conspecific and how this changes with time after bonding.

Conclusions: Technological advances have made in vivo imaging possible in a wide range of species. Our use of this technology in prairie voles has the potential to elucidate the role of reward systems in the encoding of social bonds.

Keywords: calcium imaging, prairie voles, pair bond

Disclosures: Nothing to disclose.

W132. Comparison of Antipsychotic Medication Related Cardiometabolic Risk Factors in Patients with Psychotic vs Non-Psychotic Disorders and a Control Group

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Background: Use of atypical antipsychotic (AP) medications has been on the rise secondary to increasing on and off label use of these medications in patients with psychiatric illnesses. Use of APs has been associated with weight gain and metabolic syndrome. Cardiometabolic risks have been well documented in patients with primary psychotic disorders. It is unclear if patients with non-psychotic disorders receiving APs carry differential risks of cardiometabolic complications. It is also unclear how these complications compare with cardiometabolic complications in patients carrying a psychiatric diagnosis but not receiving APs.

Cardiometabolic complications in patients with primary psychotic disorders have been associated with use of APs. However other factors (e.g. lifestyle, nutrition) related to having a serious mental illness have also been known to increase risk of cariometabolic complications. The objective of the present study is to investigate cardiometabolic risks in patients receiving APs for psychotic and non-psychotic indications in comparison with a control group of patients with psychiatric illness not receiving these medications.

Methods: A retrospective, cross-sectional chart review was conducted by randomly selecting records of active patients in the outpatient psychiatry clinic over a 1 year period. Inclusion criteria: Patients 18-79 years of age receiving APs for at-least 8 weeks for the Psychotic Disorder (PD) and Non-psychotic Disorder (NPD) indications & patients with psychiatric illness but not receiving any antipsychotic medications as the Control group. Exclusion criteria: Patients with diagnosis of bipolar disorder excluded from the NPD group, patient charts missing data required for study analyses. Patients receiving typical antipsychotic medications were also excluded.

The chart review included assessment of body mass index (BMI) (primary outcome), and specific cardiovascular (BP, HR and QTc interval) and metabolic parameters (lipid panel, fasting blood glucose of HbA1c) as secondary outcomes. The data on demographic and clinical characteristics was gathered including data on medical, psychiatric comorbidities, smoking status and other lifestyle factors.

Results: A total of 211 charts were reviewed. Out of the total 211 subject records, 71 subjects had PD, 88 subjects had NPD and 72 subjects were in the control group. All three groups had predominantly white, non-Hispanic subjects, higher number of females and higher number of subjects 40 years of age or older. All three groups were similar in terms of their current and past tobacco smoking status with slightly higher number of current smokers in the control group. All three groups had similar medical comorbidities. Most frequently prescribed APs for the PD group were clozapine, olanzapine, risperidone and aripiprazole. Most frequently prescribed APs for the NPD group were olanzapine, risperidone, aripiprazole and quetiapine.

No differences in BMI were seen between all three (PD, NPD and Control) groups. However, the mean BMI for all three groups fell in the obese range. Similarly no differences were noted in the cardiometabolic health parameters, specifically lipid profile, glycemic control and other cardiovascular indices (BP, HR and QTc intervals). However, certain metabolic parameters (triglycerides, LDL) were at the higher end of the normal range in the PD group.

Conclusions: The cardiovascular risk factors did not differ between the patient groups receiving APs for psychotic and non-psychotic indications as well as a control group of patients with mental illness not receiving APs. However, all three groups showed high cardiovascular risks based on BMI data and data on metabolic parameters. The physical health all patients receiving psychiatric care need close monitoring and interventions to improve cardiometabolic health.

Keywords: Antipsychotics, Cardiometabolic Risk, serious mental illness

W133. Reduced Between-Network Connectivity Following Exercise in Overweight/Obese Adults

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Background: Understanding how exercise affects neuronal response in overweight/obese individuals may provide insight into mechanisms of weight loss and maintenance. Previous studies have found overweight/obese individuals to show altered activity in intrinsic brain networks and changes in this activity following exercise. However, the impact of exercise on between-network connectivity is unknown. The current study investigated the effects of a 6-month exercise intervention on between-network connectivity in overweight/obese adults.

Methods: Ten overweight/obese adults completed the study (5 women, 5 men; mean BMI $\pm$ SEM: 33.6 $\pm$ 1.4 mg/kg2; mean age $\pm$ SEM: 38.2 $\pm$ 3.2 years). Resting-state brain network connectivity was assessed in the fasted state at baseline and post-exercise using fMRI. The exercise intervention was a 6-month supervised treadmill-walking program. Functional images were acquired with an echo-planar gradient-echo T2* Blood Oxygenation Level Dependent (BOLD) imaging contrast technique (TR = 2000 ms, TE = 30 ms, FOV = 240 mm2, 642 matrix, 27 axial slices angled parallel to the planum sphenoidale, 2.6 mm thick, 1.4 mm gap). Participants were asked to rest quietly with eyes open for 10 minutes. fMRI data were realigned, normalized to standard space, and smoothed with an 8mm FWHM Gaussian kernel. A whole-brain between-network connectivity analysis was performed to identify hubs with high between-network interaction, i.e., areas with high levels of connectivity to large-scale networks. Networks were identified using independent components analysis (ICA). Following ICA back-reconstruction, hubs were identified through multiple regression analyses assessing correlations between

ICA component time series' and the time series for each individual voxel. Following hub identification, effects of exercise on between-network connectivity at each hub were determined using t-tests in SPM8. To explore factors driving these observed differences, granger causality analysis was used to determine changes in directed hub connectivity from pre- to post-exercise.

Results: The posterior cingulate cortex (PCC) was identified as a hub with high levels of between-network connectivity. Overall PCC between-network connectivity was reduced following exercise, compared to baseline (t = 6.43, p < .001). Granger causality analyses identified significant changes in outgoing causal flow from the PCC following exercise (change in weighted out-degree = 0.22, significant at p < .05 by permutation testing), driven by reduced connection strength to a number of networks, including a ventral DMN network, medial temporal lobe network, sensorimotor network, higher visual network, and a visuospatial network. Additionally, ingoing causal flow to the PCC was altered post-exercise (change in weighted in-degree = .07, significant at p < .05 by permutation testing), driven by reductions in causal influence from a language network, sensorimotor network, right executive control network, and dorsal default mode network.

Conclusions: This study found reduced between-network connectivity in the PCC following exercise. This change was likely driven by reductions in both outgoing and ingoing connection strength between the PCC and multiple networks. It is possible that these alterations reflect an increase in the specificity of information flow between networks following exercise.

Keywords: exercise, fMRI Functional Connectivity, Obesity

Disclosures: Nothing to disclose.

W134. Comparing Connectomes Using Anatomical Connectivity within Extended Reward Network Regions across Male and Female Obese Subjects

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Background:: Alterations in key brain regions of an extended reward network have been linked to increased ingestive behaviors in obesity. Our recent publication demonstrated that a supervised learning algorithm can discriminate overweight compared to normal weight with greater than 90% in using regional connectivity but less so in brain morphometrics (70%) (Gupta et al., Neuroimage-Clinical, 2015). Differences were observed in the extended reward network, which includes reward, somatosensory, salience, emotional arousal, and executive control network regions. These results provided the basis for further characterization of the effects of group (obese, overweight, lean) and sex on the anatomical connectivity within the extended reward network. Connectomes were used for visualization of major disease and sex differences.

Methods: White-matter was measured in 120 healthy subjects. Regional parcellation was conducted using Free-

surfer based on the Destrieux and Harvard Oxford atlases, and resulted in 74 bilateral cortical and 7 subcortical structures, including the cerebellum. White matter connectivity for each subject was estimated between the 165 brain regions using DTI fiber tractography and Runge-Kutta algorithm. Estimates of white matter connectivity between each of the brain regions was determined based on the number of fiber tracts intersecting each region, normalized by the total number of fiber tracts within the entire brain. Controlling for the main effects of age, the general linear model was applied in Matlab on connections that measured projections from regions associated with the extended reward network. Connections showing preliminary significance were then re-analyzed using SPSS (Statistical Package for the Social Sciences) using a number of customized contrasts to assess for disease differences and sex differences. The resulting p-values were corrected for multiple comparisons. Significance was set at q < 0.05.

Results: 1. Subject Characteristics: There were 57 lean (mean BMI = 22.18kg/m2, 34 females), 47 overweight (mean BMI = 29.41kg/m2, 18 females), and 16 obese (mean BMI = 34.38kg/m2, 8 females) individuals. 2. Group Differences. i) Obese vs. Lean: Compared to females, smaller differences in connectivity were found between obese and lean subjects in males in reward to reward regions (brainstem to right NAcc (β = -1.127, q = 0.031), right CaN to left OFG (β = -0.289, q = 0.030), left OFG to right OFG (β = -2.501, q = 0.026), left CaN to right OFG (β = -1.554, q = 0.020)). Compared to females, smaller differences in connectivity were found between obese and lean subjects in males in reward to emotional arousal regions (left Amyg to left OFG (β = -14.603, q < 0.001), left Amyg to right NAcc (β = -0.143, q = 0.016), brainstem to left pgACC (β = -1.855, q = 0.008)). Compared to females, smaller differences in connectivity were found between obese and lean subjects in males in reward to somatosensory regions (left Hipp to left central sulcus (β = -1.343, q = 0.012), right CaN to right motor cortex (β = -4.631, q = 0.001), right pINS to right OFG (β = -0.149, q = 0.012), and left pallidum to left pINS (β = -0.131, q = 0.025). ii) Obese vs. overweight: Compared to females, smaller differences in connectivity were found between obese and overweight subjects in males in reward to reward regions (left OFG to right OFG (β = -2.551, q = 0.040), and left CaN to right OFG (β = -1.664, q = 0.025), but greater differences in connectivity were found from left NAcc to the right OFG (β = 0.614, q = 0.041)). Compared to females, smaller differences in connectivity were found between obese and overweight subjects in males in reward to emotional arousal regions (left Amyg to left OFG (β = -14.173, q = .002), left Amyg to right NAcc (β = -0.143, q = 0.030), and brainstem to left pgACC (β = -1.733, q = .024). Compared to females, smaller differences in connectivity were found between obese and overweight subjects in males in reward to somatosensory regions (left Hipp to left central sulcus (β = -1.406, q = 0.018), right CaN to right motor cortex (β = -4.706, q = 0.002), right pINS to right OFG (β = -0.151, q = 0.022) and left pallidum to left pINS (β = -0.128, q = 0.050), but greater differences in connectivity from right CaN to right pINS (β = 0.292, q = 0.037).

Conclusions: CONCLUSION: The connectomes demonstrate that the anatomical networks of regions within the

extended reward network vary by both BMI and sex. Specifically being obese and female is associated with more local and regional connectivity between regions associated with increased dopamine production, and less information propagation was observed in the cognitive frontal regions.

Keywords: Obesity, sex differences, anatomical connectivity, connectome, extended reward network

Disclosures: Nothing to disclose.

W135. Novel Human Evidence of Psychosocial Stress-Induced Changes in IL-18: A Potent and Ubiquitous IL-1 Family Cytokine

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Background: Substantial evidence implicates increased exposure to psychosocial stress in the exacerbation, progression, and (inversely) prognosis of a wide range of medical illnesses (i.e. coronary artery disease, various arthritides, breast cancer, etc.). Similarly, evidence also implicates interleukin-18 (IL-18), a potent, ubiquitous, IL-1 family inflammatory cytokine, in the pathophysiology of these stress associated disease states. However, while animal model evidence suggests exposure to psychosocial stress can induce plasma IL-18, human studies that directly test the impact of psychosocial stress on IL-18 are lacking. Furthermore, evidence to date from human trier social stress test (TSST) studies suggests that TSST-induced inflammatory changes occur downstream of the classic TSST-induced HPA response (as quantified by plasma ACTH and cortisol). However, these TSST studies have not examined IL-18 and have focused on inflammatory cytokines downstream of IL-18. Providing evidence that psychosocial stress induces an increase in plasma IL-18 concentration will enhance understanding of biological pathways involved in somatic translation of psychosocial stress, thereby introducing novel targets for development of potent, individualized treatment strategies in illnesses associated with substantial morbidity and mortality.

Methods: In an effort to test the impact of psychosocial stress on plasma IL-18, we exposed 60 healthy human controls (both males and females) to a standardized experimental stress paradigm (TSST) over the course of 90 minutes, collecting blood samples before, during, and after exposure to stress. Time of day was constant for all studies and time since awakening was obtained from all study volunteers. Additional data collected at study entry from each subject included age, body mass index (BMI), and psychometrics (Beck Depression Inventory, BDI; State Trait Anxiety Inventory, STAI). Plasma concentrations of IL-18, IL-18bp, and classic stress hormones (ACTH, cortisol) were quantified from blood samples using standard assay techniques (i.e. ELISA, RIA).

Results: Using Spearman rank testing, we identified a lack of correlation between each of age, time since awakening, and BMI and either IL-18 or percent IL-18 bound to IL-18bp, each assessed from plasma at time of TSST initiation ($p > 0.05$ for each). However, at TSST initiation, plasma IL-18 concentration correlated with psychometrics obtained at initiation of TSST including depression (BDI: $\rho = 0.30$, $p = 0.03$) and anxiety (STAI total: $\rho = 0.31$, $p = 0.03$; STAI State: $\rho = 0.36$, $p = 0.009$; STAI Trait: $p > 0.05$). Neither cortisol nor ACTH correlated with psychometrics obtained at time of TSST initiation ($p > 0.05$ for each). Results from repeated measures ANOVA testing (repeated measure: time, dependent variables: IL-18, ACTH, and Cortisol) confirmed the presence of significant changes in IL-18 ($F_{2,94} = 7.1$, $p = 0.001$), ACTH ($F_{2,94} = 26.5$, $p < 0.001$), and Cortisol ($F_{2,94} = 35.0$, $p < 0.001$) during the TSST task. Additional Repeated Measures ANOVA testing, substituting percent of IL-18 bound to IL-18bp in lieu of Il-18 as the main dependent variable showed stress to have a significant impact on ACTH ($F_{8,320} = 26.2$, $p < 0.001$), Cortisol ($F_{8,320} = 25.6$, $p < 0.001$), and Percent IL-18 bound to IL-18bp ($F_{8,320} = 2.9$, $p = 0.004$).

Conclusions: The data we present replicates evidence in animal models showing that exposure to experimental stress can impact plasma concentrations of a potent inflammatory cytokine, IL-18, ubiquitously expressed in various cells throughout the body and implicated in the pathophysiology of a range of stress-exacerbated medical illnesses. This evidence proposes expanded research using novel, IL-18 blocking intervention strategies, to determine IL-18's role in mediating the effect of psychosocial stress on severe, stress-exacerbated medical illnesses. That novel medical treatments based on IL-18 functioning are currently under development further highlights the potential contribution of our findings. However, while initial testing suggests that stress induction of IL-18 may be independent from the effect on classical HPA hormones (i.e. ACTH, cortisol), further testing using novel approaches (i.e. time varying effects models) is needed to determine whether the stress induced changes in IL-18 we identify are in fact independent of ACTH and cortisol.

Keywords: cytokines, psychosomatic medicine, systemic inflammation, psychoneuroimmunology, psychosocial stress

Disclosures: Nothing to disclose.

W136. Linking PFC Internal Capsule Pathways with FA Values: Implications for Relating Abnormalities in Disease to Specific Connections

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Background: The anterior limb of the internal capsule (ALIC) carries the ascending and descending prefrontal cortical (PFC) and anterior cingulate cortical (ACC) fibers. These cortical areas are associated with emotion, motivation and cognition and are linked to several psychiatric illnesses including obsessive-compulsive disorder, major depression disorder, schizophrenia and addiction. Importantly, these diseases show abnormal volume, fractional anisotropy (FA), and diffusivity generally in the internal capsule with some studies demonstrating these abnormalities specifically in the ALIC. However, unlike the motor pathways through the capsule, little is known about how fibers from different regions of the PFC/ACC are organized within the ALIC,

limiting the ability to link abnormalities within the ALIC to specific connections. In this study, we used anatomical tracing experiments in nonhuman primate (NHP) to determine how fibers from the ventromedial PFC (vmPFC), dorsal ACC (dACC), the dorsomedial PFC (dmPFC), the dorsolateral PFC (dlPFC), the ventrolateral PFC (vlPFC), and the orbital cortex (OFC) reach and travel within the ALIC. We then tested how well this organization is replicated using diffusion magnetic resonance imaging (dMRI) in NHP and humans. Finally, we evaluated the FA values through the capsule and examined whether variations in those values were associated with specific connections.

Methods: Anterograde or bidirectional tracers were injected into PFC/ACC regions. Each case was charted separately, rendered in 3D (IMOD) and transferred to a standard macaque brain for comparison. All experiments were conducted according to the ILAR Guide for the Care and Use of Laboratory Animals (ILAR, National Research Council, 1996) and approved by The University Committee on Animal Resources. Injection sites were used as dMRI seeds for probabilistic tractography (dMRI data of twelve monkeys, acquired with a 4.7T Bruker BioSpin MRI system using a two-shot echo-planar imaging sequence with b-value of 40000cm2s-1, 515 diffusion directions and a 0.7x0.7x0.7mm3 resolution). We compared the topographic organization of the PFC dMRI tractography with those identified with chemical tracing. We then analyzed human data, publically available through the Human Connectome Project. Finally, we divided the ALIC in three regions of interest (ROIs) based on the underlying connections and performed statistical analyses to compare these ROIs (mean and variance characterization, to study the variation of FA values). Finally, we studied the FA profile of 35 human subjects from the Human Connectome Project and investigated, with a similar statistical approach, an FA-driven segmentation of the ALIC.

Results: Results identify the paths taken by PFC fibers to enter the ALIC and the positions they take within the bundle. PFC/ACC fibers in the ALIC adhere to specific rules of organization that are linked to their medial/lateral, ventral/dorsal and anterior/posterior cortical position. Using these rules, we can predict where, within the capsule, specific fibers are likely to travel. Moreover, we found that dMRI was able to replicate with relative accuracy most of these rules in both the NHP and the human ALIC.

Importantly, our pilot data show that FA values are not uniform across the ALIC. These differences can be linked to specific connections within the ALIC and give rise to a well-defined FA topography, which we identified in both NHP and human dMRI.

Conclusions: Taken together, these results allow us to segment the ALIC into subareas based on specific PFC/ACC –thalamic/ brainstem connections in humans. The fact that these sub-areas can be linked to differences in FA-profiles allows us to link FA values to particular connections. This information gives us a key marker for evaluating the local FA abnormalities reported in neuroimaging studies of psychiatric diseases and relating them to the underlying connections.

Keywords: psychiatry, internal capsule, alic

Disclosures: Dr. Haber has received speaker honoraria from Pfizer and Medtronic, Inc.

W137. Novel Bold Activity Dynamics in Response to Anesthesia Modulation Observed Using Wide-Spectrum Functional MRI

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Background: Functional biomarkers for neuropsychopharmacological action in vivo are critically needed for the study and development of neuropsychiatric drugs. Amongst non-invasive approaches, functional MRI based on the blood oxygenation level dependent (BOLD) signal is used to delineate brain regions affected by a drug based on differences in task activation magnitude or inter-regional correlation of spontaneous activity. However, these measures are not informative of the regional timing and parameters of drug effects, nor are they flexible in detecting novel activity dynamics beyond task responses or low-frequency resting correlations.

Here we demonstrate a BOLD imaging platform that our results indicate can support discovery science for pharmacological action in vivo, independently of particular task designs or limited frequency ranges for spontaneous activity, based on a proof-of-concept anesthesia modulation experiment. An innovative imaging strategy was used to make these observations with high signal fidelity, involving 7T MRI, customized transmit-receive MRI coils and pulse sequences, high-dimensional decomposition and denoising, and wavelet-based time-frequency analysis to identify phasic modulations – altogether called wide-spectrum (WS)-fMRI.

Methods: Primate imaging was utilized in this experiment, which was evaluated and approved by the local IACUC. Six macaque monkeys were anesthetized using isoflurane, and dose was modulated during MRI by altering the vaporizer isoflurane level as follows: 20 minutes at baseline (1.0-1.3%), 20 minutes deep (2.5%), 20 minutes of return to baseline, for 1 hour total. Tissue isoflurane concentration was monitored with a specialist gas analysis device (PhaseIn infrared sidestream analyzer; Masimo, Sweden) recording physiological changes in response to anesthesia. Measured tissue isoflurane indicated complex time courses rather than simple step changes. Monkeys were ventilated, keeping SPO2 and end-tidal CO2 within physiologically normal parameters. Imaging with a 7 Tesla Siemens Magnetom MRI (Erlangen, Germany) and a custom monkey transmit/8-channel receive array head coil yielded high signal-to-noise ratio for characterizing novel BOLD pharmacodynamics. WS-fMRI was based on a multi-echo echo planar imaging (ME-EPI) pulse sequence (Poser et al., 2006a) with 1.5mm isotropic resolution and TR $= 1.8$s for whole brain coverage. ME-EPI data was processed using multi-echo independent components analysis (ME-ICA) which decomposes EPI time series into statistical components using high-dimensional spatial ICA and denoises time series by removing non-BOLD components (Kundu et al., 2012, 2013, 2014, 2015). Since no temporal bandpass filtering is required using this procedure, BOLD activity of a wide frequency range was studied. Phasic changes in response to anesthetic manipulation were elucidated with the continuous wavelet transform (CWT), sampling a number of windowing functions

(Dolph-Chebychev, Bartlett, cosine, etc.) across several scales. A sliding window functional connectivity analysis shows dynamic connectivity changes in response to anesthesia dose modulation.

Results: Whole-brain WS-fMRI at 1.5mm isotropic spatial resolution and 1.8s temporal resolution yielded temporal signal-to-noise ratio (tSNR) for BOLD time series being 342-655 (25th to 75th percentiles) without spatial smoothing or temporal bandpass filtering, compared to conventional filtered fMRI yielding tSNR of ~ 60-100. Phasic time courses (from dorsolateral prefrontal cortex, DLPFC) indicated large effect-size (6% signal change) differences in amplitude of oscillatory activity between light and deep sedation. CWT transformation of these phasic time series produced coefficient series for wavelet scales 10-30 that correlated significantly with measured anesthesia depth ($p < 0.001$), but bandpassed BOLD time series did not ($p < 0.17$). This indicates that CWT with varying windowing functions and scales may be used to catalog a wide variety of phasic activity in response to pharmacological manipulation. A 10-minute sliding window functional connectivity analysis of the executive function network (DLPFC seed) revealed a significant decrease in correlation (Pearson's R) as depth of anesthesia increased. Isolated BOLD time courses from representative voxels in selected ROIs (DLPFC and putamen) showed distinct phasic activity in response to changes in anesthesia depth, while the control region (primary visual cortex) is unaffected.

Conclusions: We developed signal acquisition, processing, and analysis methodology and elucidated a broad range of functional BOLD activity dynamics in response to the pharmacological challenge of anesthesia modulation, which served as a preliminary experiment to modulation of brain activity using neuropsychiatric drugs. We observed that this modulation results in phasic changes in brain activity in a dose-dependent manner, seen robustly even at the level of an individual-subject, which is untenable using current filtered fMRI techniques. Further study will involve translation of these techniques to human imaging and evaluating uptake and dose modulation BOLD response dynamics of fast-acting drugs such as oxytocin, benzodiazepines, and methylphenidate.

Keywords: Pharmacodynamics, fMRI, Anesthetic
Disclosures: Nothing to disclose.

W138. Preclinical Characterization of MIN-301, a Neuregulin-1 Fragment, as a Potential Disease-Modifying Therapy for Parkinson's Disease

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Background: MIN-301 is a recombinant protein with the same amino acid sequence as the naturally secreted extracellular domain of Neuregulin-1β isoform (NRG1). NRG1 acts mainly as a paracrine trophic factor, by binding through its EGF-like domain to ErbB receptors, activating ErbB2, ErbB3 and ErbB4 through homo- and heterodimerization and tyrosine phosphorylation. Recent studies have demonstrated that ErbB4 is likely the predominant NRG-1 receptor in the brain. This interaction initiates an intracellular signaling cascade in which phosphatidylinositol 3-kinase (PI3K) and the serine/threonine protein kinase Akt are activated, leading to a range of biological effects, including differentiation, synaptogenesis, neurogenesis and anti-apoptotosis. In addition, recent studies have shown a role for NRG-1/ErbB4 signaling in the regulation of synaptic plasticity and neurotransmission. NRG-1 and its signaling pathway have been extensively studied, and dysregulation of this pathway has been linked to disorders such as Parkinson's disease (PD). These findings have provided evidence that activation of the ErbB4 pathway via MIN-301 represents a potential treatment for neurodegenerative disorders, particularly Parkinson's disease (PD).

Methods: Rat 6-OHDA (6-Hydroxydopamine) model: A unilateral lesion was induced in Wistar rats by intracerebral infusion of 8μg 6-OHDA over 8 min into the substantia nigra pars compacta. Rats were administered MIN-301 (30 or 300 ng/mL) i.p. 6 hours post 6-OHDA injection, then daily for 8 days (early treatment), or at day 5 post 6-OHDA injection, then daily for 8 days (delayed treatment). Behavioral evaluation consisted of: a rotarod test to assess balance and coordination on a rotating rod with increasing speed; a cylinder test to evaluate locomotor asymmetry; and turning behavior to assess rotational behavior before and after injection of 0.1 mg/kg of the dopaminergic agonist apomorphine.

Mouse MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) model: C57/BL6J mice were treated with MPTP 10-50 mg/kg i.p. for 18 consecutive days. MIN-301 was administered at 30, 100 and 300 ng/kg i.p. for 31 days, just before (on Day 1) or 1 hour before (on the other days) MPTP injection. Behavioral evaluation consisted of a rotarod test and open-field test to measure the general locomotor activity and willingness to explore.

Marmoset MPTP model: A chronic slow induction protocol involving 4 weekly s.c. injections of 1 mg/kg MPTP followed by 3 weekly s.c. injections of 1.5 mg/kg MPTP was used as a model of PD. A MIN-301 analog (Q to R exchange at position 38) was administered daily at 10 μg/kg/day during 8 weeks, starting 1 week prior to the first MPTP injection. Read-outs were: daily clinical score of PD-like signs, 24-h home cage activity, sleep, motor function, pathology of substantia nigra, and immunological profiling.

Preliminary toxicology study in rats: MIN-301 toxicity was assessed in Wistar rats following daily i.v injection of 5μg/kg for 4 weeks. Systemic exposure, heart and liver organ toxicity, and bone marrow micronuclei were evaluated.

Results: In the rat 6-OHDA model, MIN-301 attenuated behavioral deficits induced by 6-OHDA treatment. At the lower dose of 30 ng/kg i.p., MIN-301 administered early significantly improved rotarod performance and reduced the contralateral circling produced by apomorphine. When MIN-301 administration was delayed, the dose of 300 ng/kg MIN-301 produced significant ipsilateral turning behavior and reduced apomorphine-induced circling.

In the chronic mouse MPTP model, MIN-301 at 30 ng/kg/day was found to significantly reduce motor function impairment induced by MPTP.

The MPTP study in marmosets showed an improvement in the parkinsonian clinical signs on the abnormal involuntary movements scale (AIMS) and a beneficial effect on

behavioral readouts, as well as a trend toward protection of TH-positive cells in animals treated with the MIN-301 analog. There was also a trend towards decreases in the levels of pro-inflammatory markers in brain such as IL-1β, TNF-α and a statistically significant reduction of inducible nitric oxide synthase (iNOS) by MIN-301 analog treatment. These preliminary results confirm the beneficial effect of MIN-301 observed in rodent models and raise the possibility that MIN-301 may have additional clinical beneficial effects alongside motor improvement and encourages the exploration of blood iNOS and other neuroinflammatory surrogate markers in PD clinical trials.

Given the activity of MIN-301 in these animal models, we believe the compound has a strong potential as a symptomatic as well as disease modifying treatment of PD.

The 4-week preliminary toxicology study in rats revealed a benign safety profile for the drug, with no apparent clinical signs and no histological abnormalities in the organs studied (heart and liver). Moreover, no statistically significant increase in micronucleated polychromatic erythrocyte frequency was observed.

Conclusions: Clinical studies are now justified, given the excellent efficacy and safety profile of MIN-301 seen to date. IND-enabling studies with MIN-301 in preparation for clinical studies are being planned. These will involve pharmacokinetic and toxicology studies in rat and primates, GLP safety pharmacology, and GMP manufacture. Furthermore, data aimed at identifying biomarkers and/or surrogate endpoints (e.g. cytokines and iNOS) for future clinical studies will be collected.

Keywords: Parkinson's disease, Neuregulin, 6-OHDA, MPTP

Disclosures: All authors are either employees of, consultants for, or contractors for Minerva Neurosciences, Inc. Dr. Luthringer is a major equity holder.

W139. PRC2 Regulates Transcriptional and Behavioral Phenotypes Induced by Mutant Huntingtin

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Background: Huntington's disease (HD) is the most common inherited neurodegenerative disease and is caused by CAG trinucleotide repeat expansions in the huntingtin (HTT) gene. Besides symptom management, there are no known treatments for HD. Previous studies have demonstrated prominent transcriptional repression in both mouse models and human post-mortem tissue, but it is not known if transcriptional repression is causally linked to HD pathogenesis and whether it therefore represents a target for therapeutic intervention.

Methods: We used chromatin enrichment analysis (ChEA) of publically available transcriptional profiles from HD post-mortem tissue and HD mouse models to identify major regulators of transcriptional repression in HD. We then used traditional biochemical techniques (Western blotting, immunohistochemistry, quantitative PCR) to validate our findings. For some experiments, cell-type specificity was

obtained through the use of translating ribosomal affinity purification (TRAP). To verify and explore the functional role of these changes, we have used adenoassociated virus (AAV) to alter gene expression in vivo.

Results: Through bioinformatic analysis, we found that targets for multiple components of the polycomb repressive complex 2 (PRC2) are enriched among genes changed in multiple HD mouse models and in human HD post-mortem tissue. We show that levels of the repressive PRC2-dependent trimethylation of lysine 27 on histone H3 (H3K27me3) are increased across three different HD mouse models and in human HD striatum, providing a mechanism for the observed transcriptional repression in HD. Finally, by artificially increasing PRC2-dependent H3K27me3 levels in wild-type striatum, we are able to mimic behavior phenotypes characteristic of HD.

Conclusions: We show through bioinformatic and biochemical methods that PRC2-dependent H3K27me3 levels are a master regulator of transcriptional repression in HD. We also show that increased H3K27me3 levels are sufficient to produce molecular and behavioral phenotypes of HD in mouse models. Future goals will be to characterize the therapeutic potential of decreasing H3K27me3 levels in mouse models.

Keywords: Huntington's Disease, chromatin, neurodegeneration

Disclosures: Nothing to disclose.

W140. Functional Characterization of a Novel 'Reader' of Neuronal Chromatin, BRWD1, in Down Syndrome Associated Neurological Impairment

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Background: Down syndrome (DS) is the most common chromosomal abnormality disorder in humans and is caused by triplication of all or part of chromosome 21. DS is associated with physical growth delays, characteristic craniofacial abnormalities and intellectual disability. Although much is known regarding physiological aberrations associated with DS, far less is clear concerning the molecular mechanisms mediating this disease. Although DS is clearly marked by deficits in neural plasticity and increased neurodegeneration, few treatments exist that adequately reverse neuronal impairments and/or cognitive deficits. Given that several neurological disorders associated with cognitive impairment result in disruptions in gene regulation, in part, via epigenetic processes, further mechanistic studies of neuronal specific histone function promise to provide clues into the underlying causes of DS associated neuropathology. We recently identified BRWD1, a neuronally enriched 'reader' of eukaryotic chromatin, as a putative mediator of DS associated transcriptional and cognitive impairment.

Methods: Employing a wide range of biochemical, biophysical and molecular approaches, we are fully characterizing functions for BRWD1 in the central nervous system to

uncover novel molecular phenomena contributing to neurological impairment and intellectual disability in DS. These techniques range from 'basic' biochemical assays to identify BRWD1 protein complex associations and interactions with combinatorial histone posttranslational modifications (PTMs), to genome-wide ChIP-seq profiling of BRWD1 in normal vs. DS neurons. Using both mouse models of DS and human induced pluripotent stem cell (iPSC) derived neurons from DS subjects, we plan to directly manipulate BRWD1 in diseased cells to investigate its functions in DS associated neuronal dysfunction.

Results: We recently described a novel mechanistic role for histone turnover in the regulation of neuronal transcription, synaptic connectivity and cognition. To understand the interplay between nucleosomal turnover and histone PTMs, we employed SILAC (Stable Isotope Labeling of Amino acids in cell Culture), coupled to mass spectrometry, to identify PTMs 'marking' dynamic histones in embryonic vs. adult neurons. We identified H3K4me1, a classic genomic enhancer-enriched PTM, as the most abundantly enriched mark on dynamic nucleosomes.

To investigate the contribution of H3K4me1 to neurological plasticity, we next set out to identify neuronally enriched binding proteins ('readers') associating with H3K4me1 in brain. We found the chromatin effector molecule BRWD1 to be a robust H3K4me1 interacting protein. BRWD1 is a neuronally enriched WD40 repeat and bromodomain-containing protein that is encoded within the DS critical region 2 on chromosome 21 in humans. BRWD1 has previously been suggested to act as a transcriptional transactivator; however, its functions in the contexts of both normal neurodevelopment and in DS have yet to be delineated. BRWD1's two bromodomains share significant homology with other bromodomain-containing proteins, which have been demonstrated to bind to genomic enhancers through transient associations with concomitant histone acetylation marks (e.g., H3K27ac). Since WD40 domains have previously been shown to interact with histone methylation signatures, we investigated whether BRWD1 might function as a multivalent binding protein that can recognize and simultaneously associate with multiple enhancer-enriched histone PTMs. Recombinant human BRWD1 WD40 and bromodomains were purified, and custom histone PTM peptide arrays confirmed H3K4me1/H3K27ac interactions with high affinity. Surprisingly, however, BRWD1 was also found to interact with distinct subsets of repressive histone PTMs (e.g., H3K27me3 and H4K20me3), often binding with greater affinities than those previously observed with H3K4me1 and/or H3K27ac. These data indicate that BRWD1 can function as either an activator or repressor of gene transcription depending on the genomic abundance/distribution of these marks in vivo. BRWD1's preference for active vs. repressive histone PTM interactions also appears to be dependent on its expression within a given cellular context, with higher expression levels favoring associations with repressive H3K27me3/ H4K20me3. Along with confirming BRWD1's increased neuronal expression in both a mouse model of DS (Ts65Dn), as well as in human induced pluripotent stem cell (hiPSC)-derived neurons from DS subjects, we also identified selective increases in the abundance of BRWD1 associated repressive PTMs in trisomy neurons, suggesting

that alterations in BRWD1 expression may contribute to transcriptional abnormalities in DS. Thus, we hypothesize that dosage imbalance of BRWD1 in DS results in aberrant interactions between BRWD1 and associated histone PTMs (shifting from active to repressive histone PTM binding), thereby contributing to reduced transcriptional plasticity and neurological impairments.

Conclusions: Employing a unique combination of chromatin biochemistry, chemical/structural biology and translational neuroscience, we provide novel insight into the consequences of BRWD1 dosage imbalance in the mediation of aberrant patterns of gene expression and cognitive impairment in DS. This work promises to accelerate the development of improved therapeutics aimed at ameliorating DS associated deficits.

Keywords: Epigenetics, Down Syndrome, Histone

Disclosures: Nothing to disclose.

W141. Gut-Derived Metabolites Involved in Tryptophan Metabolism are Associated with Brain Morphology

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Background: A growing body of preclinical literature has demonstrated bidirectional signaling between the brain and the gut microbiome, involving multiple neurocrine, endocrine and inflammation-related signaling mechanisms. In turn, the brain can influence microbial composition and function via endocrine and neural mechanisms.Tryptophan depletion has been purported to affect mood by altering serotonin levels and decreased brain-derived neurotrophic factor in the brain and has been associated with negative mood. The aim of this study is to perform a discovery based analysis to examine the association between the brain structure and concentration of gut-derived metabolites involved in tryptophan morphology.

Methods: Global stool metabolome was profiled in 23 healthy controls (14 females) and 30 (22 females) subject with chronic abdominal pain. T1 weighted images were acquired on a Siemens Allegra 3T scanner TR = 2200 ms, TE = minimum, TI = 750 ms, flip angle = 20 degrees, FoV = 220 × 220 mm, resolution = 256 × 256, slices per volume = 176, slice thickness = 1 mm, voxel size = 0.86 × 0.86 × 1 mm. Using the structural MRI, each subjects brain was segmented and parceled into 165 regions (74 cortical structures, 7 subcortical structures, the cerebellum and the brain stem based on Destrieux and Harvard-Oxford Atlases using Freesurfer on the USC Laboratory of Neuroimaging pipeline. Next we calculated the volume, surface area, mean curvature and curvature of the brain regions. We utilized a unique global metabolomics and bioinformatics platform to characterize altered biochemical pathways that are evident in stool. The Kyoto Encyclopedia of Genesand Genome and The Human Metabolome Database were used to identify metabolites involved in tryptophan metabolism. The concentrations of these

metabolites were correlated with the measures of brain morphometry using the R software and thresholded at $r \geq .40$, $p<.003$ uncorrected. Cytoscape was used to visualize and construct a brain morphometry-gut-derived metabolite interaction networks based on the thresholded correlations.

Results: Several metabolites involved in tryptophan metabolisms were found to be associated with brain morphometry. For example, Tryptamine, a common precursor molecule to many hormones and neurotransmitters, was negatively correlated with anterior segment of the circular sulcus of the insula, orbital sulci and gyri, and the posterior dorsal part of the cingulate gyrus. Picolinic Acid a metabolite involved in tryptophan metabolism and produced under inflammatory conditions, was subcallosal area, subcallosal gyrus, medial orbital sulcus, fusiform gyrus, and amygdala. 5-hydroxyindoleacetic acid (5HIAA), an indicator or serotonin synthesis was associated with parahippocampal gyrus, inferior occipital gyrus and sulcus, middle posterior part of the cingulate gyrus and sulcus, middle anterior part of the cingulate gyrus and sulcus, and the opercular part of the inferior frontal gyrus.

Conclusions: Preliminary results provide tentative support for the association between gut-derived metabolites involved in tryptophan metabolisms and brain morphometry. Data-driven discovery methods such as bipartite networks may provide new insights and a basis for generating future hypothesis for research on the brain-microbiome association.

Keywords: Gut Microbiome, brain structure, Tryptophan
Disclosures: Nothing to disclose.

W142. Use of Inhibitory Conditioned Cues to Reduce Alcohol-Seeking: Pre-Exposure Blocks Enhancement of Alcohol-Seeking Produced by an Excitatory Conditioned Cue

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Background: Conditioned cues can stimulate drug craving which can lead to relapse to substance abuse/use. Conditioned cues can be associated with the availability (excitatory; CS+) or abstinence (inhibitory; CS-) of a reinforcer. Past research from our laboratory has indicated that presentation of a CS+ results in neurochemical changes (increase in dopamine and/or glutamate) in the basolateral amygdala or nucleus accumbens shell (AcbSh) that is opposite of the effects observed following presentation of a CS- (decrease in dopamine and/or glutamate). In addition, presentation of a CS+ can result in unique neurochemical changes (reduction in serotonin levels in the AcbSh). The efficacy of using CS- as a conjunct for the treatment of addiction is currently being examined. The current experiments were designed to determine if pre-exposure to a conditioned cue in a non-drug paired environment would alter the ability of a condition cue presented in the drug-paired to alter EtOH-seeking.

Methods: In all experiments rats were conditioned to a CS+, CS-, and a neutral conditioned cue (CS0). The first experiment examined whether CS- pre-exposure in a non-drug paired environment would alter the ability of a CS+ to enhance EtOH-seeking if present in the drug-paired environment. Rats were given 30 min pre-exposure to no odor, CS+, CS-, or CS0 and then were brought to the drug paired environment (10 weeks of daily operant EtOH self-administration in operant chamber) in which the CS+ was present. The converse experiment was then performed (pre-exposure to no odor, CS+, CS-, or CS0 and then EtOH-seeking performed in the drug-paired environment in the presence of the CS-) in a separate group of rats.

Results: The data indicated that rats pre-exposed to no odor or the CS0 readily displayed an increase in EtOH-seeking caused by the presence of the CS+ in the operant chambers, which was increased by pretreatment with the CS+ (45% increase). In contrast, pre-exposure to the CS- effectively blocked both context-induced EtOH-seeking and the ability of the CS+ to enhance EtOH-seeking. Pre-exposure to no odor, CS+, CS-, or CS0 did not prevent the presentation of the CS- in the drug-paired environment from blocking the expression of EtOH-seeking.

Conclusions: The data indicate that pre-exposure to the CS- resulted in a physiological response that prevented both context and a CS+ from stimulating EtOH-seeking. It is currently unknown if pre-exposure to a CS- would alter the neurochemical response to a CS+, but this is being assessed. From multiple convergent data sets, we are establishing that inhibitory conditioned cues results affects the CNS in opposing (compared to CS+) and unique manners. Understanding the neurological basis of the CS- ability to block EtOH-seeking could lead to the development of potential pharmacotherapeutics for the treatment of drug craving.

Keywords: Alcohol-seeking behavior, conditioned cue, behavioral inhibition
Disclosures: Nothing to disclose.

W143. Nociceptive Neural Transcriptional Plasticity in Rat Dorsal Horn in a Persistent Pain Model

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Background: State-dependent changes in neural activity provoke modulation of gene transcriptional processes. Understanding persistent pain states and how chronic pain can alter CNS neural circuitry to produce central sensitization is of fundamental importance in pain neurobiology. Furthermore, such studies can yield results that extend to or overlap with neural processes such as reward and motivation and neuropsychiatric disorders like depression and addiction. For pain, transcriptional modulation can occur at the level of the spinal cord dorsal horn. Modulation begins rapidly after the start of a persistent nociceptive input and can last as long as the persistent pain state is maintained. In order to understand the full range of alterations we have used next-gen RNA-Seq to quantitatively measure every

transcript in spinal cord dorsal horn during early and sustained nociceptive input.

Methods: For many manipulations gene expression is incompletely or inaccurately assessed. RNA-Seq uses highly parallel sequencing reactions to determine the sequence of all transcripts, polyA + selected in the present study, in a tissue sample. the sequenced transcript fragments are digitally aligned to the genome and the number of reads per transcript determined. This process yields a precise, complete and quantitative measurement of the dorsal horn transcriptome. We examined the dorsal horn transcriptome at baseline and at 2 hrs and 2 days following peripheral inflammation. The two time points correspond to activation of immediate early gene and up-regulation of potential target gene the may contribute to sustained physiological or structural modifications within nociceptive circuits.

Results: Within 2 hrs of persistent inflammation of the hind paw there was a notable elevation in multiple transcription factors. The known transcription factors c-Fos and JunB, were in this group as were 4 paralogs in the NGFI-B steroid receptor-superfamily. A return to baseline occurred by the 2 day time point. Transcripts coding for two opioid neuropeptide precursors prodynorphin and proenkephalin displayed rapid induction and sustained elevation as dit the preprotachykinin precursor. All of these were expected from earlier studies using methods such as northern blot and/or in situ hybridization. We detected fifty-five genes that exhibited a 1.5-fold or higher elevation; lowering the threshold to 1.25-fold yielded another approximately 150 significantly elevated transcripts. Comparison to other manipulations of the nociceptive circuit such as the DRG following axotomy or the directly in the inflamed hindpaw the number of genes and degree of change is dorsal horn is low. The somatotopic innervation of the dorsal horn, its functional and cellular may all be factors that dilute or attenuate the measured degree of change. Multiple temporal patterns of transcriptional modulation were noted that included short-term elevation, delayed elevation, elevation followed by decrease and delayed decrease. All are indicative of engagement of an integrated, temporally orchestrated regulatory process that modulates spinal excitability and the supporting, underlying structural remodeling. The latter process is exemplified by changes in multiple immune and complement genes coding for proteins involved in synaptic modification and microglial participation.

Conclusions: Persistent pain states engage new gene expression programs that coordinate modulation of the neurotransmission process at spinal nociceptive synapses. These include opioid neuropeptides that can modulate pain locally and processes to reinforce or reduce synaptic strength on second order neurons. This range of downstream effector molecules is hypothesized to modify local inhibitory and excitatory transmission thereby shaping acute and persistent changes in nociceptive sensory circuits. Such modulation may also occur at higher levels of the neuraxis and contribute to the range of behavioral and affective changes that accompany chronic pain states.

Keywords: chronic pain, gene expression, Transcriptomics, transcription factors, Synaptic Plasticity

Disclosures: Nothing to disclose.

W144. Amygdala-Independent Pathways Involved in the Generation of Interoceptive Fear

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Background: Much of the extant evidence, in both humans and other animals, highlights the amygdala as playing a central role in the generation of fear and pathogenesis of anxiety. This amygdala-centered model of fear and anxiety has recently been challenged in a study involving a rare group of human patients with focal bilateral amygdala lesions. A single vital capacity inhalation of air containing 35% carbon dioxide triggered a panic attack in all of the amygdala-lesioned patients characterized by an intense fear of suffocation, heightened physiological arousal, concomitant thoughts of death, and prominent signs of escape behavior. These findings stand in sharp contrast to previous research demonstrating that these patients show a marked absence of fear in response to threatening stimuli from the external environment. Thus, there appears to be an important neural distinction between threats conveyed through exteroceptive sensory channels (e.g., visual and auditory pathways) versus threats conveyed through interoceptive sensory channels (e.g., respiratory and chemoreceptive pathways).

Methods: In order to elucidate the brain regions supporting the amygdala-lesioned patients' preserved experience of interoceptive fear, a functional neuroimaging experiment was conducted using a non-hypercapnic interoceptive breathing challenge that induces a transient state of dyspnea. Two monozygotic twin sisters with bilateral amygdala damage (patients AM and BG) and a group of healthy female comparison participants underwent alternating periods of loaded inspiratory breathing during fMRI. Data were analyzed using an apriori region-of-interest approach.

Results: The findings revealed that the amygdala lesion patients showed aberrant activation patterns within the insula and ventral anterior cingulate cortices during loaded breathing. Specifically, patient BG displayed a pattern of hyperactivation within this network, whereas patient AM showed the opposite pattern, one of profound hypoactivation.

Conclusions: The preserved experience of interoceptive fear evident in patients with bilateral amygdala damage may be mediated by viscerosensory and visceromotor pathways with central hubs in the insula and ventral anterior cingulate cortices. Patient AM's deficiency in insula activation is consistent with recent evidence pointing to a specific impairment in her interoceptive awareness for respiratory sensations. This work helps to advance our knowledge with regard to how interoceptive fear is implemented in the human brain, illuminating amygdala-independent pathways involved in the generation of anxiety.

Keywords: Anxiety, Amygdala, fMRI, lesion

Disclosures: Nothing to disclose.

W145. Interaction of Childhood Trauma and COMT Genotype on Hippocampal Activation during Inhibition: Potential Mechanism for Psychiatric Risk or Resilience?

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Background: Risk factors for mental illness such as posttraumatic stress disorder (PTSD) include childhood trauma and polymorphisms in specific genes, including the Val158Met polymorphism in the gene coding for catechol-o-methyltransferase (COMT), which regulates dopamine and norepinephrine levels in the brain. Although both childhood trauma and COMT polymorphism have been associated with PTSD, it is still unclear whether the two interact and how this interaction may result in long-term sequelae. COMT is mainly expressed in the prefrontal cortex and hippocampus, regions important for both fear and response inhibition. Impaired inhibition, in turn, has been implicated as a putative biomarker for PTSD. The current study used magnetic resonance imaging (MRI) during a response inhibition task to examine interaction effects of childhood trauma and COMT on the inhibitory neurocircuitry.

Methods: The participants were women with a history of civilian trauma. Functional and structural MRI scans were available from 73 participants. A Go/NoGo procedure in a 3T MRI scanner was used to measure response inhibition. The left and right hippocampus and ventromedial prefrontal cortex (vmPFC) were used as regions of interest (ROI). In addition, whole brain analyses were performed. Freesurfer was used to analyze hippocampal and vmPFC volume. DNA was extracted from saliva and used to determine COMT genotype (Val/Val, n = 38, Met carriers, n = 35). Self-report measures of childhood trauma exposure and adult trauma exposure, PTSD and depression symptom severity, and a measure for resilience were collected on all participants.

Results: ROI analyses showed a main effect of COMT group in the left (F(1,71) = 6.03, p = 0.02) and right hippocampus (F(1,71) = 4.67, p = 0.03), with individuals in the Val/Val group showing more hippocampal activation than individuals in the Met carrier group. No group differences were observed in the vmPFC. When childhood trauma was included as a continuous predictor, a significant interaction with COMT group was observed in the left (F(1,68) = 6.73, p = 0.01) and right hippocampus (F(1,68) = 6.93, p = 0.05). Left hippocampal activation correlated positively with childhood trauma in the Val/Val group, but correlated negatively with childhood trauma in the Met carrier group. No association between childhood trauma and the right hippocampus or vmPFC was observed. A significant negative correlation between left hippocampal activation and PTSD symptoms (R2 = -0.27, p = 0.02) and depression symptoms was observed (R2 = -0.39, p = 0.02). Both correlations were still significant after controlling for age and childhood trauma. Correlations of clinical measures with the right hippocampus were only marginally significant, and no correlation was observed with vmPFC activation. The measure for resilience correlated significantly with left (R2 = 0.34, p = 0.01) and right hippocampal activation (R2 = 0.29, p = 0.02) and also remained significant after controlling for age and childhood trauma. Whole brain analyses (p < 0.05, corrected) were performed to investigate group differences outside the ROIs and showed a positive correlation between number of Met alleles with the right precuneus, right cuneus, and superior occipital gyrus, and a negative correlation with activation in the left hippocampus and parahippocampal gyrus, and the cerebellar vermis. Childhood trauma and COMT genotype showed an interaction effect on activation in the left hippocampus, posterior cingulate, and right sensorimotor areas. Finally, no structure-level COMT group differences or group by childhood trauma interaction effects in the hippocampus or vmPFC were observed.

Conclusions: In this study, we showed an effect of COMT genotype on inhibition-related hippocampal activation. Moreover, we found an interaction between childhood trauma and COMT genotype, in that increased childhood trauma and presence of Met alleles was associated with decreased hippocampal activation, while childhood trauma and Val/Val genotype was associated with increased hippocampal activation. Furthermore, decreased hippocampal activation was associated with more PTSD and depression symptoms. These data suggest that hippocampal activation during inhibitory tasks may be a mechanism by which childhood trauma and genotype increase risk for trauma-related psychopathology. The hippocampus is essential for capturing contextual cues during learning processes, and providing cues for inhibition. This structure has been associated with PTSD and with childhood trauma, but this is the first study to examine G x E effects. The Val/Val genotype may lead to resilience in that childhood trauma in these individuals increased hippocampal activation. Indeed, hippocampal activation correlated positively with self-reported resilience in this study of traumatized women. Therefore, this study contributes to a better understanding of genetic and environmental risks for development of psychiatric disorders or resilience.

Keywords: Hippocampus, COMT gene, Inhibition, Childhood trauma, Resilience

Disclosures: Nothing to disclose.

W146. Oxytocin Receptor Gene (OXTR) Variation, Fear and Reward Sensitivity in Rhesus Macaque and Non-Traditional Model Animals

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Background: The oxytocin system plays roles in reward, social attachment, and stress response. Studies performed in primates, including humans, have found evidence of both positive and balancing selection at the oxytocin receptor gene (OXTR), and some studies demonstrate human OXTR variation to influence social cognition, anxiety and alcohol response. Here, we investigate the role of OXTR variation in rhesus macaque and, additionally, in non-traditional model animals.

Macaques are a well characterized model species and, though they have been maintained in captivity for research

purposes for almost a hundred years, they are non-domestic animals maintaining behavior adaptive in a free-living context. To extend OXTR analyses across species with different evolutionary histories, we examined the degree to which OXTR variation exists in recent domesticates and whether that variation correlated with behavioral measures under selection (whether artificial or natural).

At its most basic, domestication is a suite of heritable traits affecting behavior. There are intriguing phenotypic commonalities among domesticates, the most important among them being the ability to coexist with humans. The systems that permitted early domestication likely range from those involving fear and impulse control to those involving reward and sociality. Fearfulness among horses and sheep is the exception rather than the rule; they are prey species, relying on social cognition/social alliances and vigilance for survival. However, they are also domestic animals, in which levels of fear and sociality have been manipulated by selective breeding.

We wanted to determine whether OXTR variation predicted individual differences in fear and sociality/reward. As such traits can moderate risk for developing alcohol problems, we also wanted to determine if OXTR variation predicted alcohol use in macaque.

Methods: OXTR primers were designed using reference sequences available on UCSC Genome Browser. Sanger Sequencing (ABI 3100) was used in screening for variation. Blood drawn from macaques (mother- and peer-reared, MR and PR) maintained at the NIH Animal Care Center was provided for DNA extraction. ANOVA was performed in order to determine effects of genotype on intranasal oxytocin response in infants (N = 24) and on ethanol self-administration in adult animals (N = 133).

A semi-feral herd of Shetland ponies (N = 121) at the University of Pennsylvania Veterinary School were rated by veterinary behaviorists on their degrees of gregariousness, aggression, and fear. Archived blood samples were provided for DNA extraction.

Because ewes with deficient mothering skills tend to be culled among farm stock, sheep provide an excellent opportunity to study genetic variation as it relates to social attachment and caregiving, both of which are reward-dependent processes. Blood samples from sheep (N = 22) were obtained by a veterinarian during seasonal monitoring for infectious disease, and a sample was provided for DNA extraction. Behavioral scientists from the Section of Comparative Behavioral Genomics (NIH/NIAAA) observed the sheep 2-3 times/week for 12 weeks and were given a subjective score on maternal caregiving.

Results: In macaque, we identified 13 SNPs, including a non-synonymous SNP in the first exon. Based on Manhattan distances weighted by minor allele frequency and marker average LD, haplotypes for rhesus macaque were clustered hierarchically using R. The ancestral sequences at each SNP site were estimated with multiple alignments of human, chimp, and rhesus macaque. The cladograms also demonstrate the existence of alternative (yin-yang) haplotpe clades and may suggest selective pressure for markers 6/7 (in high LD) and/or marker 8 (M8).The persistence of the divergent haplotypes over time may suggest that they have been subject to selection such that at least one allele on each background is being selected—possibly in a particular environmental context.We found that carriers of M8 exhibited increased OT-induced lip-smacking behavior, suggesting a gain-of-function role for this SNP. PR macaques that were M8 carriers exhibited higher levels of alcohol self-administration.

In horse, nine polymorphisms were identified (8 SNPs and 1 indel) at the eqOXTR gene, generating 5 haplotypes. One marker was unique to a single haplotype, and we found that this marker predicted extreme fearfulness among individuals in this herd.

In sheep, we found 18 SNPs and 15 haplotypes. Sheep carrying ovOXTR haplotype H4 displayed higher levels of maternal caregiving, and this occurred in a dose-dependent manner.

Conclusions: Notable variation was found in OXTR in macaque, horse, and sheep. Among the variants identified in rhesus was a gain-of-function non-synonymous SNP, and we are currently expanding our analysis of rhOXTR variation in rhesus to determine whether other functional variants may also be contributing to individual differences in behavior. One would expect M8 carriers to be more responsive to endogenous oxytocin and, therefore, less anxious. Our data may suggest that the mechanism by which rhOXTR mediates increased alcohol consumption among PR macaques is at least, in part, through effects on reward. These studies provide validation for a role for OXTR variation in contributing to behavioral phenotypes that are likely under selection across species and suggest human alcohol use may be moderated by polymorphism at the OXTR gene.

Keywords: domesticate, OXTR, Animal Models, oxytocin, fear
Disclosures: Nothing to disclose.

W147. Endocannabinoids and Neuropeptides in CSF and Serum from Borderline Personality Disorder Patients

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Background: The endocannabinoid system plays an important role in the pathophysiology of psychiatric disorders. Due to its neuromodulatory potential, its role in emotion regulation and in control of aversive memory, the endocannabinoid system might be a potential candidate system, affecting a broad range of psychopathology in both, posttraumatic stress disorder (PTSD) and/or borderline personality disorder (BPD).

Methods: The study was conducted in two parts in a clinically well characterized population of patients and healthy controls. In the first part, we collected analyzed human serum samples from patients suffering from BPD (n = 23) or PTSD (n = 21) as well as matched healthy volunteers (n = 34). Based on our previous approach we developed and validated a specific and sensitive method using liquid chromatography/tandem mass spectroscopy (LC-MS/MS) and quantified the endocannabinoids anandamide and 2-arachidonyl-sn-glycerol (2-AG) and the related fatty acid ethanol amides (FAEs) oleoylethanolamide and palmitoy-lethanolamide.

To further test the hypothesis that the endocannabinoid system is deeply involved in emotion regulation and potentially also in pain perception serum and cerebrospinal fluid (CSF) levels of the four FAEs as well as the neuropeptides oxytocin (OXT) and vasopression (VPA) were analyzed in an independent cohort of 27 BPD patients and 26 matched controls.

Results: In our first cohort, serum levels of anandamide were significantly elevated in BPD (p = 0.001), while oleoylethanolamide was significantly elevated in PTSD (p = 0.004) when compared to controls. In BPD, 2-AG was significantly elevated when compared to PTSD patients (p = 0.001).

In our second cohort, the findings in serum of BPD did not reach significance although a numeric alteration in the respective direction was observed.

In CSF, levels of anandamide as well as palmitoyletnanolamide were significantly decreased in BPD, while elevation of 2-AG did not reach significance. CSF 2-AG levels where significantly and inversely correlated to CSF OXT (p = 0.008). Both parameters where significantly correlated to self-reported symptom severity (SCL-90-R) as well as dissociative symptoms (Questionnaire for dissociative symptoms, FDS).

Conclusions: Our data rise evidence that the endocannabinoid system may play a highly relevant functional role in the pathophysiology of BPD in orchestration with e.g. neuropeptide systems and/or independently. In addition, it may also play a role in PTSD as indicated by others (e.g. Neumeister et al. 2013), which is however not reflected in our study.

Keywords: Endocannabinoids, neuropeptides, Borderline Personality Disorder, PTSD

Disclosures: Nothing to disclose.

W148. Exome Sequencing of Borderline Personality Disorder Patients to Identify Functional Rare Variants

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Background: Borderline personality disorder (BPD) is a serious psychiatric disorder characterized by an inability to appropriately control emotions, reckless and impulsive behavior, and unstable relationships with others. In some instances individuals exhibit extreme reactions to perceived abandonment by people to whom they feel close, and patterns of unstable relationships which oscillate from extreme closeness to intense dislike. Inappropriate anger directed at self or at others and reckless impulsive behaviors such as unsafe sex, gambling and substance abuse are also observed in BPD patients. Self-harm or suicide is a significant risk for patients with BPD.

Often BPD symptoms are accompanied by depression, anxiety disorders, substance abuse, eating disorders or antisocial personality disorder. These co-morbid disorders potentially result in under diagnosis of BPD.

BPD is estimated to have a prevalence of 1.4% amongst adults and usually appears in late adolescence to early adulthood with approximately equal numbers of males and females affected.

In twin studies BPD has been shown to have a high heritability (~60%) and variation at genes involved in serotonin signaling, (TPH1, 5HTTLPR) have been associated with the disorder. However, the genetic underpinnings and the etiology of BPD remain largely unknown. The International Borderline Disorder Consortium has performed exome sequencing on a cohort of BPD patients and matched controls to identify genes or pathways in which functional coding variants are over represented in BPD patients.

Methods: Recruitment and ascertainment of BPD cases and control subjects was carried out by the BPD Research Group at the Psychiatry Department of the Hospital Vall d'Hebron, Barcelona. Exome sequencing was performed on 102 BPD cases along with 100 controls.Target enrichment was performed using Ampliseq Exome kit which targets 33Mb of coding exons through highly multiplexed amplification. Sequencing was performed on the Ion Proton platform (Life Technologies). The reads were mapped to UCSC hg19 with tmap-f3. The variants were called with TVC plugin in Torrent Suite. The genotypes at the variant positions were called by a pipeline consisting of mpileup function within samtools (version 1.2) along with calls function and vcfutils.pl in bcftools (version 1.2). AB genotypes were generated by parsing the counts in the VCF files. The variant functional annotation was performed with an in-house tool (SNP.to.ucsc-functions.pl) and Ensembl's VEP.

Results: Subjects were sequenced to an average depth of 89.3x (89.4x cases, 89.2x controls) and on average 54,690 variants (29,837-74,325) were detected in each sample. Analysis of the variant data in VEP identified a total of 254,166 SNV, 158,255 substitutions (where the length of change in the variant is the same as the reference length), 10,381 insertions, 4,719 deletions and 2,064 sequence alterations (variants that alter sequence and length). Approximately 10% of all coding variants identified are predicted to have functional consequences.

Conclusions:

Keywords: exome, borderline personaility disorder, sequencing

Disclosures: Nothing to disclose.

W149. Early Postnatal GABAa Receptor Modulation Reverses Deficits in Neuronal Maturation in a Conditional Neurodevelopmental Mouse Model of DISC1

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Background: γ-aminobutyric acid type A (GABAa) receptors are responsible for the majority of fast synaptic

inhibition in the mature central nervous system. During pre and early postnatal periods, GABA exerts a depolarizing and excitatory action, regulating multiple processes of neuronal maturation, including dendritic development. Notably, dendritic abnormalities and deficits in GABA signaling, including alteration of GABAa receptors have been implicated in multiple neurodevelopmental psychiatric disorders, such as autism spectrum disorder, epilepsy, and schizophrenia. Accordingly, developmental GABAa receptor-mediated signaling is a unique molecular target to explore novel pharmacological treatment for these devastating conditions. Given that therapeutic interventions initiated after brain development are unlikely to result in complete functional recovery from a neurodevelopmental psychiatric condition, it is critical to explore treatment strategies based on maturation mechanisms during the developmental phase.

Methods: We explore the effect of early postnatal intervention of subtype-selective positive allosteric modulators of GABAa receptors on developmental deficits caused by knockdown of Disrupted-in-Schizophrenia-1 (DISC1), a genetic risk factor for major mental disorders. DISC1 is involved in multiple cellular processes in the developing cerebral cortex. In order to explore the specific role of DISC1 for developmental GABAa receptor-mediated signaling in the prefrontal cortex during early postnatal periods, we have developed a modified conditional knockdown method by using in utero electoroporation. This system allows us to suppress DISC1 expression, specifically in the prefrontal cortex during postnatal periods. We examine whether altered prefrontal cortex maturation and resultant behavioral abnormalities induced by postnatal knockdown of DISC1 can be reversed by subtype-selective positive allosteric small molecule modulators of GABAa receptors during the early postnatal period.

Results: We found that prefrontal cortex-specific postnatal knockdown of DISC1 drives deficits in synaptic GABAa function and dendritic development in pyramidal neurons, as well as abnormalities in sensorimotor gating, albeit without profound memory deficits. We show that DISC1 is involved in regulating cell surface expression of GABAa receptors specifically in immature developing neurons, but not after full maturation. Notably, pharmacological intervention with subtype-selective GABAa receptor positive allosteric modulators during the early postnatal period ameliorates dendritic deficits and behavioral abnormalities induced by knockdown of DISC1.

Conclusions: Our findings highlight a critical role of DISC1-mediated disruption of postnatal GABA signaling in aberrant prefrontal cortex maturation and function. It also suggests that modulating developmental GABAa signaling might ultimately be considered as a therapeutic approach in the postnatal phase for neurodevelopmental disorders.

Keywords: GABAA, Prefrntal cortex, DISC1, Early intervention, Neurodevelopmental Disorders

W150. A New Platform to Improve Quality of PANSS and MADRS Administration

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Background: Strategies are needed to improve quality of rater administration of many subjective psychiatric instruments. The SCI-PANSS and the SIGMA (MADRS) are two commonly used measures of psychopathology. To improve quality of rater administration, real-time clinical guidance and scoring assistance were developed for a new electronic source (eSource) data capture and monitoring investigative study platform. To analyze the benefits, we chose one type of intervention, incompatible item scores, and tested them against recently collected clinical trial data. The PANSS is a complex scale with different scoring rules and conventions for each item and requires raters to consult several sources during administration and scoring, a cumbersome process that can impede the interview and is prone to errors. Similarly, administration of the MADRS is often performed in an inconsistent manner because raters must consider six possible levels of intensity and frequency for every item, each with unique description, rating guidelines, scoring anchors and conventions. A strategy that simplifies the interview process for raters holds the potential to decrease errors and increase interrater reliability in SCI-PANSS and SIGMA administration.

Methods: Extensive training experience (over 25,000 SCI-PANSS and over 39,000 SIGMA central assessments completed) has demonstrated the most common sources of error in administration. An e-platform was developed with automated scoring alerts triggered by these sources of error in addition to links to scoring anchors, item descriptions, and bases for rating to provide clinical guidance as the interview is being administered and scored. One type of error, incompatible item score pairs (16 pairs for PANSS and three for MADRS) was selected to analyze a subset of SCI-PANSS assessments (n = 288 from 79 raters) and SIGMA assessments (n = 1200 from 111 raters) that were recorded in previous studies. The scores were analyzed to determine how many items would have triggered at least one alert to incompatible scores.

Results: 135 of the SCI-PANSS assessments (47%) would have triggered at least one alert to a potential scoring inaccuracy affecting subscale and total scores. 58 raters (73%) would have seen at least one alert with the use of the eSource platform. 77 of the SIGMA assessments (6.4%) would have triggered alerts and 44 raters (40%) would have seen at least one alert.

Conclusions: The PANSS and the MADRS are significantly different instruments in terms of number of items and construct overlap, but the scoring accuracy of both may improve if incompatible item scores are flagged in real time. These and many other such interventions can reduce scoring errors that contribute to poor interrater reliability, which can compromise the ability to detect a positive signal. Neuropsychological test publishers have begun to utilize tablet PC platforms for test administration and scoring, enabling the inclusion of additional clinical guidance during administration as well as automated scoring. The adaptation of this technology to clinical trials has the potential to

improve the quality of rater administration of subjective semi-structured scales such as the PANSS and the MADRS.
Keywords: PANSS, MADRS, Scoring Accuracy, eSource
Disclosures: All authors are current or former employees of MedAvante; some are also major equity holders.

W151. DRD2- AKT1/ AKT3 Epistasis Effects on Neuroimaging Complex Phenotypes Add Evidence for the D2 Receptors' Signaling via AKT Pathways

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Background: While the D2 receptor is experimentally and clinically confirmed as the major antipsychotic drug target, the mechanisms by which it exerts this role are not fully understood. Recently, several studies have shown that in addition to their action via G-protein coupling signaling, the D2 receptors function via a protein kinase B (AKT) - GSK3 signaling cascade (Beaulieu et al 2007). Previous unpublished data from our group showed complex effects of the GWAS schizophrenia risk DRD2 locus (Ripke et al 2014) on fronto-striatal networks investigated with fMRI, in healthy controls and schizophrenia patients. In this study, we extend our previous observations by showing complex and sometimes opposite interaction effects between DRD2 genotypes (rs6589377) and AKT1 (rs4983559) and AKT3 (rs6656918) brain eQTLs on fronto-striatal networks, in healthy controls during working memory explored with fMRI.
Methods: 273-308 healthy controls genotyped for DRD2 rs6589377, the GWAS positive SNP in the PGC2 dataset (Ripke et al 2014), AKT1 (rs4983559) and AKT3 (rs6656918) participated in an fMRI working memory study (n-back). The AKT1, AKT3 SNPs were selected because postmortem human DLPFC poly A RNA seq data rs4983559 was the strongest AKT1 gene level eQTL, while rs6656918 was the strongest AKT3 gene level eQTL. For both SNPs, minor allele means more AKT isoform expression. During the fMRI n-back protocol, all subjects had performance accuracy $\geq$ 70% for the 2-back condition. Individual first-level GLM contrast images (2-back > 0-back) were analyzed with SPM8 (http://www.fil.ion.ucl.ac.uk/spm/software/spm8/) using full factorial designs and regression 'additive' models. For the 'additive' models, the DRD2-AKT1, DRD2-AKT3 genotypes were coded in a manner dependent on the dose of the minor allele in either gene: DRD2/ AKT1: 0 = AA/ AA; 1 = AA/ AG or AG/ AA; 2 = AG/ AG or AA/ GG; 3 = AG/ GG or GG/ AG; 4 = GG/GG. DRD2/ AKT3: 0 = AA/ TT; 1 = AA/ TC or AG/ TT; 2 = AG/ TC or AA/ CC; 3 = AG/ CC or GG/ TC; 4 = GG/CC. Age, IQ, performance accuracy, reaction time were used as covariates of no interest. We also explored right dorso-lateral prefrontal cortex (R-DLPFC) - basal ganglia functional coupling ("connectivity") using psycho-physiological interaction (PPI) analyses. Results were corrected for multiple comparisons at the whole-brain level and within pre-defined regions of interest (ROIs). The ROIs masks were constructed with MRI brain atlases (e.g. Wake-Forest pick-atlas: http://fmri.wfubmc.edu/software/pickatlas).
Results: We found a statistically significant additive effect of DRD2/ AKT1 on the right dorso-lateral prefrontal cortex

(R-DLPFC) activation during 2-back > 0-back, consistent with the classical intermediate phenotype, in that greater (i.e. less efficient) activation was associated with increasing minor alleles in either genotype (MNI: x = 27, y = 39, z = 33; p < 0.001, corrected for multiple comparisons at ROI level). No interactions were found in DLPFC. In contrast, we found opposite interaction effects, DRD2 X AKT1 and DRD2 X AKT3, on striatal engagement for 2-back > 0-back: in homozygotes of AKT1 allele associated with lower expression (AA) there was a higher activation for DRD2 AA > AG > GG in left Putamen (MNI x = -24, y = 15, z = 6; p < 0.001, corrected for multiple comparisons at ROI level), while in carriers of AKT1 allele associated with higher expression (AG + GG), there was lesser activation for DRD2 AA < AG < GG in the same locale. Conversely, in homozygotes of AKT3 allele associated with lower expression (TT), there was lower activation for DRD2 AA < AG < GG in right Putamen (MNI x = 24, y = 12, z = 0; p < 0.001, corrected for multiple comparisons at ROI level), while in carriers of AKT3 allele associated with higher expression (TC + CC), there was a higher activation for DRD2 AA > AG > GG in the same locale.
In the PPI analysis, we also found opposite DRD2-AKT1 vs. DRD2-AKT3 additive effects on the R-DLPFC- striatum connectivity: higher coupling R-DLPFC- L-Putamen was associated with more minor allele DRD2-AKT1, while lower coupling R-DLPFC- L-Putamen was associated with more minor allele DRD2-AKT3 (MNI: x = -27, y = -9, z = 9; p < 0.001, corrected for multiple comparisons at ROI level).
Conclusions: Our findings suggest that DRD2 genotype has a consistent effect on prefrontal- striatal networks; however, this is modulated at least in part by AKT1 and AKT3, and presumably downstream molecular interactions (e.g. the AKT-GSK3 signaling cascade). Interestingly, opposite interactions of DRD2 with AKT1 and AKT3 were found in network dynamics, perhaps analogous to antagonism of AKT1 and AKT3 also seen in cancer. Our data also support the notion that intermediate phenotypes associated with risk for schizophrenia may identify biological markers for monitoring the effects of current and new antipsychotic drugs.
Keywords: D2 receptor, DRD2 locus, AKT1/ AKT3, fronto-striatal networks, working memory fMRI
Disclosures: Nothing to disclose.

W152. Relationship of Frontal Dopamine D2/3 Receptor Blockade to Cognitive Functions in Initially Antipsychotic-Naïve First-Episode Schizophrenia Patients

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Background: Previous data from our group have suggested that cortical D2/3 receptor function is more involved in cognitive functions (i.e. attention, fluency and planning) in patients with schizophrenia compared to healthy controls, hereby suggesting that information processing in schizophrenia may be characterised by lower signal-to-noise

ratios1. In the present study we examined the influence of frontal dopamine D2/3 receptor blockade on cognitive deficits in the same initially antipsychotic-naïve schizophrenia patients after their first antipsychotic treatment.

Methods: The present study was a longitudinal case-control study using Single-Photon Emission Computerized Tomography (SPECT) with the D2/3-receptor ligand [123I]epidepride, co-registered with structural magnetic resonance imaging, and correlated to cognitive measures. Twenty-two first-episode schizophrenia patients who had never previously received any antipsychotic medication were examined with the cognitive test battery Cambridge Neuropsychological Test Automated Battery (CANTAB) and SPECT before and after 3 months of treatment with either the second-generation antipsychotic compound, risperidone (N = 13), or the first-generation antipsychotic drug, zuclopenthixol (N = 9).

Results: Blockade of frontal dopamine D2/3 receptors was, as hypothesized, correlated with decreased attentional focus (r = -0.615, p = 0.003) and planning time (r = -0.436, p = 0.048).

Conclusions: The finding of a negative influence of frontal dopamine D2/3 receptor blockade on attention and planning in patients with schizophrenia is highly clinically relevant. Combined with our previous data, the present results further support the involvement of D2/3 receptors in cognitive processes in patients with schizophrenia and suggest an optimal range of D2/3 availability for at least some cognitive processes in the patients.

1. Fagerlund B, Pinborg LH, Mortensen EL, Friberg LL, Baaré WF, Gade A, Svarer C, Glenthøj BY. Relationship of frontal D2/3 binding potentials to cognition: a study of antipsychotic-naïve schizophrenia patients. Int J Neuropsychopharmacol 2013 Feb;16(1):23-36.

Keywords: SPECT, extrastriatal dopamine D2/3 receptors, occupancy, antipsychotic-naive, schizophrenia

Disclosures: Dr. Glenthøj is the leader of a Lundbeck Foundation Center of Excellence for Clinical Intervention and Neuropsychiatric Schizophrenia Research (CINS), which is partially financed by an independent grant from the Lundbeck Foundation based on international review and partially financed by the Mental Health Services in the Capital Region of Denmark, the University of Copenhagen, and other foundations. All grants are the property of the Mental Health Services in the Capital Region of Denmark and administrated by them. She has nothing else to declare. Dr. Ebdrup has received lecture fees from Bristol-Myers Squibb, Otsuka Pharma Scandinavia AB, and Eli Lilly and Company and is part of the Advisory Board of Eli Lilly Danmark A/S and Takeda Pharmaceutical Company Ltd. All other authors declare no conflict of interest.

W153. Immune Involvement in the Pathogenesis of Schizophrenia: A Meta-Analysis on Post-Mortem Brain Studies

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Background: Although the precise pathogenesis of schizophrenia is unknown, there is increasing evidence for involvement of the immune system. Genetic and neuroimaging studies suggest the presence of alterations in the immune system in schizophrenia. Which immune-components are involved has yet to be determined.

Methods: In this study we analyzed evidence from studies assessing immune-factors in post-mortem brains of schizophrenia patients versus healthy controls. Thirty-eight studies were included, reporting on 665 patients and 628 controls. We divided these studies into those pertaining to cells and those assessing molecular parameters and meta-analysis was performed on alterations in cell numbers in schizophrenia.

Results: Our pooled estimate showed a significant increase in microglia (p = 0.0014) in the brains of schizophrenia patients compared to controls. Meta-regression on brain regions demonstrated this increase was most pronounced in the temporal cortex. Numbers of macroglia, such as astroglia or oligodendrocytes were not significantly different between groups. There was substantial heterogeneity of molecular parameters, prohibiting the formulation of a unifying summary. Sub-analyses on pro-inflammatory cytokines, which are expressed by microglia in an activated state, such as interleukin-1-β (IL-1β), interleukin-6 (IL-6) and tumor-necrosis-factor-alpha (TNFα), did not reveal any significant differences between patients and controls.

Conclusions: In conclusion, the significant increase in microglia density in postmortem brains of schizophrenia patients as compared to controls provides new evidence for neuro-inflammation as a component of the pathogenesis. This finding is clinically relevant as establishing immune-involvement in schizophrenia will facilitate the development of novel and may possibly lead to more effective treatment approaches, for instance with immune-modulating drugs.

Keywords: schizophrenia, Postmortem Brain Tissue, immune mechanisms

Disclosures: Nothing to disclose.

W154. Effects of Aripiprazole Once-Monthly and Paliperidone Palmitate in Patients with Schizophrenia and Concomitant Substance Use: A Post-Hoc Analysis of QUALIFY, a Head-To-Head Study

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Background: The QUALIFY study directly compared the treatment effectiveness of the dopamine D2 receptor partial agonist aripiprazole once-monthly 400 mg (AOM 400) against the D2 antagonist paliperidone palmitate once-monthly (PP) in patients with schizophrenia. The primary analysis showed superior improvements with AOM 400 vs PP on the Heinrichs-Carpenter Quality-of-Life Scale (QLS) assessing health-related quality of life and functioning (Naber et al. 2015). Substance abuse is a common problem in the treatment of patients with schizophrenia in clinical practice. Patients with concomitant substance abuse are harder to treat and are usually excluded from randomized

clinical studies. However, in the QUALIFY study, patients with substance use disorder were excluded from participation only if substance use was judged to compromise compliance with the study procedures. The objective of these post-hoc analyses was to investigate effects of AOM 400 and PP treatment in the sub-population of patients with a positive urine drug screen during the study.

Methods: QUALIFY was a 28-week, randomized, open-label, rater-blinded, head-to-head study (NCT01795547) of two atypical long-acting injectable anti-psychotics (LAIs), AOM 400 and PP (flexible dosing, per label, with 50-150 mg/month as paliperidone [EU and Canada], 78-234 mg/month as paliperidone palmitate [US]) in patients with schizophrenia. Included patients were ages 18-60 years needing a change from current oral antipsychotic treatment and, in the judgment of the investigator, would benefit from LAI treatment. Urine drug screens for drugs of abuse including, but not limited to opiates, cocaine, and cannabinoids, were conducted at screening, baseline, and completion/withdrawal visits, however patients with a positive urine drug screen were only excluded if further study compliance was judged to be compromised. Primary endpoint of QUALIFY was change from baseline to week 28 on QLS total score rated by a blinded clinician, with higher QLS scores indicating improvements in functioning. Changes in QLS total scores of 5.3 points or more were considered clinically relevant (Falissard et al. 2015). Secondary endpoints included change from baseline on the Clinical Global Impression–Severity (CGI-S) scale; and Work Readiness Questionnaire (WoRQ) total score was used to assess changes in patients' functional capacity. A mixed model for repeated measures was used to analyze changes from baseline to week 28 on QLS total scores, CGI-S scores, and WoRQ total score.

Results: Analysis of the primary endpoint showed superior improvements with AOM 400 (n = 136) vs PP (n = 132) on QLS total score in the full analysis set (FAS). The least square mean (LSM) changes from baseline to week 28 in QLS total score were 7.5 ± 1.5 after AOM 400 and 2.8 ± 1.6 after PP treatment, and the difference between treatments was 4.7 (95% CI: [0.3;9.0], p = 0.036). The number of patients with a positive urine drug screen at any time during the study were 26/136 (19.1%) in the AOM 400 and 29/132 (22.0%) in the PP groups. Patients with a positive urine drug test during the study showed LSM changes from baseline to week 28 on QLS total score of 6.4 ± 5.9 after AOM 400 and -4.7 ± 5.4 after PP treatment, with LSM difference between treatments of 11.1 (95% CI: [-5.2;27.4], p = 0.174). For secondary outcomes, LSM differences in change from baseline to week 28 were numerically better with AOM 400 vs PP: -0.1 (95% CI: [-0.5;0.3], p = 0.657) for CGI-S and -1.4 (95% CI: [-3.3;0.4], p = 0.126) for WoRQ total scores.

Conclusions: Among patients receiving AOM 400, those with a positive urine drug screen showed numerical improvements in QLS total score that were similar to the total treatment group. Patients receiving PP who had a positive urine drug screen displayed worsening in the QLS total score. The results suggest that the treatment effectiveness of AOM 400 on health-related quality of life and functioning is not compromised by concomitant recreational drug use. The magnitude of the difference, with AOM 400 improving and PP worsening QLS total scores, may be related to the dopamine partial agonist activity of AOM 400 that might spare the dopamine reward system. Since the study was not powered to detect differences between treatments in the sub-group of patients with a positive urine drug screen, these results warrant further investigation into potential benefits of dopamine partial agonists in patients with schizophrenia using recreational drugs.

Keywords: aripiprazole once-monthly 400 mg (AOM 400), paliperidone palmitate, head-to-head clinical trial, health-related quality of life, Substance use

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RAB, PS, TPS: Employee of Otsuka Pharmaceutical Development & Commercialization, Inc.
AE: Employee of H. Lundbeck. A/S, CF, KH, CP, MB, AGN, PH, HAS: Employee of Lundbeck
JYL: Employee of Otsuka. SGP: has received grant support, funding, or honoraria or served as a consultant for the following companies that conducted scientific or medical research and/or marketed medications related to psychiatric and neurodegenerative disorders: Alkermes, Amgen, Baylor University, Eisai, Eli Lilly and Company, Forest, FORUM Pharmaceuticals, Lundbeck, Merck & Co., Inc., NIH, Novartis, Otsuka, Roche/Genentech, Sunovion Pharmaceuticals Inc., Takeda Pharmaceutical Company Ltd., Toyama, University of Southern California, UCSD, UCSF and Vanda Pharmaceuticals.

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W155. Fibroblast Growth Factor 14 is an Essential Element of the Inhibitory Circuit that Controls Cognitive Function Associated with Schizophrenia

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Background: Cognitive processes require gamma-amino-butyric acid (GABA) interneurons. Via complex synaptic connections, these cells regulate cellular excitability and synaptic plasticity of principal neurons, balancing the excitatory/inhibitory (E/I) tone in cortical networks. Loss of and impairment in function of parvalbumin (PV)

interneurons and GABAergic synapses is associated with cognitive impairment in schizophrenia and other psychiatric disorders. Despite efforts to identify the molecular factors leading to E/I imbalance and impaired PV interneuron functioning, much remains to be learned. Additional knowledge of key regulatory nodes that control PV neuronal integrity and function, and GABAergic synapses is essential, especially to develop effective treatments for cognitive impairment. With a combination of animal model studies and post-mortem transcriptomics analysis, we provide what we believe are breakthrough results demonstrating a novel potential link between cognitive decline in schizophrenia and expression of fibroblast growth factor 14 (FGF14), a regulator of intrinsic excitability, synaptic transmission and plasticity. We show that Fgf14-/- mice have significantly reduced number of PV interneurons, decreased expression of the presynaptic GABAergic markers, GAD67 and VGAT, reduced inhibitory connections, decreased gamma frequency oscillations in cortical areas, and impaired working memory. Bioinformatics analysis of schizophrenia transcriptomics from human post-mortem tissue revealed functional co-clustering and correlative decreased expression of FGF14, PVALB, GAD67 and VGAT. Together these results provide evidence that FGF14 is a new risk-factor associated with schizophrenia and perhaps related disorders.

Methods: We employed confocal microscopy and image analysis to quantify changes in the PV interneuron population and GABAergic markers, single-cell patch clamp electrophysiology to assess inhibitory synaptic transmission in an ex-vivo brain slice preparation, in vivo local field potential recordings to analyze EEG spectra, and behavioral studies to evaluate working memory deficits in Fgf14-/- mice. Computational network analysis of FGF14 co-expression genes was conducted using the SEEK, a computational gene co-expression search engine. Functional annotation and pathway enrichment was obtained using GO and KEGG terms. We also evaluated the gene expression of FGF14 and other genes in two independent data sets, which derived from post-mortem DLPFC(BA46) of schizophrenic and controls subjects; the differential gene expression was analyzed by R Limma package.

Results: We show that genetic deletion of Fgf14 leads to loss of PV interneurons in the CA1 hippocampal region (74.21% ± 5.02 in Fgf14-/- mice versus 100% ± 4.89 in Fgf14+/+ littermates; p < 0.001, n = 4 littermates per group). These changes were associated with a statistically significant reduction in both GAD67 and VGAT expression in PV somas and in synaptic puncta on CA1 pyramidal neurons (p values were from < 0.01 to < 0.0001 for each phenotype, n = 3 littermates per group). Whole-cell patch clamp recordings from CA1 pyramidal neurons revealed a shift in the frequency and amplitude distribution of spontaneous and miniature inhibitory synaptic events consistent with a loss in a subset of synaptic events (n = 8-10 and n = 6-7 respectively, p < 0.001, Kolmogorov-Smirnov test). In vivo local field potential recordings showed a significant (p < 0.05) reduction in gamma frequency oscillations in Fgf14-/- mice (2.94 ± 0.11 µV2 n = 7) compared to wild type controls (7.92 ± 0.17 µV2 n = 7), while behavioral tests revealed impaired working memory in Fgf14-/- mice compared to wild type animals (n = 19-20 per group,

p < 0.001). Through the SEEK-based gene co-expression search engine with pathway enrichment by Gene Ontology (GO) terms, and KEGG orthologue analysis, we found that FGF14 was enriched within the 'GABAergic synapse' pathway and its expression profile correlated with that of PVALB (p = 0.004 in hippocampus; p = 0.0059 in prefrontal cortex), GAD67 (p = 0.0009 in hippocampus; p = 0.0003 in prefrontal cortex) and VGAT (p = 0.04 in hippocampus; p = 0.069 in prefrontal cortex). Furthermore, analysis of transcriptomics data from post-mortem tissue data sets showed significantly decreased expression of and correlation between FGF14, PVALB, GAD67 and VGAT in two independent schizophrenia data sets and matched controls.

Conclusions: The array of phenotypes associated with loss of Fgf14 in mice along with the complementary human studies provide knowledge to generate new hypotheses on the biology and the risk factors associated with disrupted GABAergic signaling in schizophrenia and other complex brain disorders.

Keywords: GABAergic circuitry, parvalbumin interneurons, synaptic connctions, Cognition

W156. Metabotropic Glutamate Receptor 5 Binding in Individuals with Schizophrenia

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Background: The implication of N-methyl-D-aspartate (NMDA) receptors in schizophrenia has received increasing interest over the last years. NMDA receptor function is modified by metabotropic glutamate receptors subtype 5 (mGluR5) suggesting that mGluR5 can also be involved in the pathogenesis of schizophrenia. This notion is corroborated by genetic studies showing that allele frequency distribution of an intragenic microsatellite of the mGluR5 gene, GRM5, is associated with schizophrenia. Here, we report the results of a study investigating mGluR5 in schizophrenia in vivo via positron emission tomography (PET) with the mGluR5-specific radiotracer 3-(6-methyl-pyridin-2-ylethynyl)-cyclohex-2-enone-O-11C-methyl-oxime ([11C]ABP688).

Methods: [11C]ABP688 PET was carried out in 15 individuals with schizophrenia (6 female) and 15 healthy controls (6 female). Psychopathology in cases and controls was assessed by the SCID-I, PANSS, BAI, BDI, and the Bern Psychopathology Scale (BPS). In subjects with schizophrenia, antipsychotic medication was transformed into chloropromazine equivalents. Exclusion criteria comprised current psychiatric (for subjects with schizophrenia additional

current psychiatric disorders), medical, or neurological disorders, history of substance dependence, pregnancy, and breastfeeding.

We applied a bolus/infusion protocol, previously evaluated for PET with [11C]ABP688, which allows reliable measurement of the relative distribution volume (DVR) and reduces potential bias due to arterial blood sampling needed for absolute quantification. With this protocol equilibrium between the tracer in tissue and blood is achieved 40 min after the start of radioligand infusion. A total of 600–800 MBq of [11C]ABP688 in a 50-mL volume was administered using an infusion pump. Individual dynamic uptake curves were checked for suitability. PET data was analyzed with PMOD (Version 3.4, www.pmod.com, PMOD Technologies, Zurich, Switzerland).

Results: Groups were age-matched (t28 = 0.172, p > 0.86): subjects with schizophrenia were 38.2 ± 10.7 years old (mean ± standard deviation), healthy controls were 37.5 ± 10.6 years old. Individuals with schizophrenia reported an average illness duration of 15.6 ± 11.6 years with an illness onset at 22.6 ± 7 years. They were under stable medication with atypical neuroleptics (risperidone, paliperidone, quetiapine, or clozapine) except for one subject who was treated with fluanxol. Individuals with schizophrenia scored higher than healthy controls in both BDI (t28 = 5.365, p < 0.001) and BAI (t28 = 2.648, p < 0.05).

No significant difference in mGluR5 DVR between subjects with schizophrenia and healthy controls was found in a repeated measures analysis of variance with 12 brain regions as a within-subjects factor and diagnostic group as a between-subjects factor (F(28,1) = 0.11; p > 0.9). Furthermore, no significant difference in mGluR5 DVR between patients and controls was found when accounting for sex, age, BDI scores, and BAI scores as covariates (F(24,1) = 0.082; p > 0.7). Adding smoking status as a second between-subjects factor in the statistical model we found a highly significant difference between smokers and non-smokers (F(22,1) = 185.632; p < 0.0001) but no significant interaction between smoking and diagnosis (F(22,1) = 0.320; p > 0.5).

We found a small potential effect of antipsychotic medication on mGluR5 DVR levels in only one region, the medial orbitofrontal cortex (mOFC), and only for non-smokers: higher chlorpromazine equivalent was associated with increased mGluR5 DVR in the mOFC of non-smokers (rho = 0.9, p < 0.02). Focusing on the same region we identified an interaction between diagnostic group and gender (F(22,1) = 8.706; p < 0.01), as well a non-significant trend for a triple interaction group-by-gender-by-smoking (F(22,1) = 3.884; p < 0.1). Schizophrenia was associated with higher mGluR5 DVR in female non-smokers, but with lower mGluR5 DVR in male non-smokers.

Within the schizophrenia group no significant correlations were found between clinical and demographic variables and mGluR5 DVR (PANSS sum score p ≥ 0.26; PANSS negative subscale p ≥ 0.49; PANSS positive subscale p ≥ 0.21; PANSS general psychopathology subscale p ≥ 0.09; BPS language subscale p ≥ 0.07; BPS affection subscale p ≥ 0.6; BPS motor subscale p ≥ 0.45; BDI p ≥ 0.24; BAI p ≥ 0.15; onset of illness p ≥ 0.13; duration of illness p ≥ 0.05).

Conclusions: We did not find differences in mGluR5 binding between individuals with schizophrenia and controls. Because antipsychotic drugs such as clozapine appeared to affect mGluR5, our findings may be clinically relevant. They also provide further insights into the high comorbidity between schizophrenia and tobacco addiction, e.g., smoking may counteract the potential upregulation of mGluR5 by antipsychotic drugs.

Keywords: schizophrenia, mglur5, pet, smoking, antipsychotics

Disclosures: Nothing to disclose.

W157. The Neurobiology of Simulated Real-World Effortful Behavior Deficits in Schizophrenia

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Background: Motivational deficits are a central feature of the negative symptoms of schizophrenia, and are linked to the significant functional impairment experienced by individuals with the illness. Effective treatments, however, for these motivational deficits remains elusive. Through efforts to advance treatment, recent work has focused on examining specific facets of motivation, from hedonic capacity and reward prediction, through reward and effort/cost valuations that guide cost-benefit analyses, to goal-directed action planning. While some aspects of motivation such as hedonic experience and reward prediction have been extensively investigated, there has been limited examination of the impairments in effort/cost valuation and the reduction in effortful behavior seen in individuals with schizophrenia. The few behavioral studies in this area have demonstrated consistent reductions in patients' willingness to pursue goals in the face of increasing effort requirements, with emerging evidence suggesting a potential role of the ventral striatum in promoting such effortful behavior. Moreover, most investigations to date have relied on abstract effort tasks with limited direct relevance to the everyday lives of individuals with schizophrenia. In contrast, this study used a novel virtual reality effort task to investigate the neurobiology of impaired effortful behaviour in schizophrenia in the context of simulated real-world tasks.

Methods: Stable outpatients between 18 and 55 years old with schizophrenia (SZ) and matched healthy controls (HC) were recruited for this study. All participants underwent clinical and neurocognitive assessments, including assessment for severity of positive and negative symptoms, apathy, depression, hedonic capacity, and cognitive function. Participants subsequently underwent functional MR imaging while performing our Virtual Reality Progressive Ratio (ViPR) task. In this task participants worked for reward in a virtual city in the face of increasing effort requirements. Our main analyses examined group differences in brain activity associated with increasing effort demands and with reward attainment after increasing effort requirements, as well as the neural correlates of task performance.

Results: 38 participants (19 SZ and 19 HC) completed this study. In addition to significant differences in task performance, whereby SZ subjects exhibited impairments in effortful behavior, our analyses revealed impaired activation across several brain regions in SZ subjects compared to controls. Specifically, we found reduced activation in the right dorsolateral prefrontal cortex (DLPFC), bilateral supplementary motor area (SMA), and left inferior frontal gyrus in response to increasing effort requirements. Further, during reward attainment following progressively increasing effort demands, SZ participants exhibited reduced ventral anterior cingulate (dACC) and left dorsomedial prefrontal cortex (dmPFC), and bilateral SMA activation. Moreover, task performance was significantly correlated with right DLPFC and left putamen activity.

Conclusions: Using a virtual reality-based effort task this study investigated the neurobiology of impaired effortful behaviour in schizophrenia in the context of simulated everyday activities. Our findings suggest that right DLPFC and left inferior frontal activation is impaired in individuals with schizophrenia during effort expenditure, along with impaired dACC and dmPFC engagement during eventual reward attainment. Impaired SMA activation in both contexts may reflect impaired cost-benefit analysis that deters the effortful pursuit of goals. In addition, across all subjects right DLPFC and left putamen activity was linked with more effortful task performance. Overall, these findings provide some insights into the neurobiology of impaired effortful behaviour in schizophrenia, and may offer important targets for therapeutic brain intervention to treat motivation deficits in schizophrenia.

Keywords: Effort, Virtual Reality, Motivation, Reward-based decision-making, fMRI

Disclosures: Nothing to disclose.

W158. Diagnostic Specificity in Adult Patients with Schizophrenia and Autism Assessed with Regional MRI Brain Volume Inter-Correlations and Dendritic Tree Analysis

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Background: Autism and schizophrenia are considered distinct diagnostic categories but share a number of dimensional symptom phenotypes. The two disorders may also appear together in patients at a higher rate than expected based on population prevalence. Social interaction deficits are one common symptom dimension, a behavioral skill which may be associated with cingulate and orbitofrontal functionality. We examined the similarities using two statistical methods. First we used dendritic tree analysis to test whether regional volume change divided followed diagnostic or phenotypic similarity. Second, interregional volume correlations were compared to test whether patterns of volume associations differed between groups. Previous volumetric contrasts showed dorsal prefrontal decreases in schizophrenia more marked than in autism but cingulate changes more marked in autism.

Methods: We obtained structural MRI findings from 108 subjects: 49 subjects with schizophrenia, 20 subjects with autism, and 39 healthy volunteers. Volumetric measures were made for gray matter for frontal and cingulate Brodmann areas in each lobe and expressed relative to total brain volume. Patients with schizophrenia were divided into good (n = 24) and poor outcome (n = 25) groups based the poor outcome criteria: 1) continuous dependence on others for obtaining food, clothing, and shelter 2) no useful employment and 3) no symptomatic remission.

Results: Analysis of interregional connectivity was assessed by examining the product-moment correlation coefficients calculated between all frontal and cingulate Brodmann area volumes and z-transformed for statistical comparisons (BA 4, 6, 8, 9, 10, 11, 12, 32, 44, 45, 46, 47, 25, 24). For each Brodmann area we computed the mean z-transformed correlation with all other frontal areas. The square correlation matrices from the healthy volunteers and patients with autism (patient and healthy volunteer groups were compared with the Steiger multivariate test (R package psych, routine cortest.normal) and significant matrix differences confirmed (chi-square = 161, df = 91, p = 0.0000087). Patients with autism and patients with poor-outcome schizophrenia also showed significantly different matrices (chi-square 165, df = 91, p = 0.0000037). Healthy volunteers and patients with good outcome schizophrenia did not show whole matrix significant differences. However, the mean z scores for the correlations between Brodmann areas 6, 8, 11, 46, and 47 were significantly higher in patients with schizophrenia by t-test. Patients with autism and patients with poor-outcome schizophrenia showed significantly diminished interregional correlations for areas 25, 10, 11, 12 and 4 and these were also areas with diminished correlation in poor outcome schizophrenia.

Hierachical dendritic tree analysis (R package rpart) was applied to the four groups of subjects. The first node dividing the four groups was the volume of Brodmann area 10 with 34/39 healthy and 20 of 20 patients with autism having relatively larger BA 10 volumes and 42/49 patients with schizophrenia having smaller BA 10 volumes. Patients with poor outcome were divided from good outcome by having very small BA 10 volume. Patients with autism were divided from patients with good outcome schizophrenia by having small Brodmann area 11 (orbital frontal cortex).

Conclusions: The regional inter-correlation data reveal communality in cingulate disconnection between poor outcome schizophrenia and autism although other volume measures differed in size and connectivity. Thus connectivity analysis appears sensitive to detecting brain organizational changes that may possibly parallel limited symptom similarities. In contrast, the cladistics approach of hierarchical tree analysis appears to more closely parallel established diagnostic divisions which themselves are developed in a cladistic viewpoint. It is of interest that connectivity analysis more closely relates to the Research Domain Criteria (RDoC) project goal of "developing "a research classification system for mental disorders based upon dimensions of neurobiology…" while hierarchical dendritic tree analysis tends to more closely match "current heterogeneous disorder categories" (Cuthbert and Insel, BMC Medicine, 2013).

Keywords: cladistics, diagnostic specificity, Research domain criteria (RDoC)

Disclosures: Nothing to disclose.

W159. Early Life Exposure to Metals and Schizophrenia: A Proof-of-Concept Study of Tooth as a Novel Biomarker

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Background: The theory of fetal origins of adult disease posits that sensitive windows exist as early as pregnancy for environmental exposures that induce maladaptive neurobehavioral phenotypes. Metals have well-known effects on neurodevelopment in children, some acting as essential nutritive elements and some others as neurotoxins. However, we know little about the impact of early life metal exposures on adult-onset psychiatric disorders. There are multiple intriguing links between several metals and psychosis. To our knowledge, no prior studies have robustly studied the link between fetal or early childhood metal exposure with risk of psychotic illness in adulthood. Our primary objective was to determine whether the timing and dose of prenatal and early childhood metal exposure influences the later development of schizophrenia and psychotic experiences.

Methods: In a proof-of-concept study, we analyzed shed teeth from nine individuals from the Genetic Risk and OUtcome of Psychosis (GROUP) study with a DSM-IV diagnosis of schizophrenia. We investigated the association between exposure to five metals [Manganese (Mn), Lead (Pb), Cadmium (Cd), Copper (Cu), and Zinc (Zn)] and schizophrenia. We also studied the relation between exposure to five metals and psychotic experiences (as assessed by the Community Assessment of Psychic Experiences (CAPE) scale) as well as intelligence quotient (IQ). Baby teeth from each subject were evaluated for pre- and postnatal metal exposure. We reconstructed the dose and timing of fetal and childhood metal exposures using a novel biomarker method. The method combines sophisticated histological and laser-based chemical analyses to precisely sample dentine layers corresponding to specific life stages, generating integrated, longitudinal, 1- to 2-week metal exposure estimates in pregnancy and during early childhood. The time-varying difference between early life (-4 to 10 months) metal concentrations, as measured in the tooth biomarker, and case/control designation was evaluated using a Distributed Lag Model (DLM).

Results: The study subjects were representative of the total GROUP cohort. The longitudinal differences in log Cd and Pb were generally estimated above zero, showing higher early life exposure to Cd and Pb among subjects with schizophrenia than controls. The differences in log Mn and in log Cu increased relatively linearly over time to postnatal negative values, indicating lower postnatal exposure to Cu and Mn in subjects with schizophrenia than controls. Differences in Zn were curvilinear with decrease after birth. There was a positive correlation between IQ and Zn concentrations both pre- and postnatally. A negative correlation between Cu concentrations and psychotic experiences was evident postnatally, where higher concen-trations were associated with more severe psychotic experiences.

Conclusions: In this proof-of-concept study we demonstrated a relationship between prenatal and early life exposure to metals and risk for schizophrenia. There was evidence of differential relationships over time and for both prenatal and postnatal critical windows of susceptibility. New biomarkers represent a potential major advance, as we can now objectively determine early-life environmental exposures in psychiatric disorders.

Keywords: schizophrenia, psychosis continuum, metals, Biomarker

Disclosures: Nothing to disclose.

W160. Acute Inhibition of ErbB4 Tyrosine Kinase by a Chemical-Genetic Approach Impairs Synaptic Plasticity and Causes Schizophrenia-Associated Behavioral Deficits

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Background: Neuregulin-1 (NRG1) and its receptor ErbB4 are both risk genes of schizophrenia. The NRG1/ErbB4 signaling has been implicated in neural development such as the assembly of the GABAergic circuitry. It has also been shown to promote the release of GABA in adult animals and thus regulate synaptic plasticity. Mutation of either NRG1 or ErbB4 leads to behavioral deficits that are associated with schizophrenia. However, current methods or models are unable to determine whether these deficits are due to ErbB4 deficiency in development or in adulthood, or both. To address this question, we generated a mouse, B4T796G, which allows for acute inhibition of ErbB4 by the specific, bulky inhibitor 1NMPP1. It enables temporal and spatial control of ErbB4 tyrosine kinase in vivo and in vitro. We studied the effect of acute inhibition of ErbB4 in adult animals. Results suggest that ErbB4 kinase activity is required for proper neurotransmission and behavior in mice. 1NMPP1-treated B4T796G mice showed behavioral deficits that are relevant to schizophrenia.

Methods: First, we mutated threonine 796 (T796) to glycine and determined whether the mutant ErbB4 was inhibited by 1NMPP1 in vitro. Next, we generated a knock-in mouse, B4T796G, where T796 was converted to glycine. Third, this mouse was injected without (control) or with 1 μg/g body weight 1NMPP1 and characterized for ErbB4 activity, neurotransmission, synaptic plasticity and behavior.

Results: NRG1 was unable to stimulate the T976G ErbB4 mutant in transfected HEK293 cells in the presence of 1NMPP1. The inhibition was reversible. The B4T796G mouse was viable and fertile and showed no apparent behavioral deficits. To study the effect of acute ErbB4 inhibition on neurotransmission and synaptic plasticity, hippocampal slices were isolated from B4T796G mice and recorded for miniature inhibitory postsynaptic currents (mIPSCs) in CA1 pyramidal neurons. 1NMPP1 (10 μM)

reduced mIPSC frequency, but not amplitude, in B4T796G slices. This effect was specific because it was not observed in control wild type slice. Moreover, pre-inhibition of ErbB4 with PDxxxXXX, an inhibitor of EGF receptor kinases prevented 1NMPP1 from inhibiting sIPSC frequency, which ruled out the off-target effect of 1NMPP1. These results indicate that ErbB4 kinase activity was necessary for proper GABA release in the brain. In support of the notion was the observation that the paired-pulse ratio (PPR) was increased after 1NMPP1 treatment, which suggested a decrease in GABA release probability. Finally, long-term potentiation (LTP) was enhanced in 1NMPP1-treated hippocampal slices of B4T796G mice, but not those of control mice. These in vitro studies indicate that acute, specific inhibition of ErbB4 impairs neurotransmission and synaptic plasticity.

To determine whether ErbB4 activity is necessary for proper behavior, we injected 1NMPP1 into B4T796G mice. Tyrosine-phosphorylation level of ErbB4 in B4T796G mice was reduced within 15 min of 1NMPP1 injection. The inhibition peaked at 30 min after the injection, after which ErbB4 activity gradually recovered and returned to normal 60-75 min after injection. Remarkably, 1NMPP1-injected B4T796G mice were hyperactive in open-field and were impaired in pre-pulse inhibition (PPI). We recorded neuronal activity in the hippocampus and prefrontal cortex in free-moving mice by multi-channel recording. A cannula was implanted in the lateral ventricle to administrate NRG1 or 1NMPP1. We found that the rhythm power of local field potential (LFP) increased by NRG1 (5 μl 20 nM NRG1), and the rhythm power decreased after infusing 5 μl 50 μM 1NMPP1. Moreover, the synchrony between hippocampus and prefrontal cortex decreased in gamma band and increased in theta band in 1NMPP1-treated B4T796G mice, suggesting a role of NRG1-ErbB4 signaling in homeostatic brain activity.

Conclusions: Acute inhibition of ErbB4 in adult mice causes deficits in neurotransmission, synaptic plasticity and behavior, some of which were observed in ErbB4 null mutant mice. These observations not only indicate that ErbB4 kinase activity is crucial for these events, but also demonstrate that the brain activity requires proper NRG1/ErbB4 signaling in adult animals. The B4T796G mouse, which enables acute, reversible, in vivo inhibition of ErbB4, may serve as a valuable model to reveal physiological function of NRG1/ErbB4 and pathophysiological mechanisms of brain disorders including schizophrenia.

Keywords: Neuregulin-1, ErbB4, Schizophrenia, 1NMPP1
Disclosures: Nothing to disclose.

W161. Assessing Reward Learning in Macaques Using a Probabilistic Selection Task

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Background: The Probabilistic Selection Task (PST) is a paradigm that evaluates whether subjects learn better from positive or negative outcomes of probability-related choice behavior mediated through cortical-striatal dopaminergic pathways. The PST has been utilized to evaluate several neuropsychiatric and neurodegenerative disorders, and has revealed reward-related learning impairments in selected patient populations (e.g., schizophrenia, autism). The intent of our studies was to assess probabilistic reward learning in non-human primates (NHPs) for evaluating potential novel therapeutics in a species with close homology to humans.

Methods: Eight adult male Cynomolgus macaques were housed individually under a 12-hr light/dark cycle in a temperature-controlled room. They had access to water ad libitum and were fed a full daily ration of food in addition to enrichment (e.g., fruit, vegetables, nuts). Animals were trained initially on a 1- and then 3-pair concurrent discrimination (CD) task in which selection of one stimulus in a pair resulted in delivery of a food reward 100% of the time (100 trials/session). The animals learned to identify which stimulus in the pair was associated with the reward and to reliably select it with $\geq$ 80% accuracy for $\geq$ 2 test sessions before moving to the next level of training. Stimulus pairs were novel in each test session and presented first as blocked then randomized trials during the training phase. Following CD training, animals began PST training, modeled after the work of Waltz et al., 2007 (Biol Psych 62; 756-64). For this task, animals were presented with 3 unique stimulus pairs (A/B; C/D; E/F) in each session, each rewarded with a different probability (A-80%/B-20%; C-70%/D-30%; E-60%/F-40%). On each trial, the NHPs made a two-alternative forced choice and learned to choose the most frequently rewarded stimulus from each set based on the identified probabilities. Animals received blocks of 60 trials of pseudo-randomized presentations of the pairs until 1) they met the criterion of selecting 'A' 65% of the time, 'C' 60% of the time, and 'E' 50% of the time, or 2) they had completed 6 blocks of trials (360 trials, 120 of each pair). Once animals reliably met the performance criterion, the transfer test, consisting of novel combinations of stimuli, was introduced to test whether NHPs learn better from positive (rewarded, i.e., A/C; A/D; A/E; A/F) or negative (non-rewarded, i.e., B/C; B/D; B/E; B/F) events.

Results: Macaques acquired the rules of the CD task quickly and reached proficiency at each level within 5 test sessions. Our early results indicate animals readily learn the PST through a combination of positive and negative events, with evidence of learning being driven more strongly through positive feedback, in line with reported behaviors in healthy human subjects.

Conclusions: In this study, we have adapted the parameters of the human PST for use in non-human primates. Our results suggest macaques are able to acquire the task contingencies of the PST showing evidence of learning through both positive and negative feedback with a preference for selecting the more frequently rewarded stimuli and avoiding the non-reinforcing stimuli. These data suggest that the probabilistic selection task may be a useful translatable animal paradigm to assess probabilistic reward learning between species.

Keywords: Reinforcement learning, Probabilistic reinforcement, Reward, schizophrenia, monkey

W162. Lurasidone Is an Effective Treatment for Treatment Resistant Schizophrenia

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Background: ~30% of patients with schizophrenia have persistent psychotic symptoms despite treatment with two or more antipsychotic drugs (APDs) for at least six weeks and constitute treatment resistant schizophrenia (TRS). The approved pharmacologic treatment for TRS is clozapine, with major improvement in psychosis beginning within six weeks for 30%, and between 6-24 weeks in another 20-30%. We have previously reported that other atypical APDs, including melperone, olanzapine, and risperidone Consta, may also be effective for psychotic symptoms in some TRS patients, possibly requiring doses that are higher than standard doses. The purpose of this study was to determine whether lurasidone, 80 mg/day, a novel atypical APD, with a unique receptor profile, which is effective in non-TRS patients at 40 mg/day producing improvement in 2-6 weeks in most such patients, 1) is also effective in the treatment of TRS (defined per the US Clozaril trial); 2) whether 6 weeks is an adequate time to achieve response to an 80 mg/day dose of lurasidone in TRS patients; and 3) whether higher doses of lurasidone are superior to the 80 mg/day dose in patients with TRS.

Methods: In phase 1, 133 putative TRS outpatients not receiving clozapine, referred from CMHCs provided written informed consent and were screened to identify 101 who met entry criteria for TRS. The sample size was based on three prior trials of atypical APDs in TRS patients directed by the PI. All met the TRS criteria of the US Clozaril trial. After thorough review of medical records and assessment of diagnosis using SCID, summary data were evaluated by the PI prior to acceptance. Current APDs were tapered over a 1-2 week period and lurasidone, 80 mg/day was given for 6 weeks, to verify TR status. Visits were scheduled weekly to assess response, side effects and adherence. Those who showed ≤20% improvement in phase 1 and still met TRS criteria of moderate-severe positive symptoms were randomized to receive either 80 or 240 mg/day for 24 weeks (N = 67).

Results: Of the 101 TRS patients who entered phase 1, only 4 improved sufficiently during open treatment to no longer meet TRS criteria, and were not enrolled in phase 2. CGI-S was 4.7 and 4.55 at the beginning and end of phase 1 (p = 0.16). The mean age of the randomized patients was 46, with duration of illness of 26 years and 6 previous hospitalizations. There were 56% African Americans, 36% Caucasians, and 8% other. 44%% were male. Twenty one patients in each dose group (~64%) completed the six month phase. Other psychotropic drugs patients prescribed prior to phase 1 were continued. Discontinuation of these medications during the course of the study was discouraged. No other APD was permitted to be utilized. All adjunctive supportive treatments available to patients from their referral sources were permitted. All but 12 patients in phase 2 received some adjunctive psychotropic drug treatment. The primary outcome measure was the change in PANSS Total score during phase 2. A mixed model with LOCF was used to identify significant improvement of PANSS Total scores from baseline to 6 months across the two treatment groups, adjusting for baseline measures. These analyses revealed no significant group x time interaction effect. However, improvement in PANSS Total over time was highly significant [F = 50.59, p < 0.0001; effect size (ES)1.17]. There were no significant differences between dose groups. The LS mean change in PANSS total between baseline and 6 months scores in the low and high dose groups were 12.7 and 14.1, respectively. Significant time effects were found for PANSS Positive (ES = 0.86), Negative, Cognitive and General factors.

The improvement in psychopathology occurred between baseline and 12 weeks in phase 2 (week 18 of lurasidone treatment), with little further improvement between weeks 12 and 24 of phase 2. 31% of the 67 randomized patients and 50% of the completers improved by ≥20% in PANSS Total. Significant improvement for both groups was found in the digit symbol substitution test (ES = 0.49) and the Wisconsin Card Sorting Test-categories (ES = 0.43), and in WISC-R mazes for the 80 mg dose group only (ES = 0.38). These improvements were independent of improvement in psychopathology. There was significant improvement in the CGI (4.6 to 4.0, F = 14.70, p < 0.0001) and the Personal and Social Performance scale [46.1 to 50.2, F = 13.22, p < 0.0001(ES = 0.65)] in both groups. There was no increase in BMI or EPS measures in both groups. There was a small increase in plasma prolactin in the high dose group. Overall, there were no tolerability differences between doses. One subject death occurred during the course of the study of non-study related causes.

Conclusions: The efficacy of both 80 and 240 mg/day lurasidone for improving PANSS scores in TRS patients are similar to our previous published findings with clozapine, olanzapine and long acting risperidone, and superior to published findings with typical APDs, e.g. chlorpromazine or haloperidol. A weakness of this study was the absence of a concomitant clozapine-treated group. Future studies should include comparisons of lurasidone with clozapine and typical APDs to rule out possible biases in the current studies. We tentatively conclude that lurasidone, as well as several other atypical APDs, e.g. risperidone and olanzapine, may be useful to treat some TRS patients, particularly when the duration of treatment is 2-6 months. Further study is needed to determine if non-responders to lurasidone would respond to clozapine and vice versa.

Keywords: treatment resistance, schizophrenia, Cognition, psychosis

W163. 7T Proton Magnetic Resonance Spectroscopy of the Anterior Cingulate Cortex in Schizophrenia

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Background: Functional neuroimaging studies have consistently provided evidence of impairment in anterior cingulate cortex (ACC) function in people with schizophrenia. Additional studies have linked ACC function to positive symptoms and response to treatment with antipsychotic medication. Proton magnetic resonance spectroscopy is a technique that provides in vivo measurements of multiple metabolites, such as N-acetylaspartate (NAA), glutamate, and GABA, which underlie brain function. Previous MRS studies have shown reduction in NAA in the frontal cortex as well as disruption of the glutamatergic system that may be related to illness stage or medication status. In this preliminary study, we sought to replicate and extend the previous studies by examining these metabolites at 7T, which offers better spectral resolution and signal-to-noise ratio compared to 3T.

Methods: 12 individuals with schizophrenia (3 female / 9 male; age: 24.3 +/- 5.1) and 12 healthy controls (2 female / 10 male; age: 24.3 +/- 3.7) were recruited from the University of Alabama at Birmingham psychiatric clinics and the general community. Imaging was performed at the Auburn University MRI Research Center on a Siemens MAGNETOM 7T MRI scanner equipped with a 32-channel head coil. 3D MPRAGE structural images (0.7 mm isotropic resolution) were obtained for voxel placement. Single-voxel spectra were acquired using an ultra-short TE STEAM sequence (TR/TE/TM = 10,000/5/45 ms, 32 averages), FASTESTMAP shimming, and VAPOR water suppression. Following localized adjustments and optimization of parameters, spectra were acquired from the dorsal ACC (27x20x10 mm). MRS analysis was performed in LCModel using a simulated basis set provided by the software developer. Spectra were eddy current corrected and quantified using the unsuppressed water signal (4 averages). Cramer-Rao lower bounds (CRLB) were used as a measure of fit, and only metabolites with CRLB < 20% were included in further analysis. Univariate ANOVA covarying for age was used to compare metabolite levels between the groups. Independent-samples t-tests were used to compare linewidths (FWHM) and signal-to-noise ratio (SNR) between the groups.

Results: Total NAA (NAA + NAAG) was significantly reduced in the schizophrenia group (6.64 +/- 0.65, institutional units) compared to the control group (7.15 +/- 0.34, institutional units) [% difference = 7.4%, p = 0.028]. No differences were observed in levels of glutamate, glutamine, GABA, total creatine, or total choline. Spectral fitting was good as indicated by mean CRLB (NAA + NAAG: 2.5%; glutamate: 2.2%; glutamine: 7.1%; GABA: 10.4%; Cr + PCr: 2.1%; GPC + PCh: 2.1%). Linewidths and SNR did not significantly differ between the groups.

Conclusions: In this preliminary study, we measured metabolites in the dorsal ACC, a region that is known to be functionally abnormal in schizophrenia. We observed reduced NAA in the schizophrenia group, which has been found in other MRS studies of the frontal cortex. NAA is generally considered to be a marker of neuronal health or integrity. Reduced NAA is consistent with postmortem evidence of reduced cortical thickness, neuronal density, and dendritic density in the ACC of people with schizophrenia. Furthermore, the lack of significant differences in linewidths and SNR suggests the NAA group effect was not due to inconsistencies in spectral quality between the groups. Taken together, these findings suggest that abnormal neuronal integrity or physiology underlies the functional alterations observed in schizophrenia.

Keywords: schizophrenia, MR spectroscopy, anterior cingulate cortex

Disclosures: Dr. Nouha Salibi is employed by Siemens Healthcare (MR R&D).

W164. Oxytocin Modulation of Neural Circuitry for Trust in Schizophrenia: A fMRI Study

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Background: Trust in another human being is a critical component of social decision making and complex social interactions. Previous studies report lower levels of trust in schizophrenia patients and their relatives. Despite the importance of social decision-making abnormalities in schizophrenia and their significance in real-world functioning, few studies have examined the neural basis of trust deficit in schizophrenia. Neuroeconomics approach uses experimental paradigms derived from economics which better approximate many real life situations including trust in human interactions. Several lines of evidence suggest a role for oxytocin in enhancing trust. However, no study has examined the effect of oxytocin on the neural circuitry of trust in schizophrenia. In this study we examined the effect of intranasal oxytocin vs placebo on fMRI activation pattern while performing a trust game task.

Methods: Eight male patients with schizophrenia (age -33.12 +/-8.54 years; education - 14.87 +/-2.41 years) underwent three fMRI scans while performing an iterative version of trust game. Trust game is a validated neuroeconomics paradigm previously used in fMRI studies. fMRI Scans were acquired using a 3T MRI scanner. Task was designed and delivered using E-prime software. After an initial baseline scan, we administered 24 IU of oxytocin or a saline placebo intranasally 30 minutes before the scan. The scans were conducted on separate days and patients were blind to the drug administered. The order of administration of oxytocin and placebo were counterbalanced. fMRI scans were analysed using statistical parametric mapping (SPM 12). First linear contrasts were computed at the individual subject level comparing investment in human vs investment in lottery trials and random effect analysis was carried out comparing oxytocin vs placebo conditions.

Results: We found a significant activation in the Right medial prefrontal cortex (Broadmann area - BA 10), Right posterior cingulate cortex (BA 31), Right caudate body, and

left prefrontal cortex (BA 9) with oxytocin compared to placebo. We also found a significant deactivation in the Right Insular cortex (BA 13) with oxytocin compared to placebo (all values significant at p < 0.001 uncorrected).

Conclusions: These preliminary findings if confirmed in a larger sample, provide the first direct evidence for oxytocin's effect in modulation of neural circuits of trust in patients with schizophrenia. Oxytocin's effect on regions involved in social cognition suggests its possible therapeutic role in treatment of trust deficits in schizophrenia. Findings of the study could potentially pave way for novel treatment options for schizophrenia in the future.

Keywords: schizophrenia, oxytocin, trust, neuroeconomics
Disclosures: Nothing to disclose.

W165. General Intelligence and Associated fMRI Networks in Schizophrenia

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Background: General intelligence, or Spearman's g, has been linked to dorsolateral prefrontal cortex (DLPFC) (Duncan et al., 2000). As impairments in g characterize schizophrenia, we asked whether the regions and systems associated with g would differ between healthy subjects (HS) and patients with schizophrenia (SCZ).

Methods: We studied 556 HS and 123 SCZ subjects using fMRI at 3T during the Nback task with g obtained as previously (Dickinson et al., 2011). We analyzed fMRI data using SPM5 in three ways: multiple regression using g, PPI as multiple regression using g, and in exploratory factor analysis (EFA) in SPSS using AAL extracted individual maps.

Results: In both groups, we found that areas within left DLPFC correlated significantly with g in an efficiency manner – less activation predicting greater general cognition. Small differences between groups were found. Using PPI in HS, we found higher g associated with greater coupling with right parietal cortex and lower coupling with left hippocampus. In SCZ, higher g was associated with greater coupling with bilateral hippocampi and reduced coupling with anterior cingulate and precuneus. EFA in both groups yielded 4 similar factors – prefrontal-superior parietal, SMA-inferior parietal, cerebellar, and anterior cingulate-basal ganglia factors. In HS, g correlated with the prefrontal-superior parietal factor, but in SCZ with the anterior cingulate-basal ganglia factor.

Conclusions: These data suggest that while g correlated with DLPFC activation in both groups wherein diagnostic differences in coupling arose the PPI and EFA analysis. SCZ may utilized a different network with PPI and EFA suggest that patients rely more heavily on anterior cingulate and basal ganglia, perhaps as compensation for prefrontal dysfunction or from abnormal prefrontal connectivity.

Keywords: fMRI, Connectivity, DLPFC, schizophrenia, Cognition
Disclosures: Nothing to disclose.

W166. Clinical Development of ITI-007 for the Treatment of Schizophrenia

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Background: Schizophrenia is a devastating and serious mental illness afflicting approximately 1 percent of the population resulting in high rates of disability to patients and a high burden to their caregivers. It also exerts an enormous toll in terms of healthcare costs. Schizophrenia ranks in the top 10 leading causes of disability in the world. Despite the introduction of neuroleptics in the 1950s and the advance of atypical antipsychotic therapy since the introduction of clozapine, there still remains an unmet need for newer treatments which address a broad spectrum of schizophrenia symptoms including positive, negative and depressive symptoms without concomitant high rates of motor disturbances, metabolic syndrome, and/or cardiovascular risk.

ITI-007 is an investigational new drug in late-stage clinical development for schizophrenia. Through synergistic actions via serotonergic, dopaminergic and glutamatergic systems, ITI-007 represents a novel approach to the treatment of schizophrenia and other neuropsychiatric disorders. ITI-007 is a potent antagonist at 5-HT2A receptors, a mesolimbic/mesocortical dopamine phosophoprotein modulator (DPPM) with activity as a pre-synaptic partial agonist and post-synaptic antagonist at dopamine D2 receptors, a mesolimbic glutamate GluN2B receptor phosphoprotein modulator and a serotonin reuptake inhibitor. This unique pharmacology has been predicted to translate clinically, in a dose dependent manner, into broad antipsychotic efficacy for the treatment of positive and negative symptoms with improved cognition, affective symptoms, and sleep.

Methods: Available data is presented and we report on the progress to date from the ongoing late-stage schizophrenia development program for ITI-007. This program includes three randomized, double-blind, placebo-controlled clinical trials in patients with acute schizophrenia: ITI-007-005, ITI-007-301, and ITI-007-302.

In the Phase 2 trial ITI-007-005, patients were randomized to receive one of four oral treatments once daily for 4 weeks: 60 mg ITI-007, 120 mg ITI-007, 4 mg risperidone (positive control) or placebo in a 1:1:1:1 ratio. In the first Phase 3 trial ITI-007-301, patients were randomized to receive one of three oral treatments once daily for 4 weeks: 60 mg ITI-007, 40 mg ITI-007, or placebo in a 1:1:1 ratio. In the second Phase 3 trial ITI-007-302 (clinical conduct ongoing), patients are randomized to receive one of four oral treatments once daily for 6 weeks: 60 mg ITI-007, 20 mg ITI-007, 4 mg risperidone (positive control) or placebo in a 1:1:1:1 ratio.

The Phase 2 trial ITI-007-005 was completed in November 2013 with 335 patients randomized. The first Phase 3 trial ITI-007-301 was completed in July 2015 with 450 patients randomized. The second Phase 3 ITI-007-302 trial is ongoing. In all studies the primary efficacy endpoint is change from baseline in the total PANSS score versus placebo at end of treatment.

Results: In Phase 2, ITI-007 60 mg significantly improved schizophrenia symptoms on the primary endpoint (least squares [LS] mean change -13.2 points versus -7.4 points; P = 0.017, MMRM, ES = 0.4). ITI-007 120 mg did not significantly separate from placebo on the total PANSS at Day 28 (LS mean change -8.3 versus -7.4; P = 0.708). Risperidone (4 mg) differed from placebo on the total PANSS demonstrating assay sensitivity (least squares [LS] mean change -13.4 points versus -7.4 points; P = 0.013, MMRM, ES = 0.4). ITI-007 was safe and well-tolerated, comparable to placebo on safety measures in this trial. Secondary analyses indicated improved negative symptoms and symptoms of depression, particularly in pre-specified subgroups with prominent negative symptoms and depression at baseline. Data analysis for the first Phase 3 trial ITI-007-301 are ongoing and will be presented.

Conclusions: ITI-007 represents a new approach for the treatment of schizophrenia with unique pharmacology as well as a differentiating clinical profile. Data from the ongoing late-stage schizophrenia program for ITI-007 continue to further characterize ITI-007's novel mechanism of action as well as the potential clinical benefits, in terms of efficacy and safety for patients.

Keywords: Schizophrenia, Dopamine, Antipsychotics, Clinical trial, 5-HT2A receptor, D2 receptor, SERT

Disclosures: K.E. Vanover, C. O'Gorman, J. Saillard, M. Weingart and S. Mates are all full-time employees of Intra-Cellular Therapies Inc. R. Davis is a paid consultant to Intra-Cellular Therapies Inc.

W167. Dimensional Traits of Psychosis Associated with Nmda Receptor GRIN2B Polymorphism

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Background: Dimensional schizotypy is a construct believed to capture the underlying genetic vulnerability to schizophrenia. However, few studies have investigated the genetic underpinnings of dimensional schizotypy and the closely related categorical diagnosis of schizotypal personality disorder. This is an exploratory candidate gene study examining the relationship between single nucleotide polymorphisms (SNPs) and multiple schizotypy dimensions in a sample enriched for dimensional schizotypy and schizotypal personality disorder.

Methods: 472 cases and controls were screened using the Schizotypal Personality Questionnaire (SPQ). 1347 SNPs were analyzed using a custom Illumina SNP array chip. Ancestry markers were used to include subjects with Caucasian heritage. Principal component analysis was used to cluster SPQ variables. Linear regression was performed using PLINK, for association between schizotypy dimensions and candidate SNPs. Logistic regression was used to analyze the association of SNPs between schizotypal personality disorder cases versus controls.

Results: A significant relationship was found between rs4763361 SNP that is positioned within the NMDA receptor (GRIN2B), for dimensional schizotypy traits related to disorganized symptoms (p = 1.358e-05), which remained significant after Bonferroni Correction. There were no significant associations between any of the SNPs and the categorical diagnosis of schizotypal personality disorder.

Conclusions: This study indicates that polymorphisms related to NMDA pathways may have an impact on dimensional traits of psychosis, particularly related to disorganized symptomatology.

Keywords: schizophrenia genetics, SNP, NMDA receptors, schizotypy

Disclosures: Nothing to disclose.

W168. Antipsychotic Pharmacogenomics: Neuronal Development, Neurotransmission, and Glutamate Gene Associations with Treatment Response

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Background: Response and tolerability to antipsychotic treatments in psychotic disorders vary across individuals. Genetic factors may predispose some individuals to not tolerate or respond to pharmacotherapy. Patients early in their course of disease with little prior treatment exposure may be useful in identifying pharmacogenetic relationships.

Methods: We examined 86 first episode patients (schizophrenia, bipolar disorder with psychotic features and, major depressive disorder with psychotic features) who had little to no prior antipsychotic exposure in a 6 week pharmacogenomic study of antipsychotic treatment response. Risperidone was the preferred antipsychotic (n = 70) with others chosen as secondary options when clinically indicated. Whole genome genotyping was used in a series of analyses to conduct an exploratory genome-wide association study with symptom response using the BPRS change score from baseline as a primary outcome measure. Ingenuity Pathway Analysis was used to profile networks and biological systems associated with response.

Results: Two SNPs in the human glutamate receptor delta (GRID2) gene (rs9307122 and rs1875705) reached genome-wide significance (-log10p = 7.96). Associations exceeding –log10p = 3.0 represented 229 genetic loci associated within genes or non-coding RNAs. The top physiological system represented was nervous system development and function related to 87 of these genes. Within this category n = 42 genes were associated with neuronal development (p = 2.11E-10), n = 28 genes related to neurotransmission (p = 1.0E-09), and n = 23 genes related to synaptic transmission (p = 2.15E-08) represented the most significantly over-represented networks observed. GRID2 was identified as a member of each of these categories.

Conclusions: These findings represent a rare pharmacogenomic study of first episode psychosis patients. The findings implicate networks of genes related to neuronal development and signaling with symptom response to

antipsychotic drugs, with the most significant SNPs identified in a gene related to glutamate signaling.

Keywords: pharmacogenomics, antipsychotic, first-episode psychosis, treatment response

W169. Open Translational Science in Schizophrenia

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Background: The OPTICS Project is a pilot initiative designed to provide a forum for translational science in schizophrenia. This is the first time data about the disorder with associated genomic information and data from therapeutic clinical trials is being made publicly available to interested investigators in one place. The project is based on data from Janssen clinical trials and NIH-funded studies about schizophrenia. Data are made available through the National Institutes of Health Database of Genotypes and Phenotypes (dbGaP), the NIMH, and the clinical trial data through the Yale Open Data Access Project (YODA).

This effort is distinct from other initiatives in that: a) it is a time-limited proof of concept for an open-science analytic collaboration based on both clinical trial and natural history data sources; it is not the development of a data resource to be used in perpetuity. b) Participant researchers have agreed to address at least 1 of the 3 project objectives (below). c) Cross-sector collaboration is encouraged in the project, leveraging analytic and therapy area strengths that exist in different disciplines – e.g. pharma/academic/bioinformatics/commercial (beyond pharma).

The Harvard Catalyst Reactor (NCATS funding) has issued a request for applications (RFA) to fund researchers to participate in the project. This program awards grants to support innovative clinical and translational research projects that have a high potential to impact human health.

Aims: The aim of this project is to conduct a pilot to demonstrate the value of an open-science approach using pharmaceutical clinical trial and federally-funded data about schizophrenia and associated genomic information to: advance efficacy and safety of medicines for schizophrenia; increase understanding of schizophrenia, including disease natural history, subtypes, and etiologies; and contribute to the development of design and analytic methods for disparate data types, including novel statistical methods and research designs.

Methods: Advisory Board: Members of the project's scientific advisory board include researchers from Yale University School of Medicine, Rutgers University, Harvard T.H. Chan School of Public Health, the National Institute of Mental Health (Genomics Branch), and Janssen. The role of the board is to manage the project and adjudicate the scientific merit of initial proposals and long abstracts submitted at the conclusion of the analysis period.

Process: An open invitation has been issued to researchers worldwide to collaborate in the analyses. All researchers must: 1) meet the data access and use requirements of the data holders; 2) agree that Intellectual property generated from this project will be dedicated to the public and free for everyone to use; and 3) agree that all publications related to this project will first be published in the OPTICS volume.

Data: Two collections of data will be used: Clinical trial data: A collection of 17 paliperidone clinical trials is available to qualified investigators through the Yale Open Data Access Project (a.k.a. YODA, link below) as the "OPTICS Bundle". The primary therapies in the trials were the atypical antipsychotic medications, paliperidone extended release and paliperidone palmitate. Comparators included placebo, risperidone, quetiapine, olanzapine, lithium and valproate. Study participants included patients diagnosed with schizophrenia, schizoaffective disorder, and bipolar disorder. The study designs are varied and include patients with acute episodes, randomized treatment, double-blinded treatment, and placebo-controlled trials (and combinations thereof). Also included among the available trial data are relapse prevention, active comparator, and open research designs. While these data will be made available to investigators, all analyses must be done on the SAS Clinical Trials Data Transparency Safe Harbor environment.

NIH-Funded Genomic Studies: A collection of NIH-funded genomic studies is available through the dbGaP. These studies include: "Genome-wide association study of schizophrenia;" "Molecular genetics of schizophrenia – nonGAIN sample (MGS_nonGAIN)," "Genetics of schizophrenia in an Ashkenazi Jewish case-control cohort," "Sweden-schizophrenia population-based case-control exome sequencing," "Whole genome profiling to detect schizophrenia methylation markers," "Genetics of neuropsychiatric and neurodevelopmental disorders," and "Joint genome-wide gene expression and GWAS mapping in the MGS dataset."

Results: At the conclusion of the analysis period (Q4 2016), researchers will meet to discuss results and prepare the publication. Participants who have completed analyses will be invited to attend a meeting at which results are presented and discussed. Groups with similar strategies or research topics will be encouraged to integrate efforts in combined or companion manuscript(s). All results passing peer review will be published in an open-access online journal. Finally, the pilot will be evaluated with the goal of replicating it for other neuropsychiatric disorders.

Conclusions: This is the first time data about the disorder and clinical treatment trials are being made available to researchers in one place. The ability to analyze these datasets together enables researchers to address questions about the disease, therapies, and analytic methods in ways not possible before now.

Keywords: schizophrenia, clinical trials, natural history genomic studies, open science, innovative methods

W170. Efficacy of Dopamine D2 Receptor β-Arrestin-Biased Ligands on Schizophrenia-Like Behaviors in Mutant Mice

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Background: Recent experiments have shown that ligand binding can induce conformational changes in G protein-coupled receptors (GPCRs) that lead to differential signaling events. Ligand binding to GPCRs can activate, inhibit, or exert no effects on the G protein-dependent signaling pathway while exerting similar or diverse actions on a G protein-independent pathway through β-arrestin (βArr). This property of functional selectivity has been established for a few GPCRs; however, none of the current ligands or drugs were developed with this property in mind. All current FDA-approved antipsychotic drugs bind to the dopamine D2 receptor (D2R). We have recently evaluated several βArr-biased compounds in the hypoglutamatergic NR1 knockdown model of schizophrenia-like behavior and reported that UNC9975 can reduce their hyperlocomotion in the open field, restore prepulse inhibition (PPI), improve novel object recognition memory, partially normalize social behavior, decrease conditioned avoidance responding, and elicit a much lower level of catalepsy than haloperidol (Park et al, Neuropsychopharmacology, 2015). The purpose of the present investigation was to determine whether the more potent UNC9975 and the more selective UNC9994 would show efficacy in the hyperdopaminergic dopamine transporter knockout (DAT-KO) mice.
Methods: Mice were examined in behavioral assays for positive, negative, and cognitive schizophrenia-like responses with the βArr-biased compounds. These tests with DAT mice included suppression of hyperlocomotion in the open field, restoration of PPI, normalization of sociability, and facilitation of novel object recognition memory. Antidepressant-like actions of UNC9975 were assessed in the vesicular monoamine transporter 2 (VMAT2) heterozygotes, a model for depressive-like behaviors.
Results: In the open field, both UNC9975 and UNC9994 decreased hyperlocomotion in the DAT-KO mice, with UNC9975 being the more potent. Remarkedly, neither compound fully rescued PPI in DAT-KO mice at doses of 0.5-4 mg/kg UNC9975 or 5-20 mg/kg UNC9994. In the sociability test, 0.5 mg/kg UNC9975 and 2 mg/kg UNC9994 enhanced social affiliation in these mutants. In the novel object recognition memory test 0.5 and 1 mg/kg UNC9994 augmented the preference for the novel object relative to the vehicle control in DAT-KO mice. In the tail suspension task, 2 mg/kg UNC9975 reduced immobility in the VMAT2 heterozygotes relative to their vehicle controls.
Conclusions: In the presistently hyperdopaminergic DAT-KO mice, both of the βArr-biased compounds – UNC9975 and UNC9994 – showed efficacy in reducing their hyperactivity in the open field, enhancing sociability, and normalizing long-term novel object recognition memory. Additionally, UNC9975 exerted antidepressive-like actions in VMAT2 heterozygotes. By contrast, PPI was not restored in DAT-KO mice with the doses of UNC9975 and UNC9994 that were tested. It should be emphasized that past experiments with wild-type (WT) and βArr2-KO mice have demonstrated the specificity of these βArr-biased compounds (Allen et al, PNAS 2011). Collectively, these findings indicate that βArr-biased ligands for the D2R may represent a novel approach for developing drugs to treat schizophrenia and other related disorders in humans.
Keywords: Schizophrenia-like behavior, dopamine 2 receptor, functional selectivity, Mouse models
Disclosures: Nothing to disclose.

W171. Dopamine-Dependent Working-Memory Performance is Mediated by Dynamic Connectivity Between Brain Networks

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Background: Research in the last decade has uncovered an intrinsic organization of the brain into functional networks that operate partly in parallel during rest and are thought to interact during complex cognitive processes such as working memory, an interaction that may afford maintenance and manipulation of perceptual information towards goal-directed actions. Understanding how brain networks interact during cognition to facilitate flexible adaptations to changing demands could provide new insights into the basis of interindividual differences in working-memory performance and clarify the perplexing nature of the deficit in working memory in schizophrenia. Dopamine is critical for working memory processing in the cortex and elsewhere although it is not clear how dopamine influences internetwork connectivity and whether such an action could mediate its effects on working memory performance.
Methods: We addressed these questions in healthy volunteers (HV) with fMRI (using an n-back working-memory task) and PET imaging using the radiotracer [11C]FLB457 before and after amphetamine administration to measure the change in binding potential (ΔBPND) of the radiotracer as an index of dopamine release capacity in cortical and subcortical extrastriatal regions including thalamus, midbrain, and hippocampus. MRI measures were available for 39 HV, 15 of whom also had PET measures and for 15 unmedicated individuals with schizophrenia. Brain networks were defined by group spatial independent component analysis using GIFT software. Networks with working-memory-load-dependent activity were selected for analysis of functional connectivity including left and right fronto-parietal networks (lFPN, rFPN), cingulo-opercular network (CON) and anterior default mode network (aDMN). Load-dependent connectivity between pairs of networks was determined via a modified psychophysiological interaction analysis.
Results: For most pairs of networks, connectivity significantly changed with working-memory load, particularly

amongst subjects with good task performance (5 of 6 pairs showed significant load-dependent connectivity, one-sample t-test, p-value range: 0.00003-0.022). Working-memory performance (adjusted hit rate in the higher load condition) was predicted by load-dependent connectivity between lFPN and rFPN (Δ connectivity lFPN-rFPN; R2 = 0.30, standardized β = 0.55, p = 0.0003) in a step-backwards regression including all network pairs; no other network pairs predicted performance independently of this pair. Additional step-backwards regressions found that working memory performance was not predicted by the more conventional fMRI measures of load-independent internetwork connectivity or load-dependent activation of individual networks. Dopamine release capacity in subcortical, extrastriatal regions (but not in cortical regions) was related to working memory performance (standardized β = 0.55, p = 0.033) and also to Δ connectivity lFPN-rFPN (standardized β = 0.58, p = 0.025). Moreover, Δ connectivity lFPN-rFPN fully mediated the relationship between subcortical dopamine release capacity and working-memory performance (Zab = 2.61, p = 0.009, bootstrap test for mediation). Compared to matched controls, unmedicated individuals with schizophrenia did not significantly differ in load-dependent connectivity but showed a significantly weaker relationship between Δ connectivity lFPN-rFPN and working-memory performance (group x Δ connectivity interaction, standardized β = 0.45, p = 0.025). Furthermore, patients showed a significant correlation between Δ connectivity lFPN-rFPN and positive symptoms measured with the SAPS (standardized β = -0.66, p = 0.020).

Conclusions: Our findings indicate that interactions between brain networks dynamically adapt to fluctuating environmental demands and that these dynamic adaptations underlie successful working memory performance. Furthermore, we provide evidence that this adaptive communication between brain networks could also be a mechanism through which dopamine signaling influences working memory performance. Such a mechanism would be consistent with dopamine's impact on synaptic efficacy, which could modulate the influence of projections extending across networks thereby altering internetwork communication in response to cognitive demand. Although cortical dopamine (which did not relate to our measures of interest) is most commonly thought to modulate working memory, our findings are consistent with previous evidence that dopamine release in the thalamus may facilitate the maintenance of working memory representations via thalamocortical loops while dopamine release in the midbrain may affect working memory by regulating the dopamine system more generally. Finally, the brains of patients with schizophrenia may not be properly exploiting these dynamic network interactions in the service of adaptive behavior and that psychotic symptomatology may relate to these network dynamics. In summary, load-dependent internetwork connectivity likely underlies critical aspects of cognitive processing related to dopamine and schizophrenia.

Keywords: working memory fMRI, Dopamine, schizophrenia, Cognition

Disclosures: Nothing to disclose.

W172. Safety of Lurasidone in Older Adults with Schizophrenia: A Pooled Analysis of Short-Term Placebo-Controlled Studies

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Background: Older patients with schizophrenia may be more susceptible than younger patients to adverse events associated with antipsychotic treatment (eg, extrapyramidal symptoms [EPS], sedation, and metabolic syndrome) (Jeste DV & Maglione JE. Schizophr Bull. 2013;39[5]:966-968). Lurasidone is an atypical antipsychotic agent that has demonstrated efficacy in the treatment of adult patients with schizophrenia. This pooled, post hoc analysis evaluated the safety profile of lurasidone in older adults with schizophrenia.

Methods: Individual patient data were pooled from 7 similarly designed, multiregional, randomized, double-blind, placebo-controlled, 6-week studies of fixed-dose, once-daily, oral lurasidone (20, 40, 80, 120, or 160 mg/d) conducted in adult patients (age 18-75 years) with acute schizophrenia. This analysis examined safety assessments, including treatment-emergent adverse events, laboratory measures, and vital signs in older patients (≥55 years old) and younger patients (<55 years old). Treatment group differences were summarized using descriptive statistics; changes from baseline to Week 6 were calculated using a last observation carried forward (LOCF) approach.

Results: This analysis included 243 patients who were ≥55 years old (lurasidone, n = 168; placebo, n = 75) and 1973 patients <55 years old (lurasidone, n = 1340; placebo, n = 633). Mean (SD) age was 60.4 (4.9) years and 59.7 (4.3) years in older patients receiving lurasidone or placebo, respectively, and 37.6 (9.4) years in younger patients (both treatment groups). The majority of patients were male in both the older (60%) and younger (72%) patient groups. The most common adverse events (in ≥5% of lurasidone-treated patients and at least twice the rate observed with patients receiving placebo) for lurasidone versus placebo were akathisia (10.1% vs 5.3%; number needed to harm [NNH] = 21) in older patients; and akathisia (13.3% vs 2.7%; NNH = 10), nausea (10.7% vs 5.5%; NNH = 20), somnolence (9.1% vs 3.8%; NNH = 19), and sedation (8.5% vs 3.6%; NNH = 21) in younger patients. In older patients, mean change in weight from baseline to Week 6 endpoint for lurasidone and placebo groups was -0.1 kg and -0.5 kg, respectively; median change from baseline was 0.0 mg/dL and -1.5 mg/dL, respectively, for total cholesterol; -6.0 mg/dL and -2.5 mg/dL, respectively, for triglycerides; and 3.0 mg/dL and -2.5 mg/dL, respectively, for glucose; and mean change from baseline was 1.0 mm Hg and 1.3 mm Hg, respectively, for systolic blood pressure (SBP) and 0.2 mm Hg and 0.1 mm Hg, respectively, for diastolic blood pressure (DBP). In younger patients, mean change in weight from baseline to Week 6 endpoint for lurasidone and placebo groups was 0.5 kg and 0.0 kg, respectively; median change was -5.5 mg/dL and -7.0 mg/dL, respectively, for total cholesterol; -4.0 mg/dL and -7.0 mg/dL, respectively, for triglycerides; and 0.0 mg/dL and 1.0 mg/dL,

respectively, for glucose; and mean change from baseline was 0.6 mm Hg and 1.2 mm Hg, respectively, for SBP and 1.0 mm Hg and 0.8 mm Hg, respectively, for DBP.

Conclusions: The safety profile of lurasidone was similar in older (≥55 years) and younger adult patients with schizophrenia. The most common adverse event observed with lurasidone in patients ≥55 years old was akathisia. In both older and younger patients, lurasidone was associated with minimal changes in weight, lipids, glucose, and blood pressure.

Keywords: schizophrenia, lurasidone, atypical antipsychotic, older patients, safety

W173. Mechanisms Governing Muscarinic LTD in the Prefrontal Cortex Implicated in Negative and Cognitive Symptoms of Schizophrenia: Role of mGlu5 and GABAa Receptors

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Background: Current treatments of schizophrenia have been shown to reduce only the positive symptoms of the disease. However, the negative and cognitive symptoms remain untreated and therefore represent an unmet clinical need. Activation of muscarinic acetylcholine receptors (mAchR), especially the M1 and M4 subtypes, have been shown to have efficacy in reducing symptoms of schizophrenia, including the negative and cognitive symptoms. However, due to the profound adverse effects and the absence of subtype selective mAchR agonists, these agents have failed to advance in clinical development. Recently, we and others identified positive allosteric modulators (PAMs) of M1 that provide high subtype selectivity and may provide a novel approach for treatment of schizophrenia. We demonstrated that M1 PAMs could reverse deficits in an M1-dependent form of long term depression (mLTD) and associated behavioral deficits in the cognitive and negative symptom domains, observed in a mouse model. In this study, we further elucidate the mechanism governing mLTD and unravel a unique role of metabotropic glutamate receptor 5 (mGlu5) and GABAA receptors in this form of plasticity. Additionally, we show that similar to the M1 PAMs, mGlu5 PAMs can also serve as promising candidates for the negative and cognitive symptoms of schizophrenia.

Methods: All animal studies were approved by the Vanderbilt University Medical Center Institutional Animal Care and Use Committee and were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory animals. 6 week old C57Bl/6 male mice were administered 10mg/kg phencyclidine (PCP) or vehicle (saline) subcutaneously, once daily for 7 consecutive days. After a 7-day washout period, mice underwent electrophysiology studies. Extracellular and whole cell electrophysiology were performed to record field excitatory postsynaptic potentials (fEPSPs) and inhibitory postsynaptic currents (IPSCs), respectively, from prefrontal cortical (PFC) slices. fEPSPs and IPSCs in layer V PFC were evoked following stimulation of layer II/III using a bipolar concentric stimulating electrode. mLTD in the layer II/III to layer V synapse of PFC was evaluated following a 5-10 min of bath application of carbachol (CCh) +/- concurrent electrical stimulation and +/- several selective orthosteric antagonists and allosteric modulators of GABAA and mGlu5 receptors respectively.

Results: Muscarinic LTD (mLTD) in PFC fEPSP responses, is an M1-dependent form of synaptic plasticity that can be induced by 50 µM CCh application for 10 min. Blockade of NMDA receptors with AP-V (50µM), failed to block mLTD induction. However, acute pretreatment with the mGlu5 negative allosteric modulator MPEP (30µM) led to a complete blockade of mLTD. Moreover, the selective mGlu5 PAM VU0409551 (10µM) significantly potentiated a threshold form of the M1 dependent mLTD induced by 10 µM CCh. Previously, we have shown that PCP-treated mice exhibited significant deficits in mLTD induction when compared to the vehicle treated or drug naïve mice. Acute incubation of the PCP-treated brain slices with the mGlu5 PAM VU0409551 completely rescued the mLTD deficits in the PCP-treated mice.

Interestingly, this study also provides evidence that mLTD is also dependent on inhibitory transmission in the PFC. Thus, in presence of the GABAA receptor antagonist bicuculline (20µM), CCh failed to induce mLTD in the PFC of drug naïve mice. Additionally, CCh application for 5 minutes led to a long term potentiation in the frequency of spontaneous IPSCs (sIPSC) recorded from the layer V pyramidal neurons. Finally, this effect on sIPSC was not observed, when CCh was applied in absence of any electrical stimulation.

Conclusions: Our results clearly indicate that glutamate, muscarinic, and GABA receptors intricately interact with each other for the induction and maintenance of mLTD in the PFC. Thus, although mLTD was independent of NMDA receptor activation, its induction was dependent on co-activation of mGlu5 and M1 receptors, while its induction and maintenance seems to be regulated by GABAA receptor subtype. This complex interplay between the different neurotransmitters in the PFC may be dysregulated following repeated PCP-treatment, which may serve as the mechanism underlying the loss of mLTD and behaviors associated with negative and cognitive symptoms in this mouse model. Our results indicate, that potentiating mGlu5 signaling is sufficient to reverse such plasticity deficits. Thus, both M1

and mGlu5 PAMs have potential as a promising new approach to restore complex network deficits in psychotic disorders including schizophrenia.

Keywords: PFC, muscarinic acetylcholine receptor, metabotropic glutamate receptor, GABAA

W174. Further Characterizing Brain Receptor Occupancy with ITI-007: Results from a Positron Emission Tomography (PET) Study in Patients with Schizophrenia

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Background: All medications approved in the USA to treat schizophrenia, to date, have to a varying extent, dopamine D2 receptor occupancy (D2RO) as a feature of their pharmacology. Nonetheless, different antipsychotics exhibit different threshold levels of striatal D2RO. Most antipsychotics are D2 receptor antagonists, both pre- and postsynaptically, and have demonstrated antipsychotic efficacy in association with about a 65 – 80% striatal D2RO, while only slightly higher striatal D2RO ($>80\%$) has been associated with the development of extrapyramidal side effects and hyperprolactinemia. Dopamine receptor partial agonists differ in this regard. For example, aripiprazole, which is both a pre- and post- synaptic partial agonist, demonstrates higher ($>80\%$) D2RO in association with efficacy. At therapeutic doses in patients with schizophrenia, clozapine is an exception, with efficacy associated with relatively lower D2RO occupancy ($<50\%$).

ITI-007 is a first-in-class dopamine receptor phosphoprotein modulator (DPPM), acting as a presynaptic partial agonist and postsynaptic antagonist. This allows for reduced release of dopamine presynaptically with ITI-007 compared to presynaptic antagonists along with blockade of dopamine postsynaptically for more efficient reduction of dopaminergic signaling than most other antipsychotic drugs. ITI-007 also benefits from potent serotonin 5-HT2A receptor antagonism, serotonin transporter (SERT) inhibition, and increased phosphorylation of glutamatergic N-methyl-D-aspartate (NMDA) GluN2B receptors likely downstream of dopamine D1 receptor activation in mesolimbic brain regions. Together, this unique pharmacology predicts more efficient dopamine modulation with antipsychotic efficacy at relatively low levels of D2RO.

Positron emission tomography (PET) data in healthy volunteers (Clinical trial ITI-007-003) was previously presented. The results from this PET study indicated ITI-007 (10–40 mg) was safe and well tolerated and rapidly entered the brain with long-lasting and dose-related occupancy. ITI-007 (10 mg) demonstrated high occupancy ($>80\%$) of cortical 5-HT2A receptors and low occupancy of striatal D2 receptors ($\sim12\%$). D2RO increased with dose and significantly correlated with plasma concentration. ITI-007 (40 mg) resulted in peak occupancy up to 39% of striatal D2 receptors and 33% of striatal serotonin transporters. ITI-007 (60 mg) was projected to have a 50% striatal D2 receptor occupancy.

The primary objective of the present study (ITI-007-008) was to determine the striatal D2RO of ITI-007 in patients with schizophrenia at a dose of 60 mg that has previously demonstrated antipsychotic efficacy.

Methods: Patients with stable schizophrenia volunteered and were washed off their antipsychotic medications for participation in this inpatient open-label study. After a drug-free period of at least two weeks, patients received a baseline scan followed by administration of 60 mg ITI-007 once daily for two weeks and subsequent post-treatment scan(s). Carbon-11-Raclopride was used as the radiopharmaceutical for imaging striatal D2 receptors. Brain regions of interest were outlined using magnetic resonance tomography (MRT) with cerebellum as the reference region. Binding potentials were estimated using a simplified reference tissue model. D2RO was expressed as percent change in the binding potentials before and after ITI-007 administration.

Results: Data from this present study, including the mean peak striatal D2RO levels observed with the dose of 60 mg ITI-007, will be presented. ITI-007 was safe and well tolerated in this study.

Conclusions: This PET study in patients with stable schizophrenia further adds to the information gleaned regarding brain receptor occupancy levels from a prior PET study in healthy volunteers. ITI-007 demonstrates relatively low striatal D2RO at the antipsychotic efficacious dose of 60 mg. In this regard, ITI-007 is more clozapine-like with efficacy at relatively low D2RO. Yet, ITI-007 demonstrates a more favorable safety profile. Consistent with low striatal D2RO, ITI-007 has a lesser liability for D2 mediated side effects, such as extrapyramidal side effects, including akathisia, and hyperprolactinemia. Together, ITI-007 represents an exciting new potential treatment for schizophrenia.

Keywords: Antipsychotic, schizophrenia, Dopamine, PET, ITI-007

Disclosures: K. Vanover, C. O'Gorman, J. Saillard, M. Weingart, and S. Mates are full time employees of Intra-Cellular Therapies; R. Davis is a paid consultant to Intra-Cellular Therapies.

W175. Association of Serotonin2c Receptor Polymorphisms with Antipsychotic Drug Response in Schizophrenia

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Background: Serotonin (5-HT)2C receptors (HTR2C) are widely expressed in the brain and have important region specific regulatory effects on GABAergic, dopaminergic, and cholinergic neurotransmission. 5-HT2C agonists suppress DA efflux in limbic, dorsal striatal, and cortical brain regions. Some typical and atypical antipsychotic drugs (APDs) are HTR2C inverse agonists, i.e. they block constitutive activity. Diminished serotonergic activity has been shown to enhance constitutive activity via editing of the HTR2C mRNA to produce isoforms encoding receptors with higher sensitivity to 5-HT. The HTR2C agonist, vabicaserin, has been reported to be a effective treatment of acute exacerbations of schizophrenia (SCZ). We recently reported confirmatory evidence that the HTR2C/2A agonist, MK-212, produced a blunted oral temperature response in unmedicated male schizophrenic patients, suggesting diminished 5-HT2C receptor stimulation. Further, some self-rated behavioral responses to MK-212, e.g. feelings of increased anxiety, depression, calmness, and good overall feeling, were also significantly decreased in the SCZ patients compared to normal controls. There is conflicting evidence for the association between common genetic polymorphisms in the HTR2C and response to atypical antipsychotic drug (APD) treatment in SCZ patients. The purpose of this study was to further examine HTR2C SNPs as predictors of APD response.

Methods: We tested the association between the HTR2C polymorphisms, Cys23Ser, -759C/T, and -697G/C, in 171 schizophrenic patients treated with clozapine, 78%, melperone, 7.0%, risperidone, 3.8%, olanzapine, 2.1%, or haloperidol (9.0%). To determine whether HTR2C SNPs are related to APD response, we conducted an analysis of covariance (ANCOVA) with covariates of baseline, race and gender and a categorical treatment response at 6 months, using a reduction of $\geq 20\%$ in the Brief Psychiatric Rating Scale (BPRS) total and positive and negative subscale scores to identify responders.

Results: Ser23 carriers showed significantly greater proportion of responders in male patients (positive symptoms, $X2 = 7.540$, $p = 0.01$; negative symptoms, $X2 = 4.796$, $p = 0.03$). A -759C-Ser23 haplotype was also associated with improvement in positive ($X2 = 6.648$, $p = 0.01$) and negative ($X2 = 6.702$, $p = 0.01$) symptoms. Logistic regression, after controlling for relevant covariates, also showed significant haplotypic associations. ANCOVA test on absolute change or % change in BPRS in symptom improvement after controlling for race, drugs, and the corresponding baseline psychopathology, also indicated

that male Ser carriers had a significantly greater improvement in positive and negative symptoms ($p = 0.025$ and 0.019, respectively) after 6 months treatment. Although we confirmed that the two promoter SNPs, -759C/T and -697G/C, are the cis-eQTL/methylation-eQTL for HTR2C in males by Braincloud database, neither -759C/T nor -697G/C alone were significantly associated with symptom improvement. Female Ser carriers also showed an association with better improvement in negative symptom at 6 months (F = 4.793; p = 0.035). A meta-analysis of six studies for Ser23 and treatment response to atypical APDs, mainly clozapine showed an overall odds ratio of 2.00 (95%CI, 1.38-2.91, p = 0.0003) or 1.94 (95%CI, 1.27-2.99, p = 0.0024) under fixed or random effect models. As Ser carriers increased the odds of having treatment response by 2.0, population risk (Kp) = 0.30, dominant mode of inheritance, and 216 responders/738 non responders were genotyped, the power (chance) to detect an association with significance $p < 0.01$ was over 90%.

Conclusions: These new data and the meta-analysis provide additional evidence that HTR2C polymorphisms, and Ser23 in particular, are associated with better response to APD treatment, particularly clozapine, in schizophrenic patients. The interpretation of the effects of Ser23 SNP and the other SNPS examined here on the activity of dopamine, GABA and serotonin neurons in various brain regions relative to APD response is difficult because of extensive editing of the HTR2C in region specific manners and the effect of editing on constitutive activity which is higher in the Ser23 carriers. Variations in the release of 5-HT in a regional manner will determine the net effect of HTR2C stimulation. The evidence for diminished HTR2C responsiveness in male schizophrenia patients, and that HTR2C agonists may be effective in the treatment of patients with schizophrenia must be reconciled with the ability of clozapine to block HTR2C stimulation. Clozapine response is often delayed until 2-6 months, during which time adaptive changes in the HTR2C may be occurring. Ser23 isoform would compensate for the ability of clozapine to diminish total HTR2C activity, whether constitutive or stimulated. It may be that HTR2C antagonism by clozapine may detract from its efficacy and that HTR2Cagonists, e.g.vabicaserin, may actually function to augment its efficacy.

Keywords: Serotonin 5-HT2C Receptor, schizophrenia genetics, treatment response, clozapine, meta-analysis

Disclosures: Nothing to disclose.

W176. Structural Imaging Patterns Linked to 12q24

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Background: Many previous studies have used source-based morphometry, an independent components analysis

optimized for structural MRI, to identify patterns of gray matter loss in individuals with schizophrenia. The loading coefficients for these patterns, particularly a key component including insula/temporal pole/medial prefrontal cortex, have also been shown to be heritable in sibling studies. We identified in a multi-family imaging and genetic dataset 1) whether this component is replicated, and 2) whether there is a quantitative trait locus for it.

Methods: Participants were individuals of Mexican American ancestry who took part in the Genetics Of Brain Structure and Function Study (GOBS) which is an extension of the San Antonio Family study. Individuals in this cohort have actively participated in genetics research for over 18 years and were randomly selected from the community with the only constraints that they are of Mexican-American ancestry, part of a large family and live within the San Antonio region. For the present analysis, subjects were excluded for MRI contraindications, documented medical history of neurological illness, or any major neurological event visible on the structural T1 scans. We used the SBM toolbox to perform source-based morphometry, on the final sample of N $=$ 887 to derive an insula-mPFC component and to investigate its genetic determinants. All quantitative genetics analyses were performed in SOLAR, which decomposes the variance of a trait into genetic and environmental components by modeling the covariance between individuals as a function of their genetic proximity. The significance of each variance component is assessed by a likelihood ratio test comparing the final model to the model without the variable of interest. Using the subject's weights on the insula-mPFC component as a trait, this method yielded an index of the overall heritability (h2) of grey matter concentration within the anatomical regions of the component. Prior to running SOLAR, eight extreme outliers (mean $+$ - 3*SD) were removed from the data and the remaining weights were transformed using an inverse normalization transformation.

Results: First, we replicated the insula-mPFC grey matter component as an independent source of grey matter variation in the general population, and verified its relevance to schizophrenia in an independent case-control sample. Secondly, the polygenic model in SOLAR using the weights of the insula-mPFC component as quantitative trait revealed that grey matter in this region is significantly heritable (h2 $=$ 0.59; p $=$ 1.78*10-15). Linkage analysis resulted in a genome-wide significant linkage peak on chromosome 12 at 12q24 (12q24.11-12q24.23; maximum LOD $=$ 3.76). The strongest association (p $=$ 7.71*10-4) was found for rs7133582, an intronic SNP in a transcription factor binding site of KSR2.

Conclusions: We showed that the neuroanatomical variation defined by this component is largely determined by additive genetic variation (h2 $=$ 0.59), and genome-wide linkage analysis resulted in a significant linkage peak at 12q24 (LOD $=$ 3.76). The strongest (but peak-wide insignificant) association was found for rs7133582, a SNP in a transcription factor binding site of KSR2, a functionally poorly characterized gene that is involved in the MAPK and ERK signaling pathways. This region has been of significant interest to psychiatric genetics as it contains the Darier's

disease locus and other proposed susceptibility genes (e.g. DAO, NOS1), and it has been linked to affective disorders and schizophrenia in multiple populations. Thus, in conjunction with previous clinical studies, our data imply that one or more psychiatric risk variants at 12q24 are co-inherited with reductions in mPFC and insula grey matter concentration.

Keywords: schizophrenia, multivariate, voxel-based morphometry (VBM)

Disclosures: Nothing to disclose.

W177. Is Increased Glutamate in the Associative Striatum a Reliable Biomarker in Antipsychotic-Naïve First-Episode Psychosis Patients?

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Background: Increased glutamate levels have been previously described in the associative striatum (precommissural dorsal caudate) of antipsychotic-naïve patients with first-episode psychosis (FEP). This increase has been observed by our group in two previous independent cohorts; longitudinally, we have also reported that glutamate normalized following clinically effective antipsychotic treatment with risperidone. The establishment of reliable biomarkers in psychiatry has been challenging. Therefore, the current study aimed to replicate, in a new cohort of antipsychotic-naïve FEP individuals, the finding of increased associative striatum glutamate levels in comparison to age- and sex-matched healthy controls.

Methods: Along with subset of participants (FEP n $=$ 35, controls n $=$ 35) previously reported, a new pool of subjects, consisting of 25 FEP patients and 25 age- and sex-matched controls, were included. All participants underwent a proton magnetic resonance spectroscopy scan performed in a 3T scanner using point-resolved spectroscopy (TE $=$ 35 ms) centered on the right precommissural dorsal caudate. Water-suppressed spectra were analyzed using LCModel (version 6.3-0E) and glutamate levels were corrected for the proportion of cerebrospinal fluid in the voxel.

Results: In the new cohort as well as in the whole sample, FEP patients showed higher levels of glutamate compared to the control group (t $=$ 3.7, p $<$ 0.001 and t $=$ 3.4, p $<$ 0.001, respectively).

Conclusions: The present study replicates the results of two previous cohorts of antipsychotic-naïve FEP patients, supporting that increased glutamate in the associative striatum might be a reliable biomarker in antipsychotic-naïve FEP patients. Our results still require further exploration to understand the mechanistic and clinical implications of abnormal striatal glutamate in FEP patients.

Keywords: glutamate, first-episode psychosis, dorsal caudate, associative-striatum, 1H MRS

W178. Development of a Discrete Trials Task to Assess Serotonergic Modulation on Interval Timing in Mice

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Background: The perception of time is essential for survival and is required for the precise organization of sequences of activity as well as the anticipation of behavioral outcomes and future events. One form of temporal perception is interval timing, which refers to the discrimination of durations, typically in the seconds to minutes range. A variety of reports indicate that schizophrenia is associated with timing deficits, and it has been proposed that impaired temporal processing is a core deficit of schizophrenia that contributes to cognitive dysfunction, hallucinations, and inappropriate behavior. There is also evidence that the serotonergic system, which is believed to play a role in the neuropathology of schizophrenia, regulates temporal perception and timing behavior. For example, serotonergic hallucinogens markedly alter the subjective experience of time and disrupt interval timing. Unfortunately, very little is known about the neural substrate(s) that are involved in serotonergic modulation of timing. Characterizing the mechanism through which the serotonergic system regulates timing will increase our understanding of the linkage between serotonin (5-HT) and schizophrenia, and will provide insight into the mechanism(s) of action of hallucinogenic drugs.

Methods: We have developed a discrete trials interval timing task in mice that can be used to elucidate the neural and receptor mechanisms underlying the modulation of interval timing by both endogenous 5-HT and hallucinogenic drugs. This paradigm is cross-species relevant because it is very similar to tasks used to assess timing in rats and humans. Development of the task in mice enables examination of the genetic contributions to performance, and facilitates the use of optogenetic challenges. In the discrete trials task, a lamp is illuminated for a variable duration, and then two levers are presented. Responding on lever A is reinforced if the stimulus duration is < 6.5 s, and responding on lever B is reinforced if the stimulus duration is > 6.5 s. Male C57BL/6J mice (n = 40) were trained to discriminate between short (2.5 and 5.0 s) and long (7.5 and 10 s) stimulus durations, and then challenged with a wider range of test stimuli (2.5-10.5 s). A 2-parameter logistic function was used to fit the %B responding data, yielding estimates of T50 (the point of subjective equality), slope, and the Weber fraction (WF) and difference limen (DL) (measures of timing precision). To determine whether the performance of the task is sensitive to the effects of serotonergic ligands, we also examined whether performance of the task is altered by the 5-HT2A/2C receptor agonist 2,5-dimethoxy-4-iodoamphetamine (DOI) and the selective 5-HT2A antagonist M100907. These studies were approved by the UCSD Institutional Animal Care and Use Committee and were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

Results: We found that mice can learn to reliably discriminate between the short and long duration training stimuli, responding on the correct lever > 85% of the time for the two extreme stimulus durations (2.5 and 10.5 s). Challenge studies demonstrated that the proportion of lever B responding increased with the stimulus duration and confirmed that the point of subjective equality (T50) was ~6.5 s. Administration of DOI (0.3-3 mg/kg IP) produced a flattening of the psychometric curve, especially at longer stimulus intervals (Drug x Stimulus Interval: $F(27,315) = 2.31$, $p = 0.0003$), and increased T50 from 6.4 s to 7.1 s. DOI also reduced the slope of the psychometric curve ($F(3,35) = 4.34$, $p < 0.02$) and increased the WF ($F(3,35) = 4.12$, $p < 0.02$) and the DL ($F(3,35) = 8.51$, $p = 0.0002$). Conversely, administration of M100907 (0.03-0.3 mg/kg SC) did not alter the DL, the WF or the slope of the curve, but did significantly increase T50 from 6.4 s to 7.7 s ($F(3,33) = 3.21$, $p < 0.04$). Pretreatment with 0.03 mg/kg M100907 blocked most of the effects of 3 mg/kg DOI, including alterations of %B responding (M100907 x DOI x Stimulus Interval: $F(9,279) = 4.78$, $p = 0.0001$), WF (M100907 x DOI: $F(1,31) = 10.53$, $p < 0.003$), DL (M100907 x DOI: $F(1,31) = 12.56$, $p < 0.002$), and slope (M100907 x DOI: $F(1,31) = 12.58$, $p < 0.002$).

Conclusions: These experiments demonstrate that mice can be trained to perform an interval timing task, and indicate that timing in mice is altered by the serotonergic hallucinogen DOI. We also confirmed that the ability of DOI to alter timing behavior is mediated by 5-HT2A activation. Interestingly, T50 was increased by 5-HT2A activation and 5-HT2A blockade. The latter finding indicates that the 5-HT2A receptor regulates central clock speed in a non-monotonic fashion. Development of timing tasks that are sensitive to hallucinogen effects is highly desirable because timing behavior is translatable, whereas many behavioral paradigms currently used to assess the effects of hallucinogens in rodents have no human counterpart. Our goal is to use this behavioral paradigm to investigate the regulation of interval timing by the serotonergic system, and to determine the neural site(s) involved in this effect. It is possible that the disruption of temporal perception induced by hallucinogens could be developed as an animal model relevant to schizophrenia, potentially facilitating the development of novel agents with antipsychotic activity.

Keywords: hallucinogen, timing, serotonin
Disclosures: Mark Geyer has performed consulting work for Acadia, Abbott, Addex, Cerca Insights, Merck, Omeros, Sepracor, Takeda, and Teva, and has an equity interest in San Diego Instruments, Inc.

W179. Altered Insula Between-Network Connectivity in Schizophrenia

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Background: Involvement of the insular cortex is a common finding in neuroanatomical studies of schizophrenia, yet its contribution to disease pathology remains largely unknown. Given the region's apparent prominence in the interaction of multiple large-scale brain networks, the investigation of insula function in this context may yield insights into disease neurobiology. As such, this study examined disease-related differences in between-network connectivity, or BNC, a novel, data-driven measure of communication between intrinsic, large-scale networks, and explored possible relationships of between-network connectivity and schizophrenia symptoms.
Methods: Twenty-seven outpatients with schizophrenia and twenty-seven healthy comparison subjects underwent functional magnetic resonance imaging at 3T in the resting state for ten minutes. A between-network connectivity analysis was performed to identify hubs with high between-network interaction, i.e., areas with high levels of connectivity to large-scale networks. Networks were identified using independent components analysis (ICA). Following ICA back-reconstruction, hubs were identified by correlating all ICA component time series' and the time series for each individual voxel. Specifically, between-network connectivity was calculated as the difference between summed squared correlations and the multiple correlation coefficient for each voxel. Group differences in insula between-network connectivity were determined using t-tests in SPM8. Pearson's correlations were used to examine relationships between insula connectivity to large-scale networks and illness symptoms.
Results: Across all subjects, high between-network connectivity was observed in both the anterior and posterior insula. Relative to healthy comparison subjects, schizophrenia patients showed reduced between-network connectivity in the posterior insula. Positive symptoms, as measured by the BPRS total score, were predicted by posterior insula connectivity to the default network.
Conclusions: Reduced between-network connectivity in the posterior, but not anterior insula in patients is consistent with previous suggestions that a relative response imbalance between these two insula sub-regions may contribute to positive symptoms in schizophrenia. This finding informs our understanding of possible disease mechanisms and merits study as a potential biomarker for therapeutic development.
Keywords: schizophrenia, Insula, functional connectivity
Disclosures: Nothing to disclose.

W180. Deficits in Working Memory Performance in Schizophrenia Are Associated with the Absence of an Inverted-U Relationship between Dorsolateral Prefrontal Cortex Activation and Working Memory Load

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Background: Patients with schizophrenia exhibit strong deficits on working memory (WM) tasks, but after over two decades of research the neurophysiological underpinnings of these deficits remain unknown. In spite of early findings supporting the concept of 'hypofrontality', alterations in the activation of dorsolateral prefrontal cortex (DLPFC), or other prefrontal regions, have not been reliably observed during WM task performance. However, recent work in healthy individuals has demonstrated an inverted-U relationship between DLPFC activation and WM load during a task with eight WM loads, the self-ordered WM task (SOT), suggesting that simple comparisons of the magnitude of activation between patients and healthy individuals will fail to capture a critical feature of how the brain instantiates WM. The present study employed the SOT in unmedicated and medicated patients with schizophrenia in order to examine whether an alteration in this pattern of activation is related to WM deficits in these patients.
Methods: Participants included 21 unmedicated patients with schizophrenia, 30 medicated patients with schizophrenia, and 45 healthy control participants. All three groups were matched on age, gender, and parental socio-economic status. Participants performed the SOT during functional Magnetic Resonance Imaging on a Philips 1.5 Tesla Intera scanner at the Columbia MRI Center at Columbia University Medical Center (TR = 2 s, whole-brain coverage with 3 mm isotropic voxels). In each trial of the SOT participants are presented with eight line drawings of 3D objects in an array. On each step of the trial the object positions are pseudo-randomly rearranged, and participants must select any object that they have not previously selected, thereby producing a gradual increase in WM load over the eight steps of each trial. Data were analyzed in a series of robust regression (or robust t-test) models using alphasim to correct for multiple comparisons ($P < 0.05$ in all cases); first restricted to a bilateral DLPFC region-of-interest (ROI), then in whole-brain analyses. Two outcome measures were evaluated in these models: 1) overall activation in response to the SOT (average activation across all eight WM loads), in keeping with standard analyses in the literature, and 2) the fit at each voxel in each subject (i.e. a first-level analysis) to an inverted-U shape identified in an independent sample of healthy individuals (Study 1 from Van Snellenberg et al., 2015). Performance on the SOT was analyzed using a maximum likelihood estimate of WM capacity described elsewhere (Van Snellenberg et al., 2014).
Results: Both patient groups showed a significant reduction in WM capacity relative to controls (all $P < 0.0005$). Within the DLPFC ROI, unmedicated patients showed greater

activation of right DLPFC relative to controls, but this was not related to WM capacity. Medicated patients showed a poorer inverted-U fit in left DLPFC compared to controls, a finding that was observed in an overlapping but slightly posterior region of left DLPFC in unmedicated patients in the whole-brain analysis (it did not appear in the ROI analysis because many of the cluster's voxels were posterior to the ROI). A follow-up analysis combing both patient groups showed a significant reduction in the inverted-U fit in this region in patients as compared to healthy controls. Morever, both healthy controls and patients with schizophrenia exhibited a positive relationship between inverted-U fit in left DLPFC and WM capacity. Thus, at least some of the deficit in WM capacity in patients with schizophrenia can be explained by the lack of an inverted-U response to WM load in this region. Moreover, whole-brain analyses demonstrated that both patient groups showed excess activation of two default-mode network (DMN) regions, the medial prefrontal cortex (mPFC) and posterior cingulate. Moreover, all three groups exhibited a negative relationship between activation of mPFC and WM capacity. Given that this region was suppressed by the task in all three groups, inadequate suppression of mPFC during WM performance may also account for some of the deficit in WM performance in patients.

Conclusions: These findings demonstrate two mechanisms that are associated with WM deficits in patients with schizophrenia: 1) the absence of an inverted-U relationship between WM load and activation of the left DLPFC, and 2) a failure to fully suppress activation in the medial PFC during WM performance. Notably, overall levels of activation in left DLPFC were normal in patients, and while patients exhibited abnormally elevated activation in right DLPFC, this increase was not observed to have any impact on WM capacity across individuals. These findings point to two little-studied pathophysiological mechanisms that may play a significant role in WM impairments in patients with schizophrenia, and which do not appear to be impacted by treatment with antipsychotic medications.

Keywords: schizophrenia, working memory, functional magnetic resonance imaging

Disclosures: Nothing to disclose.

W181. Activation of D2R Elicits Structural Changes in Human Neuronal-Like Cells Transdifferentiated from Blood Circulating Monocytes

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Background: Mounting evidence indicates that schizophrenia is a developmental illness with associated abnormalities in the dopaminergic system. However, the exact role of dopamine in schizophrenia is still a matter of debate. What is known, is that dopamine can elicit changes in the neuronal structure during early stages of brain development. The inability to access neurons directly from patients with schizophrenia has prevented us from establishing whether the effects of dopamine on neuronal structure are related to the complex pathophysiology of this mental illness.

Methods: We have developed a model to transdifferentiate blood circulating monocytes into neuronal-like cells in twenty days by combining different growth factors, anti-oxidants and conditioned media. This model provides a window into the neurodevelopment of adult individuals and only requires a standard blood sample. Unlike other models such as induced pluripotent stem (iPS) cells, the genome is not altered with viral insertions which can become a confounder in an illness with a strong but still misunderstood genetic component.

Results: We have transdifferentiated monocytes into neuronal-like cells from over 40 individuals and established that transdifferentiated neuronal-like cells resemble human neurons early in development, express neuronal markers such as Nestin, Neurofilament, MAP2, NMDA receptors, TH, GAD 65/67 and present spontaneous action potentials as well as postsynaptic inhibitory and excitatory currents. During differentiation, these cells undergo similar structural stages to those present in neurons while developing from rounded neuroblasts. We have also determined that when these neuronal-like cells are exposed to either dopamine or colchicine, they respond similarly to neurons by retracting their neuronal arborizations. In addition, we have established that activation of dopamine 2 receptors (D2R) by the specific dopamine 2 agonist quinpirole at doses of 100nM elicits retraction of the neuropil of neuronal-like cells during different incubation times.

Conclusions: Monocytes can be consistently transdifferentiated into neuronal-like cells that resemble human neurons during early brain development. These cells replicate structural responses found in neurons such as retraction of neuropil in the presence of dopamine and this structural response is at least in part mediated by D2R. The study of neurostructural changes elicited by dopamine in transdifferentiated cells from patients with schizophrenia could help us better understand the complex pathophysiology of this psychotic disorder.

Keywords: dendrites, Brain development, brain structure, neurons, Dopamine

Disclosures: Nothing to disclose.

W182. Imaging Translocator Protein (TSPO) in Subjects at High Risk of Psychosis and in Schizophrenia: An [11C] PBR28 Pet Brain Imaging Study

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Background: Abnormal brain immune responses have been implicated in the pathophysiology of schizophrenia and proposed as a mechanism associated with the brain volume decreases and illness-progression seen in schizophrenia. Microglial cells are the resident immune cells of the central

nervous system. Microglia activation can be measured in vivo with positron emission tomography (PET) using radioligands specific for the 18KD translocator protein (TSPO), expressed on microglia associated with inflammation (Karlstetter et al 2014) (1). Recent PET in-vivo brain imaging studies show elevated TSPO binding in patients with schizophrenia (1,2). However it remains unclear how this relates to the onset of psychotic illness. To determine whether total grey matter TSPO is altered in individuals with ultra high-risk (UHR) symptoms for psychosis. We also seek to determine how this compares with diagnosed patients with schizophrenia.

Methods: We recruited fourteen subjects with UHR symptoms and 14 patients with schizophrenia. Two groups of age, and TSPO genotype matched control subjects were recruited from the same community. All study participants underwent a PET scan with [11C] PBR28, a TSPO ligand and a high resolution MRI scan. The main outcome measure was total grey matter [11C] PBR28 distribution volume ratios (DVRs). DVR is the ratio of the Volume of distribution (V_T) in the regions of interest to (V_T) the whole brain.

Results: Multiple analysis of variance demonstrated an elevation in total grey matter [11C] PBR28 DVRs in both UHR subjects (controls 2.03 (0.02), UHR 2.09 (0.02); $F = 10.33$; $p = 0.004$) and patients with schizophrenia (controls 2.47 (0.02), patients 2.56 (0.01); $F = 20.8$; $p < 0.001$). UHR symptoms, as measured with the Comprehensive assessment of the at risk mental state (CAARMS) were positively correlated with total grey matter [11C] PBR28 DVR ($p < 0.01$) in UHR subjects.

Conclusions: Increases in [11C] PBR28 in UHR subjects and patients with schizophrenia suggest that neuroinflammatory changes are associated with the development of psychosis.

Keywords: microglial activation, high risk psychosis, schizophrenia, Positron emission tomography, TSPO and [11C]PBR-28 PET

Disclosures: Nothing to disclose.

W183. A Critical Role for the Transcription Factor Estrogen-Related Receptor-Gamma in the Regulation of Gene Expression and Function of Parvalbumin-Positive Neurons

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Background: Peroxisome proliferator-activated receptor-gamma coactivator 1-alpha (PGC-1alpha) is a transcriptional coactivator expressed in tissues with high metabolic demand. Several reports have linked PGC-1alpha to psychiatric disorders including anxiety disorder, bipolar disorder, and schizophrenia, but its involvement in the pathophysiology of these disorders is unclear. We have found that PGC-1alpha drives a transcriptional program for synchronous neurotransmitter release, calcium buffering, and axonal integrity in parvalbumin-positive interneurons (PV-INs) of the cortex and that PGC-1alpha-dependent

genes are reduced in the cortex of patients with schizophrenia. In this study, we sought to identify the transcription factors that are required for PGC-1alpha-mediated gene regulation, with the overarching goal of understanding the mechanisms underlying PV-IN transcriptional regulation and the contribution of PV-INs to disorders involving PGC-1alpha deficiency.

Methods: Bioinformatic approaches, reporter assays, and cell culture models were utilized to identify putative transcriptional regulators of PGC-1alpha-dependent gene expression, using the PGC-1alpha-dependent gene PV as a model target gene. Members of the estrogen-related receptor (ERR) family were identified as potential candidates; the neuroanatomical distributions of ERRalpha and ERRgamma were evaluated to determine the likelihood of their in vivo interactions with PGC-1alpha. The involvement of ERRalpha and ERRgamma in gene regulation in the central nervous system was determined by using gene expression, electrophysiological, and behavioral assays after whole body (ERRalpha) or cell-selective deletion (ERRgamma) in mice.

Results: Expression patterns of ERRgamma overlap highly with the previously reported expression patterns for PGC-1alpha, with a particular concentration of ERRgamma in GABAergic neuronal populations. However, ERRgamma was also expressed in some cell types with low PGC-1alpha expression, such as medium spiny neurons of the striatum. While whole body deletion of ERRalpha had limited effects on gene expression and electrophysiological properties of PV-INs, deletion of ERRgamma from PV-positive neurons caused motor coordination deficits and tremor, reductions in PV expression, and alterations in inhibitory tone in the cortex and hippocampus.

Conclusions: These data suggest that ERRgamma is a critical regulator of gene expression in PV-positive neurons and that it may work together with PGC-1alpha to control PV-IN gene expression. Agonists and antagonists exist for ERRgamma; future experiments will determine whether pharmacological modulation of ERRgamma can influence PV-positive neuron function for the potential treatment of disorders involving PGC-1alpha deficiency.

Keywords: GABAergic interneurons, parvalbumin, transcription, Mouse models, inhibitory synaptic transmission

Disclosures: Nothing to disclose.

W184. A Randomized Comparison of Aripiprazole and Risperidone for the Acute Treatment of First-Episode Schizophrenia and Related Disorders: 3 Month Outcomes

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Background: Research findings are particularly important for medication choice for first-episode patients as indivi-

dual prior medication response to guide treatment decisions is unavailable. We describe the first large scale double-masked randomized comparison with first-episode patients of aripiprazole and risperidone, two commonly used first-episode treatment agents.

Methods: One hundred ninety-eight participants aged 15 to 40 years with schizophrenia, schizophreniform disorder, schizoaffective disorder or psychosis NOS and who had been treated in their lifetime with antipsychotics for 2 weeks or less were randomly assigned to double-masked aripiprazole (5-30 mg/day) or risperidone (1-6 mg/day) and followed for 12 weeks.

Results: Positive symptom response rates did not differ (62.8% versus 56.8%) nor did time to response. Aripiprazole treated participants had better negative symptom outcomes but experienced more akathisia. BMI change did not differ between treatments but advantages were found for aripiprazole treatment for total and LDL cholesterol, fasting glucose and prolactin levels. Post hoc analyses suggested advantages for aripiprazole on depressed mood.

Conclusions: Overall, if the potential for akathisia is a concern, low dose risperidone as used in this trial maybe a preferred choice over aripiprazole. Otherwise, aripiprazole would be the preferred choice over risperidone in most situations based upon metabolic outcome advantages and some symptom advantages within the context of similar positive symptom response between medications.

Keywords: first episode schizophrenia, Antipsychotic Treatment, randomized clinical trial

Disclosures: Dr. Robinson has been a consultant to Asubio, Shire and Otsuka, and he has received grants from Bristol Meyers Squibb, Janssen, and Otsuka. Dr. Petrides has received research support from St. Jude Medical, Astra Zeneca, Proteus, Ai-Cure, Corcept Therapeutics and Amgen.

Dr. Hassoun has been a consultant and/or advisor to or has received honoraria from Otsuka, Lundbeck, Bristol-Myers Squibb and Sunovion. Dr. Zhang has received grant support from Genomind, Inc. Dr. Lopez has received grant funding from Janssen. Dr. Kellner receives royalties from Cambridge University Press, and honoraria from UpToDate, Psychiatric Times, and North Shore-LIJ Health System. Dr. Tohen was a full time employee at Lilly (1997 to 2008). He has received honoraria from, or consulted for, Abbott, Astra-Zeneca, Bristol Myers Squibb, GlaxoSmithKline, Lilly, Johnson & Johnson, Otsuka, Merck, Sunovion, Forest, Geodon Richter Plc, Roche, Elan, Alkermes, Lundbeck, Teva, Pamlab, Wyeth and Wiley Publishing. His spouse was a full time employee at Lilly (1998-2013). Dr. Correll has been a consultant and/or advisor to or has received honoraria from AbbVie, Acadia, Actavis, Alkermes, Bristol-Myers Squibb, Eli Lilly, Genentech, Gerson Lehrman Group, IntraCellular Therapies, Janssen/J&J, Lundbeck, Medavante, Medscape, Merck, Otsuka, Pfizer, ProPhase, Reviva, Roche, Sunovion, Supernus, Takeda, and Teva. He has received grant support from Bristol-Myers Squibb, Janssen/J&J, Novo Nordisk A/S, Otsuka and Takeda. Dr. Kane has been a consultant for Alkermes, Amgen, Bristol-Myers Squibb, Eli Lilly, EnVivo Pharmaceuticals (Forum), Forest, Genentech, H. Lundbeck. Intracellular Therapies, Janssen Pharmaceutica, Johnson and Johnson, Merck, Novartis, Otsuka, Pierre Fabre, Reviva, Roche, Sunovion

and Teva. Dr. Kane has received honoraria for lectures from Bristol-Myers Squibb, Janssen, Genentech, Lundbeck and Otsuka. Dr. Kane is a Shareholder in MedAvante, Inc. and the Vanguard Research Group. Dr. Malhotra has been a consultant to Genomind, Inc and Forum Pharmaceuticals. Drs. Gallego, John, Braga, Sevy, Addington and Lencz and Ms. Naraine, Bennett and Greenberg have no conflicts of interest in relation to the subject of this study.

W185. Role of Mitochondrial Uncoupling Proteins in Behavioral and Biochemical Phenotypes of Mental Illness

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Background: Major psychiatric illnesses are profound disorders of thought and emotion, which are strongly associated with underlying impairments in synaptic plasticity and cellular resilience. Mitochondria as cellular regulators of energy, in the form of ATP, support energy demanding processes like neural transmission, and synaptogenesis, and are thus promising points of broadening interest in the energetics underlying the neurobiology of mental illness. A case report of patients with mitochondrial cytopathy documented that 54% of study participants had major depressive disorder (MDD), 17% bipolar disorder, 11% panic disorder and 60% reported a family history of psychiatric illness. Evidence from functional assays, protein expression studies and linkage analyses, points to a specific role for mitochondria in psychotic illnesses. Patients with bipolar disorder have been shown to have impaired brain energy metabolism and increased mitochondrial DNA mutations. A recent report on major depressive disorder demonstrated significantly reduced mitochondrial energy production in neurons of patients compared to controls. Research presented here responds to a need for animal models to elucidate the role of mitochondrial function in mental illness.

Methods: UCP2 KO mice were generated as described previously (Zhang, 2001) on a C57/B6 genetic background. Behavioral and cognitive performance of UCP2 KO and WT mice were assessed using the following behavioral assays: open field; elevated plus maze; Lashley maze; and the tail suspension test. Locomotion in response to NMDA receptor blockade was assessed using intraperitoneal injections of MK801 (0.20 mg/kg). The number of spine synapses from the prefrontal cortex and dentate gyrus was calculated as published previously (Diano, 2006). Additional neurophysiological measures, including local field potential (LFP) and auditory evoked potentials (AEP), were recorded from the hippocampus CA1 region and primary auditory cortex of anaesthetized mice as described previously (Kiss, 2013). In all cases, differences between measures were assessed by student t-test; $p < 0.05$ was considered significant.

Results: Behavioral, pharmacological and cellular measures establish a potential endophenotype of mental illness in UCP2 KO mice. In a test of hippocampal-based behavior—the Lashley Maze—we observed deficits in the procedural

memory and spatial cognition of UCP2 KO mice. KO mice had an attenuated response to the tail suspension test along with demonstration of increased anxiety on the elevated plus maze. We have also shown increased synaptic density in the dentate gyrus of UCP2 KO mice. This increase in synaptic density may reflect dysregulation in formation, pruning, and synaptic maintenance contingent on mitochondrial support. We know that disturbances in NMDA receptor function likely account for deficits in cognition among the mentally ill. UCP2 KO animals were significantly more vulnerable to NMDA receptor blockade as measured by increased locomotion in response to an MK801 challenge. In addition, systemic administration of NMDA receptor antagonist, MK801, elicited aberrant gamma band oscillations (data not shown) and impaired auditory gating, considered potential biomarkers of mental illness. Indeed, impaired auditory gating has been observed in schizophrenia, MDD, OCD and Tourette's syndrome.

Conclusions: These results are at the intersection of a new hypothesis regarding the salience of mitochondria and cellular energy metabolism for mental illness. Insights obtained from this line of research, linking mitochondrial function with deficits in cognition, synaptic density, glutamate function and oscillatory activity, could open a new era of drug development for novel therapeutic targets offering ongoing hope for improved treatment of schizophrenia and other mental illnesses.

Keywords: Mitochondria, NMDA Receptor, gamma oscillation

Disclosures: Nothing to disclose.

W186. Dissection of the Auditory Steady State Response by Continuous Wide-Spectrum Rhythmic Stimulation at Varying Intensity Levels

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Background: The Auditory Steady State Response (ASSR) has been largely employed in clinical studies as a direct probe of cortical oscillatory activity, with potential molecular specificity. For instance it has been suggested as a translational biomarker for schizophrenia (SZ; O'Donnell 2013), allowing probing of a critical pathophysiologic mechanism, namely disturbances in the gamma range of cortical oscillatory activity. Pharmacological studies have tackled the molecular aspects of the ASSR response in order to gain critical insights into the machinery involved in the gamma cycle – the interaction between fast-spiking, parvalbumin-positive inhibitory interneurons and excitatory cells. The NMDA dependent transmission has been highlighted as a primary mediator of the response, leading to the conceptualization of ASSR as a selective biomarker for cortical NMDA function (Sivarao 2015, Sullivan 2015). However the ASSR response is also affected by dopaminergic agents (Komek 2012), and the role of the GABA modulation can be inferred by gamma cycle models rendering negative findings from pharmacological studies difficult to reconcile (Sullivan 2015). It should be noted that commonly used unidimensional measures of the response, e.g. amplitude of the 40Hz ASSR, might fail to capture more complex dynamics and refined molecular and diagnostic specificities of the ASSR.

The EEG response to rhythmically presented auditory clicks can be taken as a probe for oscillatory deficits only to the extent that it represents an entrainment phenomenon. Alternatively, the interpretation of findings is complicated if it rather derives from the superposition of successive transient early responses (Bohorquez 2008), which also have been reported to be affected in clinical populations, including SZ. The entrainment vs. superposition hypotheses have been largely contrasted on the grounds of indirect reasoning that has failed to provide a consensus and crucially would require further measures in each subject in order to interpret ASSR estimates (Zhang 2013).

Both hypotheses predict the frequency specificity of the response; however, they critically diverge in predicting its intensity dependency (Menard 2008; Stone 2009). Here we exploit this reasoning to demonstrate in healthy controls how a novel ASSR paradigm showed a complex pattern, showing the contribution of both mechanisms and providing the basis for a better direct test for oscillatory deficits in clinical populations.

Methods: High density EEG responses to auditory clicks stimuli delivered at time-varying frequencies, "sweeping" continuously from 4-90 Hz over 20 seconds, was tested at 4 intensity levels (70 dB SPL and 3 log decrements) in 10 healthy controls. Under the assumption of quasi-stationarity, wavelet analysis of the auditory cortical response allowed derivation of a continuous estimate of the frequency-specific activity profile over the broad range of frequencies, with comparison of such profiles across the varying intensities.

Results: Sweeps evoked a preferred response at a frequency matching clicks rate, peaking in the low gamma (30-40 Hz) range and diminishing with intensity. This is in agreement with observations from more standard paradigms. However as intensity decreased we also observed that: 1) the width of the peak response decreased; and 2) an additional, leftward shifted peak emerged. The first novel finding can be interpreted as an expansion of the region of synchronization across the explored parameter space in an 'Arnold tongue' pattern, in alignment with the entrainment hypothesis. The second novel observation is predicted by the superposition hypothesis, since an optimal superposition of successive transient responses would occur at a lower presentation rate as the latency to lower intensity clicks is longer.

Conclusions: Frequency-varying click sweeps at varying intensities were able to integrate and extend previous ASSR findings from various paradigms into a unique set of observations. By deriving a continuous, broad-range representation of the cortical response we showed features in agreement with both the entrainment and superposition hypotheses. This complex pattern provides grounds for the use of ASSR as a direct probe of oscillatory activity, albeit highlighting the dual nature of the response.

Standard ASSR paradigms based on responses at predefined, sparse frequencies cannot be unambiguously interpreted and underscore the potential of our more comprehensive account of the frequency and intensity

dependencies of the ASSR. Moreover, such a protocol allows a robust, highly-resolved assessment of the preferred frequency of auditory cortical responses, providing additional information that can be exploited for more refined characterizations of pathophysiologic mechanisms, testing of novel treatments, and tracking of clinical treatment responses.

Keywords: ASSR, schizophrenia, gamma oscillation

Disclosures: Nothing to disclose.

W187. Disrupting Function of GABA Neurons with a Modified Diphtheria Toxin

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Background: Several neuropsychiatric and neurodevelopmental disorders are characterized by alterations in GABA interneuron function. For example, reduced expression of GAD67, the main isoform synthesizing GABA in brain, is one of the most replicated findings in schizophrenia post mortem brain studies; however, the functional impact of alterations in GABA neurotransmission is not well understood. Although several animal models of schizophrenia risk lead to decreased GABA function, there is very little evidence that reduction in GABA function directly affects these behavioral phenotypes. We are taking a novel approach to dissecting the functions of GABA neurons by selectively expressing modified Diphtheria toxin A (DTA) gene in specific brain regions of mice.

Methods: Diphtheria toxin inhibits protein translation by inactivating elongation factor 2 (EGF2), which in turn kills cells via apoptosis. DTA tox-176, an attenuated form of DTA, was used in these studies. We generated a YFP-DTA tox-176 fusion protein to further decrease the DTA toxicity. We then inserted the fusion gene, floxed by double lox-p sites, into an AAV vector which gets expressed in the presence of cre-recombinase and confirmed expression of the control AAV-YFP in striatum of Dlx6a-Cre mice (Cre expression in only GABAergic neurons). GAD67 and dopamine D2 receptor expression were measured in striatum via immunohistochemistry. To conduct an initial assessment of the behavioral effects of modified DTA in GABA neurons, AAV-DTA-YFP or control (AAV-YFP) were infused into either the striatum or mPFC of Dlx6a-cre mice. Mice were tested in locomotor activity, startle and prepulse inhibition of startle, and spontaneous alternation in a t-maze.

Results: Expressing AAV-DTA tox-176-YFP construct in striatum of Dlx6a-Cre mice reduced dopamine D2 receptor and GAD67 expression. Expression of the YFP-DTA tox-176 with reduced toxicities did not cause cell death, however. In preliminary studies, Dlx6a-Cre mice expressing DTA tox-176-YFP in PFC GABA neurons bilaterally show behavioral disruptions relevant to neuropsychiatric disease – reduced spatial working memory, locomotor hyperactivity, and alterations in prepulse inhibition of startle. A similar pattern of behavioral effects was observed when DTA tox-176-YFP was infused into the striatum of Dlx6a-cre mice.

Conclusions: AAV-DTA tox-176-YFP was successful in transducing cells and led to reductions in both dopamine D2 receptors and GAD67, suggesting that GABA neurons were successfully targeted. Disruption of GABA neurons in both the striatum and mPFC produced behavioral effects, suggesting functional alterations produced by the modified DTA approach. We chose this approach over complementary approaches examining short-term disruptions in GABA signaling to potentially model the reorganization and chronic nature of GABAergic dysfunction in neuropsychiatric illness. This approach also allows us to obtain cell-, region-, and temporal-specificity and will be a useful tool for the neuroscience community.

Keywords: GABAergic cells, diphtheria toxin, Prepulse Inhibition, working memory

Disclosures: Dr. Powell has received a gift from Neuropore Therapies and has a service contract with ACADIA pharmaceuticals.

W188. Differential Impact of Peripheral Inflammatory Mediators on Neuropil Synthesis and Pruning in Schizophrenia: Clinical and Neurocognitive Implications

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Background: Mounting data support the association of inflammation with schizophrenia (SZ), and with gray matter loss. Correlation of enhanced progressive cortical thickness reduction in clinical high risk subjects with peripheral inflammatory mediator levels suggest a putative mechanism through which inflammation may increase SZ risk. However, gray matter consists of glia, neurons, axons, small blood vessels, intercellular space and other components, thus lacking in specificity to which cortical elements show the most impact. Phosphorus magnetic resonance spectroscopy (31P MRS) assesses neuropil (defined as synaptically dense region of dendrites, myelinated and unmyelinated axons, and glia with few cell bodies) by measuring membrane phospholipid (MPL) precursors [phosphocholine (PC) and phosphoethanolamine (PE)] and catabolites [glycerophosphocholine (GPC), and glycerophosphoethanolamine (GPE)]. Animal studies, cellular models, postmortem and human developmental studies clearly show that increasing GPC + GPE levels reflects increased synaptic pruning and decreasing PE + PC reflect decreased neuropil formation. Besides, changes in MPL metabolites may emerge well before gray matter changes suggesting greater sensitivity of MRS measures. We examined the association of peripheral inflammatory mediators (Interleukin-6, IL-6 and C-reactive protein, CRP) with variations in MPL metabolite levels in specific brain regions among SZ and healthy controls (HC).

Methods: We acquired whole-brain, 3D multi-voxel 31P MRS data at 3T on 49 subjects (SZ = 28, HC = 21). SZ subjects were clinically stable and in their early course (mean illness duration: 1.99 ± 1.33 years from the onset of first psychotic symptom). Clinical and neurocognitive data

were obtained on all subjects. The voxels of interest (VOI) included 12 grey matter voxels (namely, the dorsolateral prefrontal cortex, ventral prefrontal cortex, orbitofrontal cortex, inferior frontal cortex, ventral and dorsal hippocampus, caudate, thalamus, anterior, middle and posterior cingulate, and superior temporal gyrus). The VOI were predefined anatomically on a template brain and were co-registered to the subject space, which included shifting the 3D voxel grid prior to the Fourier Transform. The metabolite quantification (PE, PC, GPC, GPE, PCr, ATP, dinucleotides and inorganic orthophosphate) of the extracted 31P signal was 100% automated and were expressed as mole% of the total signal. IL-6 and CRP were assayed using the highly sensitive sandwich immunoassay on the peripheral blood collected around the same time of the day for all subjects. Partial correlation tests were used to examine the relationship between IL-6 and CRP levels, and $PC + PE$ and $GPC + GPE$ levels extracted from the VOI including age, sex and diagnostic group as covariates. Antipsychotic dose was not included as a covariate since existing data show changes in high energy phosphates with long-term antipsychotic administration.

Results: SZ subjects (25.2 ± 8.0 years) and HC (25.2 ± 6.3 years; NS) did not differ in age. Sex distribution differed between the groups; there were more men in the SZ group and more females among HC ($\chi2 = 7.89$, $p = 0.009$). IL6 and CRP levels, however, did not differ between the sexes. IL-6 and CRP levels correlated with $PC + PE$ and $GPC + GPE$ levels in distinct and different brain regions despite showing no differences between the diagnostic groups. Specifically, IL-6 levels correlated positively with $GPC + GPE$ levels in bilateral thalamus (Right, $r = 0.47$, $p = 0.005$; Left, $r = 0.57$, $p = 0.0004$) and with the left dorsal hippocampus ($r = 0.44$, $p = 0.009$). CRP levels, however, correlated negatively with the caudate ($r = -0.34$, $p = 0.049$). After correcting for multiple tests (12 voxels*2 metabolites), the correlation with the left thalamus $GPC + GPE$ remained significant (corrected $p = 0.0096$). The variance contributed by IL-6 to left thalamus $GPC + GPE$ variations in both SZ and HC was similar ($R2 = 0.18$). However, $GPC + GPE$ of the left thalamus was increased by approximately 6% among SZ subjects compared to HC (ANCOVA, $F(3,68) = 4.96$, $p = 0.029$). The left thalamus $GPC + GPE$ correlated positively with negative symptoms ($r = 0.44$, $p = 0.008$) and Go-No Go response time ($r = 0.26$, $p = 0.013$) but negatively with verbal learning ($r = -0.21$, $p = 0.04$).

Conclusions: Our data show differential correlation of IL-6 and CRP with neuropil content in that the IL-6 correlated with MPL breakdown whereas CRP with MPL synthesis. Increased MPL breakdown is correlated with synaptic pruning in animal, postmortem and human developmental studies. Differential correlation of IL-6 and CRP with MPL metabolites among SZ and HC without significant group differences suggest increased susceptibility of SZ subjects for peripheral inflammatory mediators. Similar variance contributed by IL-6 for increased MPL breakdown in both groups along with increased MPL breakdown in SZ suggest that other pathophysiological processes may also affect the thalamic neuropil dynamics in SZ. Increased sensitivity of other immune mediators, e.g. the role of complement cascades in regulating synaptic pruning is reported. Correlation of increased MPL breakdown with negative

symptoms and impaired sustained attention (increased response time on Go-No Go task) and verbal learning underscores the clinical significance of increased thalamic pruning. Our study highlights a potentially treatable mechanism affecting both clinical and cognitive manifestations of SZ.

Keywords: 31P Magnetic Resonance Spectroscopy, Cognitive impairments, Negative symptoms, Thalamus, Neuropil
Disclosures: Nothing to disclose.

W189. Predictors of Functional Outcome and Transition to Psychosis across the ARMS Category: A Longitudinal Comparison Study on Predictors of Functional Outcome and Transition to Psychosis in Ultra High Risk (UHR) and Non-UHR Young Patients with Comparable Axis I and II Diagnoses

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Background: Prediction, early intervention, and prevention across the full range of mental disorders are preeminent clinical and research goals.

The same key domains identified as predictors of functional decline and transition to psychosis in "At Risk Mental State" individuals, also represent poorly controlled and highly relevant dimensions of different psychiatric syndromes that cut across traditional diagnostic boundaries. Baseline functional impairments, cognitive deficits, as well as neurological soft signs, have been shown to be impaired and to seriously affect real-world functioning in both psychotic and non-psychotic disorders.

Given the lack of diagnostic-specificity of these predictive factors, we hypothesize that their expression in young patients with different psychiatric disorders could have the same predictive power on functional outcome observed in ARMS individuals and an influence on psychosis transition also in not-ARMS patients.

We conducted a longitudinal prospective study to develop a prediction model for poor functional outcome and transition to psychosis in young patients with a recent onset psychiatric diagnosis: i) independent of the ARMS categorization and ii) comprehensive of clinical information, neurological soft signs (NSS), neurocognitive, social cognitive and genetic data.

Methods: A total of 138 patients were recruited in a secondary mental health service (mean age, 24.4 years). According to the Comprehensive Assessment of the At Risk Mental State (CAARMS) interview 67 individuals resulted positive to the ultra-high risk criteria (UHR +) and 71 were negative (UHR-); 116 subjects were followed up for an average of 2.3 years. The 165599 Catechol-O-methyltransferase (COMT) and rs4532 dopamine D1 receptor (DRD1) polymorphisms were analyzed in a patients' subsample.

A binomial logistic regression was performed to identify potential predictors of functional outcome. Cox regression was used to derive a risk index for psychosis transition.

Results: The overall conversion rate to psychosis was 21% for the whole sample (UHR+ and UHR-), 9% for the UHR- and 34% for the UHR+ group. The final "psychosis transition" predictor model for the whole sample of patients, with a positive predictive validity of 85.7%, consisted of four variables: disorder of thought content, sensory integration (NSS), visuo-spatial index of the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS), and Faux Pas test performances. Poor functioning, defined as a score in the Global Assessment of Function (GAF) <60, was predicted by a reduced RBANS attention index, higher avolition levels and motor coordination impairments (NSS). The areas under the curve for the transition to psychosis and functional outcome models were 0.90 (95% CI, 0.83-0.98; P < .001) and 0.79 (95% CI, 0.71-0.87; P < .001), respectively. Functional decline was independent of the UHR status and of the baseline DSM-IV diagnosis.

Conclusions: The combination of clinical, NSS, neuro- and social- cognitive domains have the potential to improve predictive accuracy of the transition to psychosis outcome compared with UHR criteria used alone. Key predictors of functional decline resulted to be not specific to the UHR population, but they could be expressed at the early stage of different psychiatric disorders. A brief neuropsychological assessment should be administered to young patients with a recent onset psychiatric disorder in order to improve prognostic accuracy.

To the best of our knowledge, the models proposed in this study, which need replication, are the first one applied to a secondary mental health service setting.

The genetic influence on both the predictive models will be discussed.

Keywords: high risk psychosis, Predictive Models, neuro-cognition, functional outcome

Disclosures: Nothing to disclose.

W190. Metabolic Abnormalities Prior to the Onset of Psychosis: Another Risk Factor for Psychosis?

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Background: Patients with schizophrenia have a high prevalence of metabolic disorders and cardiovascular mortality. First episode patients with psychosis also have high rates of insulin resistance as well as elevated serum glucose and cortisol. It is possible that a vulnerability to metabolic abnormalities is associated with risk for psychosis. In this study we investigate cardiometabolic and oxidative stress indices in a cohort at Clinical High Risk (CHR) for psychosis from the NAPLS Omega 3 Fatty Acid Clinical Trial and compare them to First Episode Psychosis (FEP) subjects from the RAISE study (Correll et al. 2014) and the general US population (NHANES III).

Methods: Subjects met CHR criteria per the Structured Interview of Prodromal Syndromes, received physical exams and metabolic monitoring prior to randomization into the Omega 3 trial. Antipsychotic medication or history of diabetes were exclusions. Anthropometrical measures, vital signs, glucose, lipids and lipid peroxidation (TBARS, Thiobarbituric acid-reactive substances) were assessed and compared to published values from the RAISE study and NHANES III.

Results: The sample included 109 CHR subjects (46% Female) ages 12-29 (Mean/SD = 18.3/4.4). The mean calculated BMI was 24.2 (range 16-52.8) with 31% of the sample either Overweight (13.2%) or Obese (17.9%). 24.4% met criteria for abdominal obesity and 39.3% met criteria for prehypertension (>120-139/80-89mm Hg) or hypertension (>140/90mm Hg). Mean total cholesterol (Mean/SD: 157.3/30.3), LDL (88.6/23.1), HDL (51.7/12.2), and Triglycerides (82.9/41.2) were all within the normal range but 42.4% of the sample showed evidence of dyslipidemia. Low HDL-Cholesterol and Elevated Non-HDL-Cholesterol showed higher prevalence in both CHR (30% and 14% respectively) and FEP (30 and 41%) compared to NHANES III (23.3% and 12.9). The prevalence of dyslipidemia in CHR (42.4%) and FEP (56.5%) was twice the value of NHANES III (20.3%). TBARS in CHR were 11.1μM ± 5.2μM (normal 1.86-3.94 μM).The mean fasting glucose was 84.2 in the CHR sample (range 54-226) with 4.2% meeting criteria for prediabetes or diabetes. Using a proxy measure of insulin resistance (Triglycerides/HDL), 9% the CHR sample were in the insulin resistance range (>3.5). 9.5% of the CHR sample met criteria for metabolic syndrome compared to 13.2% of the FEP sample in RAISE. Within the CHR sample, metabolic parameters (BMI, Abdominal Circumference, glucose) were significantly associated with prodromal symptoms and worse role functioning (r's 0.22-0.30, p's <0.05-0.002). Greater levels of negative symptoms was associated with a diet consisting of few Omega 3 fatty acid rich foods (r -.20, p<0.05).

Conclusions: CHR subjects show metabolic abnormalities and oxidative stress similar to FEP that are associated with worse functioning and more severe symptoms, suggesting a link with a poorer psychopathological outcome. Early detection of metabolic disturbances is crucial since most of these are modifiable with the potential for significant gains in terms of quality of life and physical health. Longitudinal follow-up will provide insight into whether these indices are risk factors for future psychosis.

NANHES: National Health and Nutrition Examination Survey

Keywords: Cardiometabolic Risk, schizophrenia prodrome, Subclinical psychosis

Disclosures: Nothing to disclose.

W191. Chondroitin Sulfate Proteoglycan Abnormalities are Associated with Altered Thalamic Axonal Pathways in Schizophrenia

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Background: Extensive evidence from imaging studies has identified disrupted cortico-thalamic functional connectiv-

ity in subjects with schizophrenia (SZ). Impaired connectivity of this pathway is believed to impact several clinical aspects of this disorder, including psychosis, attention sensory motor integration, and emotional processing. Despite this evidence, the molecular mechanisms behind this dysfunction have not been identified. Extracellular matrix molecules, particularly chondroitin sulfate proteoglycans (CSPGs), represent strong candidates for molecules that contribute to cortico-thalamic disruption. CSPGs are involved in the regulation of axonal guidance, including cortico-thalamic axons. Changes in CS sulfation in these molecules have profound effects on this regulation. We previously identified abnormal CSPG expression in the amygdala, entorhinal cortex, and prefrontal cortex of subjects with SZ. Recent genetic studies, including GWAS, have reported associations of genetic polymorphisms for genes encoding specific CSPGs, such as neurocan, neuroglycan-C, and PTPRZ1, and molecules involved in the regulation of CSPGs including matrix metalloproteases, suggesting that CSPG abnormalities represent core aspects of the neuropathophysiology of SZ.

As a first step in testing the hypothesis that CSPG abnormalities contribute to cortico-thalamic disconnectivity in SZ, we used three separate approaches (histochemistry, immunofluorescence, and mass spectrometry) to test the association of abnormalities in CSPGs with altered myelinated fiber bundles in the mediodorsal thalamic nucleus (MD) of subjects with SZ.

Methods: Postmortem tissue blocks containing the complete thalamus from normal control (n = 15), SZ (n = 14), and bipolar disorder (BD; n = 15) subjects were obtained from the Harvard Brain Tissue Resource Center. Serial sections were processed for luxol blue histochemistry for myelin bundle labeling. Numbers of myelinated fiber bundles were quantified according to stereology-based methods using computer-assisted light microscopy. Stepwise ANCOVA testing was carried out accounting for several potential covariates, including pharmacological treatment and substance abuse. A separate set of sections from control subjects (n = 3) were processed for immunocytochemistry using an antibodies raised against NG2, brevican, and the axonal marker SMI-312. High-resolution confocal microscopy was used to characterize the relationship of NG2 and brevican immunoreactive (IR) structures with SMI-312-IR axons in the MD thalamic nucleus.

To test the hypothesis that abnormalities in CS are present in the MD thalamus, we performed chondroitin sulfate (CS) disaccharide analysis on lightly fixed, free floating MD tissue sections on the same cohort used for quantification of myelinated axon bundles. Following antigen retrieval and cell lysis, ethanol precipitation was performed to remove the detergent. This was followed by in solution tryptic digestion and then chondroitinase ABC or chondroitinase B digestion on top of a 10 kDa centrifugal membrane. Skyline analysis software was used to build targeted proteomics experiments to quantify levels of a subset of proteins and CSPGs. CS disaccharides were separated using 15 cm 300 μm ID columns packed with 1.9 μm HILIC-WAX (Glycan-Pac AXH-1, Dionex) material and analyzed in the negative ionization mode to determine alterations in sulfation patterns.

Results: Total numbers of myelinated axon bundles labeled with luxol blue were significantly increased in the pars fasciculata division of the MD thalamus in subjects with SZ (p < 0.02), but not in subjects with BD. Furthermore, in the human MD, we observed that NG2 and brevican were co-expressed in axonal coats surrounding individual SMI-312-IR axons within axonal fascicles. The thickness of NG2-IR axonal coats was estimated to be 800-900 nm. Glial cells expressing the same CSPGs were often found in juxtaposition with axonal fascicles, suggesting that they may contribute to the formation of CSPG-positive axonal coats. We observed a significant increase in the percentage of unsulfated CS disaccharide from CS-B/dermatan sulfate in subjects with SZ compared to healthy controls in the same subjects in which changes in myelinated axon bundles were observed microscopically. In addition, we detected significant decreases in the percentages of D0a4/D0a6 and the D2a4/D2a6 sulfation levels following chondroitinase B digestion in subjects with SZ but not in subjects with BD. No significant changes in CS percentages were observed between diagnosis groups following chondroitinase ABC digestion.

Conclusions: Increased total numbers of myelinated fiber bundles in the pars fasciculata of subjects with SZ suggests abnormal organization of axon bundles in this region. Together with increased percentage of CS-0, and decreased percentage of D0a4/D0a6 and D2a4/D2a6 sulfation levels from CS-B/dermatan sulfate in the same cohort, this suggests that decreased levels of sulfated CS may contribute to disrupted axonal organization of cortico-thalamic fibers in this region. The identification CSPG-enriched axonal coats surrounding individual axons in this region suggests CSPGs may serve key functions, including regulating myelination, fasciculation, as well as saltatory impulse conduction and synaptic functions. Taken together with changes in CS levels in the MD of subjects with SZ, and increased numbers of myelinated fiber bundles, these findings suggest that CSPG abnormalities may contribute to cortico-thalamic mis-wiring, and potentially to disrupted cortico-thalamic functional connectivity reported in subjects with SZ.

Keywords: mediodorsal thalamus, Connectivity, psychosis
Disclosures: Nothing to disclose.

W192. Association Network Disruption in Psychotic Disorders: Effects of Diagnosis, Symptoms, and Network Dynamics

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Background: Previous work using resting state functional connectivity MRI (rs-fcMRI) has identified disruption in large-scale cortical association networks in individuals with psychosis. In particular, we and others have reported disruptions to the frontoparietal control network (FCN) that cross conventional diagnostic boundaries, but much remains unknown about the relationship between associa-

tion network function and clinical presentations. Here, we characterized cortical association networks abnormalities in a large sample of individuals with psychosis to understand how (a) clinical diagnosis, (b) symptom domains, and (c) nonstationarities in the spontaneous BOLD signal (i.e., reflecting presumed cortical network dynamics) affect association network function.

Methods: A total of 243 individuals with either schizophrenia (SZ, n = 70), schizoaffective disorder (SZA, n = 59), bipolar disorder with psychosis (BPP, n = 93), or other psychotic disorders (e.g., psychosis NOS, MDD with psychotic features) were characterized clinically using SCID-IV and several clinician-reported scales, including PANSS, YMRS, and MADRS, and scanned on a Siemens 3T Trio MRI scanner at McLean Hospital to collect structural (T1, multi-echo MPRAGE) and resting-state functional (T2*, eyes-open rest, 6-12 min BOLD, TR = 3s) MRI. Healthy participants were drawn from the Genome Superstruct Project (Buckner et al. 2014), a large, existing data set that used identical imaging procedures, allowing us to match patients and controls on demographics and data quality.

Static functional connectivity was computed by projecting each individual's pre-processed BOLD time series to their own cortical surface (FreeSurfer 4.5.0) and then taking the average interregional BOLD correlation for all pairs of 122 surface-based cortical regions of interest (sROIs) distributed across both hemispheres (Baker et al. 2014). Dynamic network configurations (or "states") were computed based on k-means clustering of BOLD co-activation patterns (Liu and Duyn, 2013), limited to a subset of 56 regions within three association networks: FCN, DN, and the dorsal attention network (DAN). Frequency, dwell time, and spatial characteristics of each state were assessed as a function of diagnosis and symptom burden.

To explore symptom-connectivity relationships, symptom dimensions were derived using confirmatory factor analysis of clinical symptom scales in a larger sample of patients (n = 1137); then, for patients who had been scanned, we regressed individual participant factor loadings against measures of functional connectivity.

Results: Effects of diagnosis: As previously reported, FCN within-network connectivity was reduced across psychotic disorders, with SZ and SZA showing a more pronounced reduction than BPP. In addition, both SZ and SZA showed connectivity reductions in other networks (e.g., ventral attention network [VAN] and somatomotor network) that were largely unchanged in BPP.

Symptom-connectivity relationships: Factor analysis revealed five symptom dimensions roughly corresponding to mania, depression, negative symptoms, positive symptoms, and substance use. Mania scores were associated with tighter correlation between nodes of the DAN and the DN and FCN, which both typically anticorrelate with DAN. Negative symptoms were associated with tighter correlations in the ventral attention network (VAN) and salience network (SN), a pattern distinct from what was observed for depression. Critically, we observed similar symptom-connectivity relationships when conducting independent analyses on patients with BDD and SZ/SZA diagnoses, indicating that diagnosis alone was not the sole driver of the observed symptom-connectivity relationships.

Network dynamics: Analysis of network dynamics revealed 12 stable network configurations involving association cortical regions. Intriguingly, a single configuration, characterized by DN and FCN co-activation, showed significant differences between patients and controls, occurring less frequently in patients (p < 0.05). In contrast, configurations involving DN alone, FCN alone, and any combination of DAN regions, were equally present in patients and controls. Neither dwell times nor spatial characteristics differed significantly between patients and controls for any configuration. These results replicated when applied to two independent BOLD runs.

Conclusions: (1) Our findings further support the view that association cortex dysfunction, especially in FCN, may be a shared feature common across psychotic presentations, while specific patterns of dysconnectivity, particularly among but not limited to association areas may give rise to variation between clinical presentations. (2) We found that SZ and SZA involve connectivity changes to the somatomotor system in addition to association territories, which was not the case for BPP. (3) Manic symptoms, whether occurring as part of SZA or BPP, were associated with reduced anti-correlation between attentional and association systems. (4) Finally, we characterized association network dynamics, showing that individuals with psychosis spend less time in a state in which FCN and DN are functionally coupled, possibly reflecting a problem with network dynamics rather than connectivity per se.

Keywords: Resting State Functional Connectivity, dimensions of psychosis, cortical dynamics, mania, connectomics

Disclosures: Nothing to disclose.

W193. Effect of DISC1 Gene Structural Variants on Cognitive Potentials and Gamma Activity

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Background: DISC1 gene has been associated with P300 amplitude in a large Scottish pedigree. The aim of the present study is to investigate the effects of DISC1 haplotypes (rs3738401-rs821616) on the P300 latency and amplitude during a Stroop Task and during the 2-Back Task, using Electroencephalography (EEG).

Methods: Thirty healthy participants were tested while EEG activity was recorded. In the 2-Back Task, black capital letters were presented on a screen one at a time, in a continuous sequence. In the Stroop Task, words were presented one at a time on a screen and the participants are asked to identify, as quickly as possible, the font color (red, blue, green or yellow) of the word by pressing one of four color-coded response keys, using the four fingers of the dominant hand. "Depressive", "positive" and "neutral" emotional valences were based on semantic content of word stimuli. Event-related potentials (ERPs) were recorded from a 32 channel EEG cap following an extension of the 10-20 system and Neuroscan data acquisition system. Vertical and horizontal eye movements were also recorded for ocular artifact rejection. ERP P300 amplitude and latency were analyzed for the Stroop Task. For the 2-Back paradigm,

participants were instructed to respond by pushing a button (2; target) if the present stimulus was identical to the stimulus presented 2 trials back and by pushing a different button (1; non-target) if they were different. EEG recordings were processed using MATLAB and EEGLAB toolbox. Gamma power was analyzed for the correct target trials in the 2-Back Task, as well as accuracy and response time.

Results: The results were overall negative and P300 amplitude on Pz was not associated with the disc1 gene haplotype in the neutral condition (p = 0.908).

Conclusions: Unlike the previous studies, this investigation does not support the inference that DISC1 haplotypes effect in endogenous cognitive potentials.

Keywords: EEG, P300, DISC1

Disclosures: Nothing to disclose.

W194. Comparing DTI White Matter Integrity to Labeled Fiber Tracts in CLARITY Whole-Brains

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Background: Abnormalities in white matter integrity have been reported in several psychiatric disorders, including schizophrenia. These deficits are typically measured using diffusion tensor imaging (DTI), a technique that infers white matter microstructure based on directional water diffusion through brain tissue. While DTI has proven to be a powerful non-invasive tool in both clinical and research settings, it lacks the spatial resolution necessary to interrogate neural circuits on the microscopic level and performs poorly in regions with complex fiber trajectories. Also, because DTI is an indirect measure of white matter integrity, it cannot determine the cause of anisotropy or diffusivity changes. Recently, novel brain clearing techniques such as Clear Lipid-exchanged Acrylamide-hybridized Rigid Imaging/Immunostaining Tissue-hYdrogel (CLARITY) allow for molecular phenotyping of neural circuits on multiple biological scales, including brain-wide, mesoscopic, and microscopic. By combining DTI with CLARITY, we set out to compare inferred white matter microstructure to axon-associated proteins labeled with myelin basic protein (MBP) and neurofilament (NF).

Methods: For ex vivo DTI, we scanned six PFA-hydrogel C57BL/6J mouse brains on an 11.7 T MR scanner for 24 hrs and 15 directions. DTI data was analyzed using FSL and TrackVis software. Following MR scanning, samples were processed with the CLARITY protocol until optically transparent. Whole cleared brains were then immunostained with MBP or NF primary antibody followed by a fluorescent secondary antibody (Alexa Fluor 633). Cleared and labeled brain samples were imaged on an Olympus Fluoview FV1000 multiphoton microscope and analyzed using Imaris 8.0 software.

Results: We found that the liquid PFA-hydrogel solution did not adversely affect high-resolution DTI scanning. CLARITY whole brains labeled with MBP or NF antibodies produced intact 3-dimensional white matter tracts of the major commissures and fiber tracts in mice. We compared CLARITY brains to DTI scalar metrics of fractional anisotropy (FA), mean diffusivity (MD), axial diffusivity (AD), and radial diffusivity (RD) in specific regions of interest (ROIs) that have been associated with decreased FA in schizophrenia patients. In the corpus collosum ROI, we found that mean FA strongly correlated with mean MBP fluorescence intensity (Spearman's $R = 0.92$, $P < 0.01$), while RD ($R = -0.64$) and MD ($R = -0.70$) were negatively correlated. In examining the anterior commissure with seeded tractography, we also found positive correlations between mean FA and MBP intensity ($R = 0.66$), and NF intensity ($R = 0.40$).

Conclusions: This unique strategy of combining DTI and CLARITY provides insight into the nature of white matter integrity differences that are observed in models of psychiatric disease and in human patients. By using molecularly phenotyped CLARITY whole brains, we can now map axon-related proteins and quantify fiber tracts on several biological scales. By understanding how the structural connectivity of the brain is changed by psychiatric illness, we can begin to develop improved therapeutics and drugs to remedy these deficits.

Keywords: Diffusion tensor imaging, CLARITY, mouse brain, schizophrenia, White Matter

Disclosures: Nothing to disclose.

W195. Tryptophan Degradation and White Matter Structure in Schizophrenia

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Background: Schizophrenia is characterized by abnormalities in the structure and functioning of white matter, but the causal processes underlying this neuropathology are unclear. We hypothesized that increased tryptophan degradation in the kynurenine pathway would be associated with white matter abnormalities.

Methods: Fasting plasma samples were obtained from 37 schizophrenia patients and 38 healthy controls and tested for levels of tryptophan and its metabolite kynurenine; the ratio of kynurenine to tryptophan (KYN/TRP) was used as an index of tryptophan catabolic activity. Most of these participants also underwent a multimodal neuroimaging assessment of white matter structure, including diffusion tensor imaging (DTI) and arterial spin labeling.

Results: Patients had significantly lower levels of plasma tryptophan (M = 57.1 ± 15.2 μmol/L) compared to controls (M = 70.7 ± 15.2 μmol/L; p < .001). Patients also had a significantly higher KYN/TRP ratio than controls (p = .018). Plasma fasting tryptophan level was significantly correlated with whole brain tract-averaged fractional anisotropy (FA) in patients (r = .347, p = .038, n = 36) but not in controls (r = .182, p = .27, n = 38). Similarly, average white matter cerebral blood flow (CBF) was positively correlated with plasma tryptophan in patients (r = .431, p = .012, n = 33)

but not in controls (r = .099, p = .62, n = 27). However, KYN/TRP ratios were not significantly related to FA or CBF in either patients or controls.

Conclusions: These results add to the evidence implicating elevated kynurenine pathway activity in schizophrenia, and suggest that low levels of fasting plasma tryptophan may be a peripheral biomarker indexing more severe white matter pathology in schizophrenia. The mechanism underlying the relationship of peripheral tryptophan and white matter structure is unclear, but may reflect the influence of the kynurenine pathway metabolites on oligodendrocyte maturation and functioning.

Keywords: schizophrenia, white matter, tryptophan, kynurenine

Disclosures: Nothing to disclose.

W196. Conditional Rescue of NMDA Receptor Hypofunction to Study the Plasticity and Circuitry of Schizophrenia-Relevant Behaviours

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Background: Neurodevelopmental disorders like schizophrenia present challenges for treatment because diagnosis occurs decades after the causal insult to the CNS. It is not clear whether the neural circuits that mediate schizophrenia-relevant behaviours are sufficiently plastic to overcome developmental deficits in wiring. We addressed this question by generating a new mouse line in which NMDA receptor hypofunction occurs throughout development, and can be rescued at different stages of postnatal development. In this study, we determined the extent to which schizophrenia-relevant behaviours can be normalized by restoring NMDA receptor levels in either adolescence or adulthood.

Methods: The NR1-IR (inducible rescue) mouse line was generated by targeted insertion of a floxed neomycin cassette into intron 17 of Grin1. Rosa26-Cre-ERT2 transgenic mice (Jackson Labs) were used to achieve tamoxifen-inducible rescue at 6 or 10 weeks of age. The four experimental groups were: WT, WT-CreTg, NR1-IR, NR1-IR-CreTg. Tamoxifen was administered to all groups of mice by oral gavage (6 mg/25 g body weight in corn oil), followed by two weeks of tamoxifen diet (500 mg/kg chow, Harlan). Male and female mice were behaviourally tested either 4 weeks or 8 weeks after oral gavage and subsequently used for post-mortem biochemistry. The following behaviours were measured: locomotor activity and stereotypy in a novel environment (digital activity monitors), executive function (puzzle box), sociability (modified 3-chamber test), anxiety (elevated plus maze), and sensorimotor gating (pre-pulse inhibition of acoustic startle response). Brain biochemistry was performed to measure Cre-mediated excision (genomic qPCR), NR1 mRNA (quantitative RT-PCR), and NR1 protein (western blot).

Results: NR1-IR mice recapitulate all of the behavioural abnormalities observed in our previously studied NR1 knockdown line, including increased locomotor activity and stereotypy, impaired executive function, reduced anxiety in the elevated plus maze, reduced sociability, and impaired sensorimotor gating. In the absence of tamoxifen treatment, we did not detect excision of the mutation in NR1-IR-CreTg mice, demonstrating that the Cre-ERT2 transgene is not leaky. There was no difference in the rate of Cre-mediated DNA excision between 6 and 10 week old mice, but there were brain-region-specific differences in excision rate, with cortex and hippocampus having the greatest rates of excision and cerebellum having no detectable excision. There were also circuit-specific improvements in behaviours. Significant but incomplete normalization of locomotor activity was observed in NR1-IR-CreTg mice (54% reduction compared to NR1-IR mice). Stereotypy was not substantially improved in NR1-IR-CreTg mice (20% reduction compared to NR1-IR mice). Executive function, sociability, and sensorimotor gating were all markedly improved, with NR1-IR-CreTg mice performing similar to WT mice and significantly improved compared to NR1-IR mice. Behaviour in the elevated plus maze indicated a partial rescue, with NR1-IR-CreTg mice displaying an intermediate phenotype between WT and NR1-IR mice. Importantly, the age at intervention or length of recovery had no effect on the extent of behavioural rescue.

Conclusions: Originally we hypothesized that earlier intervention would result in a more complete normalization of schizophrenia-relevant behaviours. Instead we found that circuit plasticity was more crucial than age of intervention. While some schizophrenia-relevant behaviours were highly plastic, others were less amenable to improvement. The nearly complete rescue of cognitive behaviours like executive function and sociability is remarkable, and supports the concept of profound adult neuroplasticity. Although the cognitive and negative symptoms of schizophrenia are not improved by current antipsychotics, our work suggests that these behaviours are not refractive to improvement, even in adulthood. Ongoing studies will examine the molecular events that control behavioural plasticity. Additional studies are underway to achieve dopamine cell-selective rescue to map the contribution of dopaminergic circuits to schizophrenia-relevant behaviours.

Keywords: NMDA Receptor, mouse behavior, schizophrenia, Neurodevelopmental Disorders

Disclosures: Nothing to disclose.

W197. Association of Neuroanatomical Structures Mediating Episodic Memory Impairment and Resting State Functional Connectivity in Early-Phase Psychosis

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Background: The purpose of this study is to elucidate how neural circuitry involved in episodic memory dysfunction in an early-phase psychosis (EPP) population is related to resting state functional connectivity.

Methods: EPP subjects (N = 35) were recruited through the Prevention and Recovery Center for Early Psychosis. The control group (N = 20) was comprised of well-matched healthy subjects who were free of major psychiatric disorders as determined by a structured diagnostic interview. Imaging

studies were conducted on a Siemens 3T Tim Trio scanner while performing episodic memory tasks consisting of blocked visual scene encoding and recognition. Comparisons of activation differences within- and between-groups (EPP vs. control) were conducted on a voxel-by-voxel basis throughout the entire brain. A resting-state fMRI scan was also conducted, and time-series data were analyzed with advanced processing methods designed to minimize signal and motion artifacts that cause non-neuronal signal, including principal components analysis, non-gray matter signal regression, and time point censoring. Time-series correlations were then used to quantify functional connectivity with hippocampus seed regions of interest.

Results: Patients demonstrated significantly lower activation than controls in the right hippocampus and left fusiform gyrus during scene encoding and lower activation in the posterior cingulate/precuneus and left middle temporal cortex during recognition of target scenes (all corrected p < .05). These findings were used to establish seed regions for functional connectivity analysis. In both groups, the hippocampus had positive bilateral connectivity with the posterior cingulate during resting-state fMRI and negative connectivity with lateral prefrontal cortex. In the patient group, right hippocampus-prefrontal resting-state functional connectivity was positively associated with hippocampal activity during encoding (r = .45, p = .02), although this association did not differ between groups.

Conclusions: Results suggest altered episodic memory encoding and recognition circuitry involving frontal, temporal, and posterior cingulate regions in EPP. This circuitry is functionally connected at rest, although no significant group differences were found. While prefrontal-hippocampus resting-state connectivity was associated with hippocampus activity during encoding, the lack of group differences in this association suggests that the functional relationship between prefrontal cortex and hippocampus does not drive hippocampal dysfunction during encoding. These findings add to a growing body of research describing episodic memory circuitry in the pathophysiology of schizophrenia and describe potential associations between episodic memory circuitry and cortical circuits at rest.

Keywords: Resting State Functional Connectivity, episodic memory, first-episode psychosis

Disclosures: Nothing to disclose.

W198. Mismatch Negativity and Repetition Positivity Deficits Implicate Deficient Predictive Coding in the Auditory System in Clinical High Risk Youth who Transition to Psychosis

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Background: Mismatch negativity (MMN) is a component of the event-related potential (ERP) elicited by infrequent deviant auditory stimuli presented during a stream of frequent standard stimuli. Prior research has indicated that MMN is robustly reduced in schizophrenia and evidence is emerging that its reduction may predict conversion to psychosis among individuals exhibiting putatively prodromal symptoms indicative of clinical high risk (CHR) for psychosis. Moreover, pharmacological challenge studies with NMDA receptor antagonists have indicated that MMN is dependent on glutamatergic neurotransmission at NMDA receptors. Traditionally, MMN is measured in the difference wave obtained by subtracting the ERP wave elicited by the standard stimulus from the ERP wave elicited by the deviant stimulus. However, recent theoretical accounts of the MMN that adopt a predictive coding framework have led to separate consideration of the signals elicited by the repetitive standard stimuli, in addition to the traditional MMN. According the predictive coding framework, the MMN represents a prediction error signal when a deviant stimulus violates the prediction that a standard stimulus will repeat. Consistent with this view, successive repetitions of standard stimuli have been shown to produce progressively increasing "repetition positivity" (RP) in the same time window as the MMN response to deviants. This RP is thought to reflect repetition dependent strengthening of the memory trace for the standard, a form of experience-dependent short-term synaptic plasticity that supports predictive coding in the auditory system. We examined the MMN and the RP in a large sample of CHR individuals and healthy controls collected as part of the North American Prodromal Longitudinal Study (NAPLS).

Methods: Individuals at CHR for psychosis (n = 595), including a subgroup who transitioned to psychosis (CHR-T; n = 79) and a subgroup who did not transition during a 24-month follow-up period (CHR-NT; n = 244), as well as healthy comparison subjects (HC; n = 242), were recruited from 8 NAPLS consortium sites. Measures included EEG-based baseline duration- and pitch-deviant MMN, as well as the RP (100-200 msec post-stimulus) from ERPs to successive standard stimuli in the oddball stimulus sequence (standards in positions 2, 3, 4-5, 6-7, 8-10, > 10).

Results: CHR-T subjects, relative to CHR-NT and HC subjects, had smaller MMN amplitudes irrespective of deviant type (p < .05) and smaller RP to late standards (standards in positions 8-10 and > 10; p < .05).

Conclusions: Deficits in the late standard RP and MMN were evident in CHR subjects relative to HC, and further, predicted transition to psychosis in CHR subjects. These results implicate deficits in two aspects of short-term plasticity in the auditory system as risk factors for psychosis: 1) experience dependent strengthening of the memory trace to repeating standard tones (RP), and 2) deficits in the prediction error signal associated with the appearance of deviant tones (MMN). These results are consistent with a model in which deficits in NMDA-receptor dependent short-term plasticity diminishes experience-dependent synaptic strengthening. Excessive weak synapses may then be subject to synaptic over-pruning during the transition to psychosis, producing the gray matter deficits, neuropil reduction, and neural dysconnectivity that constitute core pathophysiological features of schizophrenia.

Keywords: schizophrenia prodrome, Mismatch Negativity, ERP

Disclosures: Nothing to disclose.

W199. Discovery of Novel Inhibitors of D-Amino Acid Oxidase

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Background: The legendary biochemist, Sir Hans Adolf Krebs discovered D-amino acid deaminase. D-amino acid oxidase (DAAO) is the first enzyme being crystalized. Its main substrate, D-serine and D-alanine are critically important co-agonist of the N-methyl-D-aspartate receptor (NMDAR). We recently demonstrated that sodium benzoate, as a competitive antagonist of DAAO, can substantially improve the positive and negative symptoms and neurocognition of schizophrenia, presumably enhance NMDA function by raising D-serine and D-alanine levels. Also, in mild dementia, sodium benzoate improves the cognition and function.

The development of glutamatergic NMDA treatments in the past two to three decades, is following a similar path of the development of aminergic and GABAergic treatments. All three lines of treatments involve neurotransmitter analogue and its precursor (e.x. chloroziapoxide, tryptophan), agonist/antagonist (L-dopa, chlorpromazine), uptake blocker (imipramine, fluoxetine, bupropion), as well as catabolism inhibitor (ip002roniazid, selegiline). In analogy to the aminergic and GABAergic treatments, the initial discovery was the therapeutic application of the co-agonists/partial agonists, including glycine, D-serine, D-cycloserine and D-alanine. The NMDA treatment options coming up later were obvious in the hindsight; first, the neurotransmitter uptake inhibition by glycine transporter-1 inhibitor; second, NMDAR antagonists; third, the inhibition of the DAAO. The treatment approach of DAAO inhibition is analogous to monoamin oxidase inhibitors (MAOI), which upregulates monoamine for CNS disorders like depression and Parkinson disease. In another word, DAAO inhibitors are similar to MAOI in raising the tone of neurotransmitter of interest, by inhibiting the catabolism enzyme.

Methods: Following the initial success of DAAO inhibition by sodium benzoate, we search for candidate drugs that can safely activate NMDAR, we had screened and identified several DAAO inhibitors that can be potential therapeutics for CNS disorders. For DAAO activity assay, recombinant DAO-6Histidines (400 nM) was added into 3% (w/v) o-phenylenediamine, 1 U horseradish peroxidase, 40 mM D-alanine with a final volume of 200 µL. The reaction at 25 °C generated hydrogen peroxide that was oxidized by the peroxidase and further converted, in the presence of o-phenylenediamine, to 2,3-diaminophenazine, which was quantified by measuring OD450. For the inhibitor assay, candidate drugs at mM-nM was applied to determine the IC50. To confirm the findings of the enzyme assay, we also perform molecular docking study of the potential DAAO inhibitors. To decide the candidate for new drug development, we test the compounds with low IC50, which were confirmed by docking study, by a battery of animal behavior studies relevant to CNS disorders.

Results: We had identified more than 20 candidate DAAO inhibitors. One of the DAAO inhibitors, SND51, botanic in origin, was confirmed to be a competitive antagonist of DAAO, with IC50 at 5 µM, which is more potent than sodium benzoate (IC50 = 61µM). Repeated administration in rodent does not affect its body weight, food intake, liver and kidney weights, nor gross and histopathology. The findings in carcinogenesis, mutagenesis studies are benign. Its safety profile is satisfactory, with LD50 > 1500 mg/Kg in most toxicology studies.

In rodent behavior models, 20 mg/kg administration of SND51 improves spatial memory. It attenuates the MK-801-induced hyperlocomotion and restores sensorimotor gating disrupted by MK-801. It also improve the anxiety performance on elevated plus maze.

Conclusions: Given its activities in both the DAAO and the favorable findings in rodent behavior assays, SND51 is chosen for the clinical development of CNS disorders. We believe DAAO inhibition is a novel mechanism that holds promise like MAOI in aminergic treatments. DAAO inhibition represents a safe approach to enhance NMDAR-mediate neurotransmission to improve the symptoms of CNS disorders.

Keywords: NMDA, D-amino acid oxidase, screening

Disclosures: Professor Tsai holds substantial equity of SyneuRx International Corp. which is developing NMDA treatments.

W200. Development and Validation of a Laser Capture Microdissection - Targeted Mass Spectrometry Approach for Cortical Layer Specific Protein Quantification in Postmortem Human Brain Tissue

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Background: Cytoarchitectonic abnormalities, such as reduced dendritic spine density, have been reproducibly observed in layer 3 of multiple brain areas in schizophrenia. These alterations are widely believed to underlie deficits in cortical function and schizophrenia symptoms. While genetic and pharmacologic studies have identified synaptic signaling pathways linked to disease, the molecular pathology of these cytoarchitectonic alterations remains unknown. Laser capture microdissection is routinely paired with microarray and qPCR to investigate cell type specific alterations in mRNA expression. However alterations in mRNA expression may not reflect changes in protein levels at neuronal processes and synapses. The goal of this study is to develop and validate a laser capture microdissection – targeted mass spectrometry approach for multiplexed protein quantification in specific cortical layers of human postmortem brain tissue.

Methods: Experiment 1: Tissue sections from PFC area 46 were obtained from five control subjects with ages ranging from 28 to 60 years and a PMI ranging from 5.5 to 23 hours. 54 million µm3 of tissue was collected, in duplicate, from cortical layers 3 and 5 of each section by laser capture microdissection. Protein was extracted from these samples

and mixed with 4 μg of a lysine 13C6 stable isotope labeled neuronal proteome standard. 386 peptides from 250 selected proteins were quantified by Liquid Chromatography – Selected Reaction Monitoring (LC-SRM) mass spectrometry on a TSQ Quantiva triple stage quadrupole mass spectrometer (Thermo Scientific) with a nanoAC-QUITY UPLC system (Waters) and a picochip column (New Objective). Experiment 2: The previously described experiment was replicated in an additional five control subjects. Experiment 3: To further investigate the effects of postmortem interval on protein stability in this assay, cortical sections were obtained from cynomolgus monkey (Macaca fascicularis) with simulated postmortem intervals of 0, 6, 12, and 24 hours. LC-SRM analysis of these samples is currently underway.

Results: The mean coefficient of variance for repeated quantification of the 250 proteins from the same subject was 4.3%, indicating excellent technical reproducibility. The mean coefficient of variance for the total assay, including biological variability across the five human subjects, was 15% in layer 3 and 14% in layer 5. We observed significant differences in protein expression between layers 3 and 5 and unsupervised hierarchical clustering clearly distinguished between the layers. Functional Gene Annotation Analysis revealed that proteins enriched in layer 3 included those involved in synaptic and mitochondrial function, while those increased in layer 5 were involved with cell projection, axon, and cytoskeletal activities.

Conclusions: These results demonstrate that laser capture microdissection – targeted mass spectrometry in human postmortem brain tissue is robust, opening the door to new studies and questions in a variety of neuropsychiatric diseases. Results from the experiments in Cynomolgus monkey tissue will reveal extent to which the laminar enrichments/segregation is stable through 24 hours.

Keywords: Postmortem Brain Tissue, Laser Capture, Proteomics

Disclosures: Nothing to disclose.

W201. Whole Genome Sequencing in a Founder Population Identifies Novel Candidate Rare Variants for Schizophrenia

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Background: Amongst the most well-replicated and robust findings in the entire schizophrenia (SZ) literature is the high heritability ($\sim$80%). While a recent large-scale genome-wide association study has identified 108 genetic loci repicably associated with SZ, effect sizes are extremely small and there remains a considerable degree of "missing heritability." It has been suggested that SZ and other serious mental illnesses most likely include a heterogeneous collection of underlying genetic loci, including rare alleles of moderate or strong effect. While studies of large copy number variants in the genome have provided proof of concept for the role of rare variants in SZ, to date there has been no clearly implicated single nucleotide variant (SNV) of large effect. Extreme allelic heterogeneity poses a challenge to identifying disease-related rare variants. Consequently, we examined a founder population (the Ashkenazi Jewish population) with relatively reduced levels of allelic heterogeneity to perform a case-control whole genome sequencing study of schizophrenia.

Methods: We performed high-depth whole genome sequencing in a total of 339 schizophrenia (SCZ) cases and 242 controls (CNTL) drawn from the Ashkenazi Jewish population. Sequencing was performed on the Illumina HiSeq x10 platform (299 SCZ/114 CNTL) or the Complete Genomics, Inc platform (40 SCZ/128 CNTL). Both platforms provided mean coverage $>$30x for $>$97% of the genome for all subjects. After strict QC, approx 3.4M single nucleotide variants from each subject were available for analysis. We restricted preliminary analyses to protein-coding changes predicted to be damaging based on PHRED score$>$15 using CADD (Combined Annotation Dependent Depletion), and that were observed in 5 or more cases but no controls. We then examined allele frequencies of these variants in two additional sources of controls: 1) n$=$1679 independent Ashkenazi Jewish controls genotyped with the Illumina exomechip; 2) n$=$60,706 unrelated individuals (all ethnicities) with publicly available exome sequence-derived allele frequencies as part of the Exome Aggregation Consortium (ExAC).

Results: We identified a total of 71 SNVs that met our initial search criteria. Of these, 49 were genotyped in the larger cohort of 1679 independent Ashkenazi controls. Five variants had allele frequency of zero in both Ashkenazi control cohorts, and were further examined in the ExAC database. All five of these variants had minor allele frequency $<$0.0005 in the ExAC database, resulting in Fisher's exact test $<$5E-8 when compared to the SCZ cohort. The strongest results were obtained for a missense variant in MANBA, the gene encoding lysosomal beta A mannosidase, with frequency of 7/678 SCZ chromosomes and 11/125254 control chromosomes (OR$=$119; p$=$3.84E-12). Three of the remaining genes produce synaptic proteins, and the fifth gene produces a calcium-binding protein.

Conclusions: Many complex diseases have conclusively demonstrated the presence of both common, low penetrance risk variants and rare, high penetrant variants (e.g., APOE vs APP in Alzheimer's; FGFR2 vs BRCA1 in breast cancer). However, the landscape of rare variation in SZ remains largely unexplored. Using a unique population, which we have previously demonstrated was founded by $\sim$350 individuals less than a thousand years ago, we sought to reduce the obstacle of massive allelic heterogeneity which otherwise makes rare variant studies so challenging. We are currently testing these variants in additional replication cohorts drawn from the Ashkenazi population. If replicated, the association with MANBA is consistent with the presentation of psychosis in several lysosomal storage disorders. The association to synaptic and calcium-related genes is also consistent with evidence from studies of common genetic variation in schizophrenia.

Keywords: schizophrenia genetics, whole genome sequencing, rare variation

Disclosures: Nothing to disclose.

W202. Disrupted Functional Connectivity of Auditory Cortex in Psychotic Bipolar Disorder Patients with Lifetime Auditory Hallucinations

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Background: Though auditory hallucinations (AH) are most commonly associated with schizophrenia (SZ), they are non-specific and transcend diagnoses, occurring also in mood disorders, trauma spectrum disorders, substance use and intoxication, neurological disorders, and even in a subset of healthy individuals. In bipolar disorder (BP), the prevalence of AH ranges from 11-34%. Though AH is not uncommon in BP, the literature examining the neurobiology underlying AH in BP has been sparse.

Building on existing literature pointing to abnormalities of the primary auditory cortex in AH pathogenesis, our group previously studied functional connectivity (FC) abnormalities associated with the primary auditory cortex, specifically left Heschl's gyrus, in SZ patients with AH and found that FC between left Heschl's gyrus and prefrontal cognitive control areas, including cingulate cortex and orbitofrontal cortex, co-varied with severity of AH. FC between left Heschl's gyrus and several brain regions important in speech/language including Broca's area was also associated with greater severity of AH, consistent with inner speech models of AH.

In this study, we sought to extend our investigation of FC abnormalities associated with the primary auditory cortex in AH, this time focusing on individuals with psychotic BP with and without lifetime AH. We hypothesized that many abnormalities, particularly those involving speech/language and cognitive control areas would be shared across individuals with AH, including BP patients with AH. We also hypothesized that BP patients with AH would additionally show unique abnormalities, most likely in brain areas that modulate affect.

Methods: Participants were 16 BP patients with lifetime AH (AH), 33 BP patients with lifetime history of psychosis but no history of AH (NAH), and 46 healthy controls (HC). Patients were recruited from both inpatient and outpatient services at McLean Hospital. Control participants were selected from an existing database of 2292 adults (aged 18-83) scanned previously using identical pulse sequences on an identical scanner, and selected to match patients on the basis of age, sex, handedness, and image signal to noise ratio (SNR).

Diagnosis was determined using the Structured Clinical Interview for the DSM-IV-TR (SCID). Patients scoring 3 (threshold/true) on SCID item B16 ("Did you ever hear things that other people couldn't, such as noises, or the voices of people whispering or talking?") were categorized as AH; all other patients were categorized as NAH.

All imaging data were collected on 3T Tim Trio scanners (Siemens) with a 12-channel phased-array head coil. Functional data were acquired using a gradient-echo echoplanar imaging sequence with the following parameters: TE/TR 30/3000ms; flip angle 85°; $3 \times 3 \times 3$mm voxels; FOV 216; 47 axial slices collected with interleaved acquisition and no gap. Participants were instructed to remain still, stay awake, and keep their eyes open. Each functional run lasted 6.2min (124 time points). Structural data included a high-resolution, multiecho, T1-weighted, magnetization-prepared, gradient-echo image.

We used FSL v5.0.6 for image analyses. After discarding the first 4 volumes, images were slice-time and motion corrected, smoothed with a 6mm Gaussian kernel, band-pass filtered (0.009Hz $< f < 0.08$Hz), and affine registered to standard MNI space. We placed a 10 mm diameter sphere on left Heschl's gyrus (LHG; -42, -26, 10), the putative location of left primary auditory cortex in humans. The BOLD time course from LHG was extracted and entered into a general linear model using FEAT, with signal from white matter, CSF, whole brain, and six motion correction parameters regressed. Data from first-level analyses were entered into a mixed-effects group analysis, with age, sex, handedness, CPZ equivalents, and a measure of head motion (mean absolute displacement) included as covariates. We used a $p < 0.01$ voxel threshold, whole-brain corrected with a $p < 0.05$ cluster threshold. We also performed connectivity analysis with a right HG seed (RHG; 46, -20, 8), using the same approach as for LHG.

Results: BP patients with AH show increased LHG-seeded FC with bilateral anterior cingulate cortex and with right cerebellum (lobule VI, crus I-II), and decreased LHG-seeded FC with bilateral orbitofrontal and bilateral occipital cortices, relative to psychotic BP patients without AH. When analysis is seeded in RHG, BP AH patients compared to BP NAH patients show increased FC with multiple areas in bilateral cerebellum.

Conclusions: These results provide evidence for disrupted auditory cortex-seeded functional connectivity in BP patients with AH. Our finding that BP AH patients have increased connectivity between LHG and anterior cingulate cortex is consistent with our earlier findings in schizophrenia patients with AH, and is in line with the idea that heightened auditory expectancy may increase susceptibility to AH. Discrepant from our prior schizophrenia AH findings, we found decreased rather than increased LHG connectivity with orbitofrontal cortex; this finding in BP AH is in line with models that propose a role for decreased top-down cognitive control in vulnerability to AH. Finally, we found abnormally increased connectivity in cerebellum with both LHG and RHG seeds; these findings are intriguing in light of growing evidence that the cerebellum is extensively connected to higher-level association cortices, and that it contributes to cognitive and affective as well as motor functions.

Keywords: Bipolar Disorder, Resting State Functional Connectivity, Auditory hallucinations

Disclosures: DO served on the Scientific Advisory Board for Lilly in 2013.

W203. Dopamine D4 Agonist Restores Novel Object Recognition in Sub-Chronic Phencyclidine-Treated Rats

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Background: Dopamine (DA) is a key neurotransmitter implicated in the pathophysiology of both the psychotic

symptoms and cognitive impairment associated with schizophrenia (CIAS). DA has also been shown to be central to the ability of typical antipsychotic drugs (APDs) to improve positive symptoms and to contribute to the ability of atypical APDs, via multiple mechanism, e.g. 5-HT2A receptor blockade and 5-HT1A partial agonism, to improve positive symptoms and CIAS. While DA D2 receptor blockade is the basis for the antipsychotic action of typical APDs, direct and indirect effect of atypical APDs to stimulate or block DA D1 and D3 receptors, respectively, contribute to their effects to improve cognition in animal models of CIAS, including the deficit in novel object recognition (NOR) produced by subchronic (sc) administration of the NMDA receptor antagonist, phencyclidine. NOR is the rodent equivalent of human declarative memory. Thus, we reported that SCH23390 and SKF83566, DA D1 receptor antagonists, can induce a deficit in NOR in normal rats, while SKF38393, a DA D1 receptor agonist, reverses the NOR deficit in subchronic (sc) phencyclidine (PCP)-treated rats with an inverted U-shaped dose-response curve. The role of the D4 receptor, which is D2-like in that the activated receptor inhibits adenylate cyclase, thereby reducing the intracellular concentration of the second messenger, cyclic AMP, in APD action is more controversial. Seeman, van Tol and colleagues postulated that DA D4 receptor blockade was central to the action of clozapine, the prototypical APD, initiating an extensive effort to discover, develop and test D4 antagonist as APDs in schizophrenia. However, none of the D4 antagonists which were tested clinically showed any efficacy as APDs, effectively ending interest in the D4 receptor as a target. However it was recently reported that a selective DA D4 receptor agonist (PD168077) reversed scPCP-induced NOR deficit in rats. The purpose of this study was to further examine the potential of D4 agonists to improve CIAS using the scPCP-model mentioned previously.

Methods: Female, Long-Evans rats received vehicle or PCP (2 mg/kg, twice daily) for 7 days, followed by a 7-day washout prior to NOR testing (3 min acquisition of the knowledge of the familiar object, and testing trials separated by a 1 min inter-trial interval). This induces an indefinite deficit in NOR. In order to determine whether D4 antagonism affect NOR, vehicle-treated rats were administered L745870, a selective DA D4 antagonist (Kulagowski et al., 1998), 30 min prior to acquisition. To test the beneficial effects of a D4 agonist, a group of scPCP-treated rats received PD168077, a selective DA D4 agonist (Gazi et al., 2000) 30 min prior to acquisition. Next, scPCP-treated rats were pretreated with L745870 (15 min) prior to an effective dose of PD168077 or lurasidone, a novel atypical APD which lack affinity for the D4 receptor, to determine whether D4 antagonism or agonism interferes the ability of lurasidone to restore NOR. We also tested sub-effective doses (SED) of PD168077 in combination with SED lurasidone and SED clozapine in scPCP-treated rats. Augmentation of the cognitive-enhancing effects of atypical APDs is a promising approach to the treatment of CIAS.

Results: Acute administration of the DA D4 antagonist, L745870, prior to acquisition induced an NOR deficit in normal rats. The D2 antagonist, haloperidol, did not affect NOR in normal rats. However, acute treatment with the DA D4 agonist, PD168077, reversed the NOR deficit in scPCP-

treated rats. Pre-treatment with L745870, a D4 antagonist, blocked the effect of PD168077 or lurasidone in scPCP-treated rats. Interestingly, SED lurasidone co-administered with SED PD168077 significantly reversed the NOR deficit as assessed by the discrimination index (DI) compared to vehicle, while SED PD168077 prior to SED clozapine tended to reverse the PCP-induced NOR deficit, but the effect was not statistically significant.

Conclusions: The major finding of this study is that D4 antagonism has an adverse effect on NOR in rats and that D4 agonism may be beneficial in an animal model of CIAS. This study demonstrated that a DA D4 receptor antagonist, L745870, alone, impaired NOR in vehicle-treated rats and blocked the effect of lurasidone to restore NOR in scPCP-treated rats. We have reported that haloperidol will also block the effect of lurasidone to restore NOR in rats. We confirmed a previous report that PD168077 reversed scPCP-induced NOR deficit (Sood et al., 2011). These results suggest that DA D4 receptor agonism shows pro-cognitive effects, while D4 antagonism induces cognitive impairment. It is interesting to note that a DA D4 receptor agonist potentiated the effect of lurasidone, but not clozapine. This may be due to the difference in DA D4 receptor affinity between the two atypical APDs as clozapine acts as an antagonist at the D4 receptor, while lurasidone has negligible affinity for DA D4 receptors. Therefore, clozapine's action as an antagonist with high affinity for the DA D4 receptor may block the potentiating effect of a DA D4 receptor agonist. In summary, DA D4 receptor agonism may provide an efficacious therapeutic approach for the treatment of CIAS. DA D4 receptor agonists may also be useful in potentiating the pro-cognitive effects of atypical APDs with no or weak affinity for the DA D4 receptor. Further study of D4 receptor agonism in animal models of CIAS and development of a D4 agonist for human use is indicated.

Keywords: phencyclideine, dopamine d4, novel object recognition, lurasidone, clozapine

W204. Interleukin-1β Alters Cortical Connectivity and Mediates the Effects of Maternal Immune Activation through Dynamic Changes in IL-1β Receptor Localization and MHCI Signaling

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Background: There is growing evidence for a critical role for neuroimmune interactions in brain development and both genetic associations and environmental risk factors implicate immune molecules in neurodevelopmental disorders. Recent genetic data from multiple large cohorts of individuals with autism spectrum disorder (ASD) and schizophrenia (SZ) demonstrate associations with genes

encoding immune molecules, including several cytokines and their receptors as well as genes within the major histocompatibility complex (MHC). Among the cytokines, the interleukin (IL)-1 family is enriched in genetic mutations associated with ASD and SZ. In addition, environmental risk factors that alter immune responses, especially maternal infection, have been implicated in both disorders. Our laboratory recently demonstrated that levels of IL-1β and its receptors are altered in the cerebral cortex in both mouse and non-human primate models of maternal immune activation (MIA), suggesting that IL-1β may mediate the effects of MIA in altering synapse development and leading to disease-related behaviors.

Methods: Cultured neurons from the frontal cortex of C57/B6 mice were treated during the peak period of synapse formation with IL-1β and/or transfected with shRNAs to manipulate IL1-R1, IL1RAcP and IL1RAPL1. For the MIA model, pregnant mice were injected with saline or poly(I:C) on E12.5 and levels of IL-1Rs and glutamatergic synapse density were detected using immunocytochemistry (ICC) and quantified. The effects of IL-1β on altering binding of IL1Rs accessory receptors to their trans-synaptic binding partners and IL1-R1 were detected using co-immunoprecipitation from neuronal cultures and quantified.

Results: IL-1β bi-directionally regulates cortical connectivity in a dose-dependent manner. At elevated concentrations similar to those found in the MIA mouse model, IL-1β decreases synapse density through altering the synaptic localization of members of the IL-1β receptor complex that double as trans-synaptic adhesion molecules. Elevated IL-1β specifically alters the synaptic enrichment and trans-synaptic interactions of IL-1 receptors with PTPδ. Surprisingly, these elevated levels of IL-1β also increase MHCI expression on neurons and MEF2 signaling, which are required for IL-1β to decrease synapse density. Both of these mechanisms underlying IL-1β signaling mediate the effects of MIA on the early development of cortical connections. We are currently studying how these two signaling pathways interact to regulate synaptogenesis.

Conclusions: Elevations in IL-1β signaling in the brains of newborn offspring activate the MHCI-MEF2 signaling pathway in neurons and decrease IL-1 receptor adhesion to cause deficits in synapse formation during development and in disease, which may be a central mechanism underlying aberrant cortical connectivity in SZ and ASD.

Keywords: Neuroimmunology, schizophrenia, autism Spectrum Disorders, glutamatergic synapses, cytokines

Disclosures: Nothing to disclose.

W205. Development of a Platform Agnostic Software Engine to Facilitate Widespread Adoption of Cognitive Remediation Therapy in Schizophrenia

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Background: Studies have shown significant disruption in cognition in those with schizophrenia; with a strong relationship to poor long-term outcomes. Many studies have focused on developing tools to not only quantify the level of deficit but to also improve function. This is called cognitive remediation therapy (CRT). The systems powering CRT are generally a set of neuroscience tools operationalized into a specific technology framework with a variety of commercial and public domain offerings. While there are many choices there has been limited success in widespread adoption in schizophrenia specifically. There are many possible explanations for the limited adoption but we posit a major cause has been lack of an open solution that is technology agnostic and platform independent. With the dramatic change in technology over the last 10 years, researchers, clinicians, and patients have new expectations about how technology fits into their lives and specifically their health. Many CRT platforms do not take these expectations into account and instead rely on old technologies or passe trends. In order to gain widespread acceptance, a new methodology is required. Our solution, called project plasticity, is a new platform that hybridizes traditional neuroscience tasks with a popular video game engine and a cloud computing backend.

Methods: Our system is based on a series of well-defined cognitive tasks alongside a fully extensible framework allowing custom tasks to be developed as needed. Tasks are written in either C# or JavaScript and utilize our framework in addition to the framework provided by the Unity game engine. The Unity engine is a leading video game development platform which provides task developers with robust 2d and 3d tools capable of being run on all modern platforms including desktop, mobile, webgl, AR, and VR. Our extensible framework sits between unity and the tasks and provides core services for many of the common cognitive engine needs (adaptive difficulty, response timing and verification, etc). It uses an Inversion of Control (IoC) paradigm to facilitate loose coupling and extensibility. Our framework also provides a pluggable cloud-based backend that is robust, resilient, and secure; capable of supporting any of the common core cloud providers. It scales elastically based on user demand and is able to allow focused management of data processing streams. Finally, the system encourages openness and transparency at every level so that patients, clinicians, and researchers can clearly document task operation and their expected outcomes; all based on research principles.

Results: The system is currently in the 4rd round of iterative development. Two tasks (reversal learning and digit span) have been developed alongside core underlying services. Tasks have been tested under android, windows, and mac but also have the capacity to run on ios and a web browser under webgl. Tasks are configurable with respect to task parameters (number of trials and task complexity) and data is being collected on timing, accuracy, and reliability and uploaded to a cloud-hosted backend.

Conclusions: Cognitive remediation is an important and relevant area with the potential to facilitate significant change in long term outcomes. While neuroscience is moving forward rapidly, the technological implementations available to support wide-spread adoption are lacking. Due to the explosion of mobile devices, the cloud, and alternative platforms, new, exciting tools have become available to developers. It is now time to use these tools to build a next-generation platform that will enable improved

access for patients, clinicians, and researchers. The goal being to evoke widespread change in an otherwise difficult to treat illness.

Keywords: cognitive remediation, first episode schizophrenia, non pharmacological interventions

Disclosures: Nothing to disclose.

W206. Preliminary Longitudinal Study Examining the Clinical Correlates of Medication Adherence Assessed via a Mobile Health Application in Early Psychosis Care

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Background: Failure to adhere to medication is associated with a fivefold increase in risk of relapse, higher rates of hospitalization, and approximately 33 more days in hospital per year in individuals with psychotic illness. A recent meta analysis (Anglada-Martinez et al., 2015) suggested that mobile health applications may improve medication adherence, but findings were mixed. We tested a smartphone application that gathers survey data on patients' clinical symptoms and medication adherence in an outpatient early psychosis program.

Methods: Early psychosis participants (N = 36), ages 14-30, were enrolled from the University of California Davis Early Psychosis Programs. Mobile data collected prospectively over 4 months via smartphone application included daily and weekly surveys assessing basic symptoms, mood, medication adherence, and social contact. Gold-standard assessments of medication adherence (MARS), symptoms (BPRS) and psychosocial functioning (GFR/GFS) were conducted in person monthly. Repeated measures analysis examined longitudinal relationships between medication adherence (smartphone data and MARS) and clinical assessments.

Results: Survey completion via the smartphone application was high (average 86%). Preliminary data indicates that 52% of the sample reported high adherence on the MARS (score of 8 or higher) at baseline. At baseline, higher self-reported adherence on the MARS was significantly associated with lower clinician-rated symptoms of aggression/mania (p < .01), depression/anxiety (p < .05) and a trend toward lower positive symptoms (p = .10) on the BPRS. Over the 4 month follow up, participants in the Low Adherence (LA, N = 17) group showed a significant increase in self-reported adherence on the MARS (p < .0001). High Adherence (HA) participants showed a small but statistically significant decline (p < .05), although this was associated with less than a 1 point change on the MARS. Improvement in MARS adherence was correlated with reductions in BPRS aggression/mania and negative symptoms (p = .05). Similarly, weekly self-report data gathered via the smartphone application also showed increased medication adherence over time, with a significant improvement observed in the LA group (p < .05). However, none of the associations between smartphone data and BPRS symptoms reached statistical significance.

Conclusions: Results indicate 1) high level of acceptance of smartphone application as a method for collection medication adherence data; 2) generally high rates of medication adherence reported by study participants in an outpatient early psychosis program; 3) participants with higher medication adherence at baseline show less severe psychiatric symptoms; 4) increased rates of adherence over time are associated with reductions in clinical symptom severity. Additional analyses in a larger sample will be presented. Impact of insight on adherence, as well as relationship to functional outcomes, will be addressed. Results will inform development of a controlled study investigating the impact of mobile health-delivered interventions to improve adherence.

Keywords: antipsychotic medication, early psychosis, Medication adherence, mobile health, smartphone

Disclosures: Nothing to disclose.

W207. Functional Imaging of Working Memory, Episodic Memory, and Social Stress

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Background: Childhoods in urban relative to rural environments have been suggested to increase risk for neuropsychiatric disorders including psychosis. The long-term goal of our China-U.S. study is to elucidate urban-rural upbringing effects on brain function and disease risk. Medial prefrontal (mPFC), striatal and amygdalar function have been implicated in mediating social and environmental stress risk mechanisms. Here, we report pilot fMRI data on these brain functions using an episodic memory paradigm with aversive and neutral pictures, and a novel paradigm engaging working memory (WM) with and without social-competitive stress.

Methods: Twenty healthy individuals were scanned in a 3T GE MRI scanner. The WM paradigm engaged events associated with WM context encoding, maintenance and computation of numerical information. These processes were performed with or without social-competitive stress featuring a similar gender competitor doing better at the same task. The episodic memory paradigm engaged incidental encoding and subsequent retrieval of aversive and neutral pictures.

Results: Behavioral performance during the WM paradigm was generally faster under social-competitive stress. Across WM events, social-competitive stress was associated with relatively increased mPFC (0,59,16; t = 5.01), and decreased striatal activation (-18,17,-5; t = 5.58). MPFC engagement under stress was accentuated during WM computation relative to maintenance (4,50,36; t = 4.51). Encoding aversive relative to neutral pictures engaged overlapping mPFC regions (10,50,20; t = 3.94), as well as bilateral amygdalae (t > 5).

Conclusions: Consistent with previous reports, we found interactions between cognition and emotion engaging mPFC, striatum and amygdalae. How these brain regions are differentially modulated by childhood environments

and genetic risk for psychotic illness will be explored in forthcoming studies with larger samples.
Keywords: Gene–environment interaction, childhood urban-rural upbring, functional magnetic resonance imaging, working memory, episodic memory
Disclosures: Nothing to disclose.

W208. Polysomnographic Characterization of Nocturnal Sleep in Cynomolgus Macaques

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Background: Macaques are a diurnal species that exhibit consolidated night-time sleep with evidence of NREM (Stages 1-4/N1-N3) and REM sleep stages similar to humans. Since many psychiatric medications can affect sleep, we sought to determine whether Cynomolgus macaques are a useful non-human primate (NHP) species in which to conduct polysomnographic sleep/wake studies with translational potential to humans. Accordingly, we determined sleep/wake and electroencephalographic (EEG) parameters in macaques by telemetry under normal conditions and following the administration of the psychostimulant caffeine.
Methods: Adult, male Cynomolgus macaques (n = 3), housed individually under LD12:12 conditions in a temperature-controlled recording room, had access to water ad libitum and were fed a full daily ration of food in addition to enrichment (e.g., fruit, vegetables, nuts). Animals were implanted with telemetry transmitters (D70-EEE; DSI, St. Paul, MN) for EEG and electromyography (EMG). Stainless-steel screw electrodes were placed epidurally above the left and right pre-frontal cortex with references in posterior parietal/occipital cortex. The EMG electrode was implanted into the trapezius muscle to detect muscle tone during sleep. Acticals (Philips Respironics, OR) were clipped onto the animals' collars to measure gross locomotor activity continuously. EEG/EMG recordings, complemented by infrared video recordings, were initiated prior to light offset and continued throughout the 12-h dark period following administration of either saline or caffeine (10mg/kg; i.m.). The EEG was scored by two trained observers who adapted the guidelines of the American Academy of Sleep Medicine (AASM, 2007) for scoring human sleep records. In addition, EEG activity during wake, NREM and REM sleep during each 30s epoch was quantified by spectral analysis.
Results: Vehicle-treated Cynomolgus macaques showed a sleep onset latency (SOL) of 14.5 ± 2.5 min after light offset, 112.7 ± 9.8 min of Wakefulness after initial sleep onset (WASO), and a Sleep Efficiency of $81.7 \pm 2.0\%$. Of the 588.5 ± 14.3 min of total sleep time (TST), N1 comprised 3.1%, N2 was 42.12%, N3 was 54.8% and REM sleep comprised the remaining 9.7%. The mean number of NREM/REM cycles in the vehicle-treated monkeys was 10.7 ± 0.7 per night. Initial observations suggest a distinct sleep architecture in which N3 sleep predominates during the first half of the dark period and REM sleep is more consolidated towards the latter part of the night. In addition,

controls showed a REM onset latency of 85.5 ± 24.4 min, similar to that observed in humans. Caffeine was administered immediately prior to the dark phase to induce wakefulness at a time of day when sleep pressure was high. Consistent with the stimulant properties of the drug, caffeine-treated animals showed a significant increase in gross locomotor activity and a prolonged SOL compared to controls (83.3 ± 31.0 vs 14.5 ± 2.5 min). Sleep Efficiency declined greatly to $27.8 \pm 6.1\%$ (p = 0.022) as TST (p = 0.020) and the amounts of both REM (p = 0.004) and NREM sleep (p = 0.032) were significantly reduced during the 12-h dark period. In particular, caffeine-treated animals showed a reduction in N3 sleep compared to controls (p = 0.041). REM onset latency increased (p = 0.044) under caffeine treatment and the number of NREM/REM cycles during the 12-h dark period greatly declined (p = 0.001). Although the large increase in WASO to 436.7 ± 64.7 min did not reach statistical significance (p = 0.061) in this small sample size, along with the other results, is consistent with the conclusion that sleep is disrupted by caffeine in macaques.
Spectral power within the conventional frequency bands was calculated for each vigilance state during the 12-h dark period. As expected, relative delta (1-4Hz) power was higher during NREM ($76.8 \pm 2.9\%$) than both REM ($49.1 \pm 5.6\%$) sleep and Wake ($55.8 \pm 5.6\%$) in vehicle-treated macaques. Similarly, the relative theta (4-8Hz) power was higher during REM ($20.3 \pm 1.6\%$) than NREM sleep ($14.3 \pm 1.4\%$) and wakefulness ($12.7 \pm 0.8\%$). Furthermore, alpha (8-12Hz), beta (15-30Hz) and gamma (30-100Hz) power during Wake were greater than during NREM sleep. Caffeine-treated animals showed significant increases in alpha (p = 0.047) and beta (p = 0.021) during NREM sleep compared to controls. There were also trends toward a reduction in delta (p = 0.067) and increases in sigma (12-15Hz; p = 0.055) power during NREM sleep and beta power during wakefulness (p = 0.072).
Conclusions: Using a telemetric approach to measure EEG/EMG, we determined basal sleep parameters and the architecture of nocturnal sleep in Cynomolgus macaques. Although the general features of human sleep are present in macaques including a REM onset latency similar to humans, the NREM/REM cycle is shorter resulting in more cycles per night. Cynomolgus macaques respond to caffeine with increased activity, longer SOL, less NREM and REM sleep, "lighter" N3 sleep and increased REM latency – all effects previously reported in humans. Together, these results indicate that Cynomolgus macaques show sleep characteristics that are similar to humans under both normal conditions and after treatment with a psychostimulant.
Keywords: sleep, caffeine, wakefulness, telemetry, EEG
Disclosures: Nothing to disclose.

W209. Jumping the Gun: Mapping a Translational Network in Waiting Impulsivity

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Background: Waiting impulsivity or premature responding is the tendency towards anticipatory responding before

target onset. In preclinical studies, waiting impulsivity, measured using the 5-choice serial reaction time task, is both a predictor of compulsive drug use and influenced by drug exposure. Preclinical studies implicate the infralimbic cortex, nucleus accumbens and subthalamic nucleus (STN). We sought to assess waiting impulsivity using a novel translational task, the 4-Choice Serial Reaction Time task (4-CSRT), in disorders of addiction and to map neural correlates using resting state functional connectivity across alcohol misuse and the influence of deep brain stimulation targeting the STN.

Methods: Using a novel translational task, the 4-CSRT, premature responding was measured across multiple disorders of addiction relative to healthy controls. Subjects were separately assessed with resting state fMRI using a novel multi-echo EPI sequence and independent components analysis. Waiting impulsivity was also tested in subjects with obsessive compulsive disorder (OCD) who had undergone deep brain stimulation targeting the STN.

Results: Waiting impulsivity was elevated in abstinent alcohol- (N = 39) and methamphetamine- (N = 23) dependent subjects, binge drinkers (N = 30) and current cannabis and nicotine users compared to healthy controls. Waiting impulsivity correlated with decreased resting-state connectivity of the subthalamic nucleus – ventral striatum – subgenual cingulate in healthy volunteers (N = 55), a network implicated in rodent studies and dissociable from a stopping network. The same network is decreased with severity of alcohol use in social and binge drinkers, and decreased in alcohol use disorders and binge drinkers compared to healthy controls. Using machine learning, STN connectivity predicted classification of alcohol misuse. In OCD subjects (N = 12), deep brain stimulation of the STN influenced waiting impulsivity as a function of baseline impulsivity.

Conclusions: Our findings emphasize the translational relevance of waiting impulsivity focusing on the role of the subthalamic nucleus and dimensional relevance across alcohol misuse.

Keywords: impulsivity, Alcohol dependence, subthalamic nucleus, Resting State Functional Connectivity, deep brain stimulation

Disclosures: Edward Bullmore is employed by GSK.

W210. Cocaine-Induced Neuroplasticity Depends on Behavioral Responding to a Natural Reward

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Background: Withdrawal from psychostimulants such as cocaine and amphetamine has been associated with reduced motivation for natural rewards in both rats and humans. Rats exhibit individual differences in their consumption of naturally rewarding sucrose. There is evidence to suggest that rats with a high preference for sweet substances differ from rats with a low preference in their response to psychostimulants. However, it is yet to be fully explored whether cocaine-related neuronal plasticity differs between these groups. This study examined the effect of a 'binge' cocaine treatment on sucrose preference and the motivation to achieve sucrose reward, and associated changes in neuronal excitability in medium spiny neurons (MSNs) of nucleus accumbens (NAc) shell.

Methods: Individual differences in sucrose consumption were identified using two behavioral tests; sucrose preference, and responding for sucrose reward on a progressive ratio (PR) schedule of reinforcement. For sucrose preference, rats were tested for their preference between a 1% sucrose solution and water over a 12 hour period on 3 consecutive days. Rats were divided in to high and low groups based on percentage sucrose preference. A separate group of rats was trained to respond for a sucrose reward on a PR schedule of reinforcement and divided into high and low responders based on their breakpoints. Sucrose preference or breakpoints were then measured over 5 days of 'binge' cocaine treatments (3 daily injections at 1 hour intervals, 15mg/kg i.p.) and for 2 days after the termination of drug treatment. Following the final sucrose preference or PR session, slices containing NAc were prepared and whole-cell patch clamp recordings were performed from NAc shell MSNs.

Results: In cocaine treated rats trained on the PR schedule for sucrose reward, breakpoints were reduced during the 5 days of cocaine treatment and for 2 days following the termination of treatment. No difference in the magnitude of the reduction was seen between the high and low breakpoint groups. In saline treated rats, breakpoints remained stable for the duration of the experiment. Whole-cell patch clamp recordings from NAc shell MSNs indicated differences between the high and low breakpoint groups on measures of action potential firing and excitatory synaptic strength. In cocaine treated rats tested for sucrose preference, the 'binge' cocaine treatment had no effect on sucrose preference either during treatment or after its termination. There were also no differences in neuronal excitability between the high and low sucrose preference groups.

Conclusions: These results demonstrate that individual differences in motivation to obtain sucrose reward are associated with broad variability in neuronal responses to cocaine. This variability appears to be specific to motivational differences as it was not apparent when rats were divided based on sucrose preference. We are now exploring whether individual motivation to achieve sucrose reward similarly co-varies with measures of membrane and synaptic excitability in the absence of cocaine.

Keywords: Neuroplasticity, cocaine, individual differences

Disclosures: Nothing to disclose.

W211. Imaging CA1-Hippocampal Neuronal Ensembles during Nicotine-Dependent Contextual Associations

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Background: Learned associations between environmental cues and the rewarding properties of addictive drugs are a major cause for relapse among drug addicts. The hippocampus (HIP), although not previously considered to play a

central role in the reward system, is therefore a likely key player in the development of addictive behaviors. The link between learning and memory systems, contextual cues, and reward circuitry is largely unknown. The conditioned place preference (CPP) paradigm attempts to model this aspect of drug reward-associations and can be useful in examining the underlying neural circuitry involved in the formation of drug-associated memories.

Methods: Here we combine in vivo calcium imaging of CaMKIIa CA1 HIP neurons with CPP to study the role of the HIP in nicotine-induced behaviors. We injected AAV5-CaMKIIa-GcAMP6f-eYFP into the HIP CA1 area and after one week, implanted a 1 mm diameter and 4 mm length GRIN lens is 100 microns above the injected region. After another 1-2 weeks, we use a small microscope to observe the neuronal activity in the CA1 area can be observed from the microscope, while the animals are awake and experiencing conditioned place preference for nicotine. We then used a standard unbiased, counter-balanced CPP protocol to observe neuronal activity during the development of nicotine CPP. Mice were pre-tested in the CPP boxes on day 1, and days 2-3, they received saline in the AM and nicotine (0.5mg/kg, s.c.) in the PM for a 20 min session. On day 5, they were tested for nicotine place preference, as determined by the time they spent in the drug-paired chamber post-test minus pre-test. After collecting all data from each session, Mosaic™ is used to preprocess the data by reducing dataset, meaning filer, motion correction. By PCA/ICA, single neuron activity was separated and sorted manually. Each session retrieved one dataset for single neuron spatial filters and one dataset set for their time course. We also generate cellular maps of calcium transient events, and can determine the activity of cells during nicotine place preference behavior.

Results: After comparing neuron activity between two CPP chamber within posttest section, we found distinct patterns of neuronal activity when a mouse enters the nicotine-paired chamber compared to the saline-paired chamber potentially indicating that spatial cue-reward neuronal activity is potentiated during conditioning and formation of a nicotine place preference. We also assessed how CA1, CAMK2 positive neuronal activity during the conditioning sessions to determine how their activity may change during the conditioning behavior. Additional studies were conducted to assess how the activity of these CA1 neurons is regulated during discrete cue associations during reward seeking, during a sucrose FR1 operant task. Finally, studies were conducted in which AAV5-CaMKIIα-HA-hM4D(Gi)-IRES-mCitrine was injected into the CA1 HIP region and we found that silencing CA1, CAMKII+ cell activity is sufficient to block the development of nicotine-induced CPP.

Conclusions: The ability to visualize network activity and calcium transients during nicotine-dependent behaviors provides a new window into how cue-reward associations are formed, and specifically provides temporal and spatial dynamics during this pavlovian behavioral task. Taken together, these data identify unique evidence for CA1 HIP region in nicotine-contextual associations and begin to further dissect the circuitry mediating the development of drug-reward cue associations.

Keywords: nicotine, calcium imaging, Endomicroscopy, conditioned place preference, Hippocampus
Disclosures: Nothing to disclose.

W212. Phasic Dopamine Release Elicited by Unexpected Presentation of Drug-Paired Cues Increases with Protracted Drug-Access

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Background: Altered dopamine transmission is implicated in most contemporary theories of drug abuse, however the timing and directionality of these changes remain a matter of heavy debate. Dopamine in the nucleus accumbens core (NAcc) seems to play an important role in producing drug "satiety" and thereby regulating drug intake. Recent work from our laboratory demonstrating a causal link between decreases in NAcc dopamine release to cues during drug-taking and the escalation of cocaine intake are consistent with this drug "satiety" theory. In contrast, there are a multitude of studies demonstrating a role for NAcc dopamine in mediating the motivation to seek drug ("craving") elicited by drug-related cues. How is it that dopamine transmission within the NAcc could serve as both a satiety signal and produce craving? We hypothesized that changes in phasic dopamine transmission that occur following chronic drug use are dependent on the mode of cue presentation and contribute to different, but equally important, core symptoms of substance use disorders. We have previously shown that decreases in phasic dopamine release elicited by response-contingent cues during self-administration promote drug-taking. Here we predict that dopamine responses elicited by unexpected presentation of the same drug-paired cues promote drug-seeking.

Methods: We used chronically implantable carbon-fiber microelectrodes to measure phasic dopamine release elicited by unexpected presentation of drug-paired cues (CS) using fast-scan cyclic voltammetry at multiple time-points throughout the course of three weeks of cocaine self-administration. Measurements were both taken prior to a self-administration session, when the animal had no drug-in their system, as well as following self-administration when there is drug present. We use both short-access (one hour daily sessions) in which animals typically exhibit very stable drug-intake over time, as well as long-access (six hour daily) sessions in which animals escalate their drug-intake over time and exhibit many other behaviors that are remniscent of the human drug addiction criteria.

Results: We find that phasic dopamine release elicited by drug-paired cues increases over the course of drug-taking, and that animals that have undergone long-access self-administration exhibit much larger cue-elicited responses than those only having short-access.

Conclusions: The observed increases in dopamine release to unexpected presentation of drug-cues may serve to promote drug-seeking. These findings together with our previous work studying behavior-contingent cue responses during self-administration may explain how dopamine transmis-

sion within the NAcc might serve as both a satiety signal and produce craving.

Keywords: cocaine addiction, Dopamine, cues

Disclosures: Nothing to disclose.

W213. Neuroepigenetic Regulation by Extra-Coding RNA

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Background: Methylation of cytosine bases in DNA is a critical regulator of the function and plasticity within the central nervous system. In the developing brain, neurons exhibit unique DNA methylation patterns that are correlated with synaptogenesis and regulate the expression of neuronal genes. In the adult brain, active DNA methylation is required for memory formation and maintenance, and neuronal activity and behavioral experiences lead to site-specific reorganization of DNA methylation dynamics. In addition, maintenance of DNA methylation patterns at specific genes is disrupted in numerous cognitive and neurodegenerative disorders. Despite the well-appreciated role of DNA methylation in neuronal function and physiology, the mechanisms by which individual genes or sequences of DNA are targeted for active methylation or demethylation are presently unclear. Emerging evidence from other systems suggests that site-specific regulation of DNA methylation can occur via non-coding RNA species, which bind to DNA methyltransferases and direct gene-specific methylation patterns. However, the extent and nature of this regulation in neurons has not been explored.

Methods: Here, we first utilized whole-genome sequencing in neuronal systems to identify and characterize the relationship between a recently described non-coding RNA species termed extra-coding RNA (ecRNA) and DNA methylation status across the genome. Next, we explored activity-dependent properties of ecRNA regulation and function using in vitro neuronal stimulation and inactivation, RNA polymerase inhibition, methylated DNA immunoprecipitation, antisense mediated ecRNA knockdown, RNA immunoprecipitation, and electrophoretic mobility shift assays. Finally, we explored a role for ecRNA in memory formation using contextual fear conditioning.

Results: Using genome-wide sequencing approaches together with in vitro neuronal culture systems, we found that the transcription of non-polyadenylated ecRNA species from protein-coding genomic loci is a widespread phenomenon in neurons. ecRNA expression is associated with decreased promoter methylation and enhanced mRNA expression, is regulated by neuronal activity, and is overrepresented at genes involved in synaptic transmission and cognitive function. Using the ecRNA arising from the Fos gene locus as a representative candidate, we found that ecRNA undergoes unique biogenesis, is sensitive to multiple forms of neuronal activity, and binds to both de novo and maintenance DNA methyltransferases. Anti-sense mediated knockdown of the Fos ecRNA locus results in gene hypermethylation and blunted Fos mRNA transcription, suggesting that ecRNAs act to inhibit DNA methylation at overlapping genes. Finally, we show that hippocampal expression of Fos ecRNA is required for the formation of long-term fear memories, demonstrating functional and behavioral relevance of ecRNAs in vivo.

Conclusions: These results suggest that extra-coding RNAs interact directly with DNA methyltransferases to inhibit neuronal DNA methylation in a gene-specific manner. As such, ecRNAs serve as fundamental regulators of the establishment and perpetuation of DNA methylation patterns in neuronal systems, and reveal a promising avenue for therapeutic targeting in neurological and cognitive disease states.

Keywords: Epigenetics, long noncoding RNA, DNA Methylation

Disclosures: Nothing to disclose.

W214. Recruitment of a CRF-Regulated Dopaminergic Projection from the VTA to the Prelimbic Cortex Results in Heightened Susceptibility to Stress-Induced Relapse

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Background: Although a strong relationship exists between stress and cocaine addiction, the ability of stressors to directly trigger cocaine craving/use in human addicts and preclinical rodent models is variable and is determined, in part, by the amount and pattern of prior drug use. Stress-triggered relapse can be studied in rodents using a self-administration/shock-induced reinstatement approach and our lab has previously reported that reliable shock-induced drug seeking is only observed in rats with a history of self-administration under conditions of daily extended access and high drug intake. In this study we examined the role of a critical corticotropin releasing factor- (CRF-) regulated dopaminergic projection from the ventral tegmental area (VTA) to the prelimbic cortex in shock-induced reinstatement and its recruitment under self-administration conditions that establish relapse vulnerability.

Methods: Adult male Sprague Dawley rats were implanted with intravenous catheters and trained to self-administer cocaine (0.5 mg/kg/inf) or provided access to saline by pressing a lever under a fixed-ratio one schedule of reinforcement during daily 2-h sessions. Once responding stabilized, rats were assigned to either long-access (LgA; 14 x 6 hrs/day), short-access (ShA; 14 x 2 hrs/day) or saline (Sal; 14 x 2 hrs/day saline) groups according to their self-administration test conditions for the next 14 days. Following self-administration, rats underwent extinction training prior to testing for reinstatement in response to electric footshock [15 min; 0.5 mA, 0.5 s duration, mean inter-shock interval = 40 s (range 10-70 s)] and/or brain excision and processing for analysis of Fos expression alone (nickel-enhanced diaminobenzide) or in cells labeled with site-specific injections of the retrograde tracer cholera toxin B subunit (ctB; diaminobenzide), or VTA CRFR1 mRNA levels using in situ hybridization (35S-labeled riboprobe). Site specific antagonist injections were used to determine

the requirement for VTA CRFR1 receptors (antalarmin, 250 ng/injection) and/or cortical D1 dopamine receptors (SCH 23390; 200 ng/injection) for reinstatement and/or Fos responses.

Results: As we have previously reported, shock produced robust reinstatement following LgA but not ShA self-administration and did not increase responding in Sal controls (n = 8-10/group; p < 0.05 LgA shock vs. LgA no shock and Sal shock). Augmented shock-induced reinstatement in LgA rats was accompanied by a heightened shock-induced Fos response in the prelimbic but not infralimbic cortex (n = 8-10/group; p < 0.05, LgA shock vs. all other group). Both shock-induced reinstatement and the shock-induced Fos response in the prelimbic cortex in LgA rats were prevented by intra-VTA administration of the CRF-R1 antagonist, antalarmin (n = 6/group; p < 0.05; antalarmin vs. veh in prelimbic and not infralimbic). Using retrograde labeling with CTb, we found that stress-induced cocaine seeking in LgA rats was associated with increased Fos expression in VTA neurons that project to the prelimbic cortex (n = 7-9/group; p < 0.05 vs. no shock). To determine if the CRF-regulated dopamine pathway from the VTA to the prelimbic cortex plays a functional role in stress-induced cocaine seeking, we used a pharmacological disconnection approach. Injection of antalarmin into the VTA of one hemisphere and the D1R antagonist SCH23390 into the prelimbic cortex of the contralateral hemisphere prevented shock-induced reinstatement (n = 5/group; p < 0.05 vs. veh). By contrast administration of antalarmin and SCH23390 in the ipsilateral hemisphere or disconnection of the CRF-regulated pathway to the infralimbic cortex failed to block shock-induced reinstatement. To test the hypothesis that the VTA-prelimbic cortex pathway that mediates stress-induced reinstatement was recruited following LgA self-administration as a result of increased CRFR1 expression, we employed in situ hybridization. Indeed, CRFR1 mRNA was increased in the posterior VTA (the site of CRF regulation of cocaine seeking) following LgA but not ShA self-administration (n = 8-11/group; p < 0.05 LgA vs. Sal).

Conclusions: Altogether, these findings implicate a CRF-regulated dopaminergic projection from the VTA to the prelimbic cortex in stress-triggered cocaine seeking and suggest that this pathway is recruited following excessive cocaine use via upregulation of CRFR1 receptors in the VTA to establish relapse susceptibility.

Keywords: relapse, stress, cocaine addiction, Corticotropin-releasing factor (CRF), prelimbic

Disclosures: Nothing to disclose.

W215. Reasons for Change in Alcohol Drinking Behaviors in a Native American Community Sample

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Background: Only 22% of people with alcohol-related problems who recover do so with medical services or self-help groups, leaving the vast majority to recover without treatment. Additionally, national surveys have revealed that women and minorities in particular are less likely to utilize available treatment options. Understanding why some problem drinkers overcome their problems with alcohol and others do not and identifying the intrapersonal and environmental influences that cause a person to increase or decrease their drinking over time are important as they can lead to a better understanding of the recovery process and may suggest new approaches that can be implemented in community based interventions. Native Americans have some of the highest rates of heavy drinking and alcohol related disability, yet little is known concerning the factors that influence changes in alcohol consumption over the lifetime in this ethnic group.

Methods: The present study collected data from 237 Native American adults residing on 8 geographically contiguous Indian reservations who were participating in a larger study on risk factors for alcoholism. Participants were assessed for demographic and medical information, substance use and other psychiatric disorders using the Semi Structured Assessment for the Genetics of Alcoholism (SSAGA). Information on their quantity and frequency of drinking and what they perceived as the "reasons for change" in their drinking behaviors over the course of their drinking history was assessed using a timeline follow-back methodology. Reasons to increase alcohol consumption and reasons to decrease consumption were both evaluated.

Results: The most commonly endorsed reasons to increase drinking were: pressure from peers, exposure to parties and celebrations, and family stress. The reasons for decreased drinking were: health/pregnancy, lost interest in drinking, family pressure, went into jail or mandated rehab, and new work responsibilities. Many individuals could not identify a reason for change in their drinking levels and this was significantly more common in males (p < 0.01). Women were more likely to decrease their drinking in response to health/pregnancy concerns and more males decreased drinking in response to being in jail/mandated rehab (p < 0.01). Having higher Native American heritage, low economic status and not having completed high school were all associated with more endorsements of increases in drinking (p < 0.01). Anxiety and affective disorders were associated with endorsing family stress and health consequences as reasons for changes in drinking behavior.

Conclusions: These results suggest that there are a range of factors that individuals believe influence their motivation to change their drinking behaviors that differ based on gender and comorbid conditions. Using data from reasons to change evaluations may prove important in tailoring intervention and prevention programs in this high risk population.

Keywords: Native American, drinking, reasons for change

Disclosures: This study was supported by 5R37 AA010201. The authors have no disclosures.

W216. Altered Neural Processing to Social Exclusion in Young Adult Marijuana Users

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Background: Marijuana use is steadily increasing among young adults. Particularly for adolescents, peer groups are

one of the most important predictors of marijuana initiation, progression to regular use, and failure to discontinue use (van den Bree and Pickworth 2005). However, the relationship between peer group influence and drug use is complex. On one hand, people tend to use marijuana in social settings among friends (Terry-McElrath, O'Malley et al. 2009). On the other hand, some studies have reported that youth who are isolated from their peers are more likely to use tobacco than those who belong to peer groups (Bailey, Ennett et al. 1993). It is possible that people who use drugs are more sensitive to social exclusion than non-users, and that their desire to avoid social rejection underlies their desire to use drugs in social settings. In this study, we examined whether young adults using marijuana had altered neural responses to social exclusion relative to non-using control participants.

Methods: We examined the neural mechanisms underlying social exclusion in young adults who use marijuana (MJ, n = 20) and in non-using controls (CON, n = 22). MJ used marijuana at least once a week, and were asked to refrain from using substances on the day of the study. To elicit feelings of social exclusion, we used Cyberball, an interactive computerized ball-toss game (Williams, Cheung et al. 2000), which has been used extensively in functional magnetic resonance imaging (fMRI) studies to examine brain responses to ostracism. As in previous Cyberball studies, participants were told that we were interested in 'mental visualization' ability, so that participants were not aware that we were studying social exclusion. They were told that they would play a game of catch over the Internet with two other players, and were asked to try to imagine the experience as vividly as possible. In reality, the actions of these 'other players' were actually pre-programmed to include or exclude the participant in different phases of the task. There were four successive blocks: (a) spectating block, in which participants were told that they would just be observing the other players; (b) inclusion, in which participants played with the other players; (c) exclusion, in which participants were then excluded from the game, and (d) re-inclusion, in which participants were re-included in the game for the remainder of the experiment. In addition to the MRI scan, participants also completed the Multi-dimensional Iowa Suggestibility Scale (MISS), which measured peer conformity as well as other indices of suggestibility. They also completed a questionnaire administered immediately after the Cyberball task to assess exclusion-related distress.

Results: Neuroimaging results indicated that during exclusion compared to fair play (inclusion), CON activated the right anterior insula and ventral anterior cingulate cortex (vACC), regions reported as regularly activated by this task in a meta-analysis (13), as well as the frontal poles and the lingual gyrus. MJ, in contrast, activated only the vACC in response to social exclusion. A direct comparison between groups indicated that CON had greater activation than MJ in two clusters in the right anterior insula, one of which also encompassed the OFC, but the groups did not differ in vACC activation.

The post-game questionnaire indicated that both groups felt mild distress following the experiences of social exclusion (CON = 17.4, MJ = 17.5) with no difference between groups (p = 0.95). MJ scored significantly greater on peer conformity on the MISS than CON (t (1,40) = 2.08, p = 0.044. Across groups, peer conformity scores were positively associated with vACC activation (r2 = 0.10, p = 0.042). The correlation across groups was driven by the MJ group (r2 = 0.35, p = 0.006); no correlation was detected in CON for peer conformity self-ratings and vACC activation to social exclusion. Neither group showed correlations between self-reported suggestibility scores and insula activation.

Conclusions: This study is the first to examine neural signals of social exclusion in young adult marijuana users, and has several novel findings. First, though both groups reported similar levels of distress following social exclusion, the MJ group did not demonstrate activation in the right anterior insula during peer rejection, yet they showed normative responses in the vACC. Second, though the groups rated similar levels of task-related distress, MJ self-reported higher levels of peer conformity than CON. Finally, vACC activation correlated with levels of self-reported peer conformity and total suggestibility in the MJ group but not the CON group. This dissociation between vACC and insula suggests that while the MJ group was cognitively aware of being excluded (a function of the vACC), they were not emotionally responsive to the exclusion (a function of the insula). vACC activation in the MJ group was also proportional to social conformity scores, indicating that those who were more likely to report social conformity experienced greater monitoring of affective states during the task. In conclusion, this study suggests a neural correlate of social exclusion that functions differently in individuals with regular marijuana use. This differential activation in response to social exclusion may be cause or a result of marijuana use.

Keywords: inclusion and exclusion, social interaction, functional neuroimaging, rejection, cannabis use

Disclosures: Nothing to disclose.

W217. Mesocortical Dopamine Encodes Cocaine Cues after Chronic Cocaine Self-Administration via Enduring Inhibition of Kv7 Channels

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Background: In human cocaine addicts, drug-associated cues induce hyperactivity in the prefrontal cortex (PFC), precipitating drug craving and relapse. While the underlying mechanisms are unknown, they may involve cellular adaptations in dopamine receptor signaling. Pyramidal cells in the PFC normally display robust spike-frequency adaptation (accommodation) that limits repetitive neuronal firing. Acute activation of dopamine receptors, however, removes this intrinsic (potassium channel mediated) brake, transiently increasing neuronal excitability. While multiple potassium channels are involved in this form of intrinsic inhibition, it is unknown whether specifically Kv7 channels in PFC neurons play a role in drug-seeking behavior and whether their role changes after a history of cocaine experience.

Methods: Using whole cell patch clamp electrophysiology we recorded accommodation and after-hyperpolarization from L5 pyramidal cells in the prelimbic PFC of rats. We prepared slices containing the prelimbic PFC from adult rats that were either: (i) naïve, (ii) had a history of chronic cocaine self-administration and extinction training, with or without re-exposure to cocaine-paired cues, (iii) were yoked to cocaine administering rats, or (iv) were trained to lever press for sucrose. In another group of rats, we stimulated ventral tegmental area (VTA) terminals in acute brain slices. Therefore, Cre-dependent AAVs were injected into the VTA of TH-Cre rats to selectively transfect VTA dopamine cells with channel rhodopsin (ChR2) or synthetic receptors (DREADDs). After ∼30d, PFC brain slices were prepared and VTA terminals were stimulated with transient pulses of blue light (to activate ChR2) or bath application of CNO (to activate DREADDs).

Results: After chronic cocaine self-administration and extinction, prelimbic neurons of the PFC demonstrated elevated firing rates, loss of spike accommodation, and reduced AHPs under baseline conditions. This adaptation was absent in slices from either yoked-cocaine rats that received identical amounts of cocaine but were not subjected to drug-related cues, or from rats trained with cues on sucrose self-administration.

We next tested if dopamine release from VTA terminals could reproduce this loss of inhibition observed after chronic cocaine self-administration. In PFC slices from cocaine-naïve rats, acute stimulation of VTA terminals using DREADDs increased the excitability of cells projecting to the nucleus accumbens core by inhibiting accommodation and the AHP. Stimulating VTA terminals with ChR2 produced similar effects. Co-application of cocaine increased the percentage of cells rendered hyperexcitable by VTA terminal stimulation, and inclusion of dopamine-D1 receptor antagonists prevented this plasticity. Furthermore, bath application of dopamine mimicked the effects of VTA terminal stimulation (EC50: 18uM), and inclusion of cocaine (1uM) in the bath shifted the dose response curve leftward (EC50: 8nM). These data suggest that by blocking dopamine reuptake, cocaine potentiates the influence of dopamine released from VTA terminals in the PFC. The observed loss of accommodation after chronic cocaine self-administration may result from chronic activation of this circuit.

We then examined whether stimulation of cortical VTA terminals alters drug-seeking behavior in rats with a history of cocaine experience. In TH-Cre rats transfected with DREADDs in the VTA, infusion of CNO into the PFC prior to an extinction day did not induce drug-seeking. However CNO infusion prior to a cue-reinstatement test potentiated reinstatement compared to cue + vehicle infusion. These data indicate that stimulation of cortical VTA terminals potentiates drug seeking in response to cocaine-paired cues.

Lastly, we found that enhancing Kv7 channel function by bath application of retigabine restored both accommodation and Kv7 channel currents (but not the AHP) in tissue from rats with a history of cocaine self-administration. Furthermore, in vivo infusion of retigabine into the PFC prior to cue-induced reinstatement testing blocked cocaine-seeking behavior.

Conclusions: Taken together these data suggest that VTA terminals utilize a DA-D1 receptor mechanism to regulate PFC excitability. In the transition from acute exposure to chronic cocaine self-administration and extinction, this mechanism becomes superactivated, resulting in reduced Kv7 channel mediated inhibition and repetitive neuronal firing in response to depolarizing (excitatory) inputs. This neuroadaptation may underlie the enhanced saliency of drug-related cues that trigger relapse in cocaine addicts.

Keywords: Dopamine, cocaine, prefrontal cortex, Ventral tegmental area (VTA), addiction

Disclosures: Nothing to disclose.

W218. Effects of DRD4 VNTR Genotype on Alcohol Cue-Elicited Brain Activation among Treatment-Seeking Alcoholics

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Background: Despite decades of research, the relationship between dopamine (DA) signaling and addictive behavior remains unclear. In animal and human models of addiction, both potentiated and attenuated DA tone and signaling have been reported. The DA D4 receptor is a G-protein-coupled receptor that is densely expressed in the prefrontal cortex (PFC) and, when bound by DA, initiates a signaling cascade that ultimately inhibits cyclic adenosine monophosphate (cAMP). A 48-base-pair variable number tandem repeat (VNTR) polymorphism in the gene that encodes the D4 receptor, DRD4, has been associated with differential cAMP inhibition, such that receptors encoded by the "long" allele (7 or more repeats), relative to those encoded by the "short" allele (6 or fewer repeats), display reduced cAMP inhibition, and thus reduced response to DA signaling. Among younger individuals (e.g., heavy college drinkers, early-stage alcoholics), the DRD4 long allele has been associated with greater craving for alcohol after cue exposure and less stimulation and arousal after drinking, suggesting that DA insensitivity may underlie these phenomena. However, the variant's effects among older individuals with more severe alcohol problems are less clear. The current study tested the effects of DRD4 VNTR genotype on alcohol dependence severity and alcohol cue-elicited brain activation in a large sample of treatment-seeking individuals with alcohol dependence.

Methods: One hundred and twenty-five treatment-seeking Caucasians with alcohol dependence (mean age = 50, 62% male, 11 drinks/drinking day, with 93% heavy drinking days in the past month) provided a blood sample for DNA analysis and completed an alcohol cue reactivity fMRI task immediately prior to medication randomization in a randomized clinical trial. Genotyping was conducted with polymerase chain reactions, using custom primers and gel electrophoresis. Alcohol dependence severity and cue-elicited activation were compared between individuals homozygous for the DRD4 short allele (S/S) and those who carried at least one copy of the long allele (L/x).

Results: Genotype frequencies were consistent with expected frequencies for Caucasian individuals (S/S = 80, L/S = 42, L/L = 3) and in Hardy-Weinberg equilibrium. Relative to the S/S group, the L/x group had lower Alcohol Dependence Scale scores (S/S mean = 15.2, L/x mean = 13.0; p = .04) and displayed less alcohol cue-elicited activation, particularly in cortical areas, including the medial PFC, orbitofrontal cortex, and inferior frontal gyrus (z > 2.3, cluster-corrected p < .05).

Conclusions: Collectively, these data suggest that, among older, treatment-seeking individuals with alcohol dependence, a genetic variant that putatively results in DA insensitivity in the PFC is associated with lower disease severity and reduced cortical activation to alcohol cues. This finding contrasts with data from younger individuals that have suggested an association between the DRD4 long allele and intermediate phenotypes of addiction. This disparity might suggest that, although cortical DA insensitivity may be a risk factor for the development of addictive behavior, among individuals who transition to more severe alcoholism, this insensitivity confers some protection, perhaps because it reduces the cortical response to alcohol cues. Further, it might indicate that DA-acting drugs used for relapse prevention might be more effective in DRD4 long-allele carriers.

Keywords: fMRI/imaging genetics, Cue Reactivity, Dopamine

W219. Sex-Differences in Grey Matter Volume in Cocaine Use Disorder: A Voxel-Based Morphometric Study

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Background: Structural imaging studies have demonstrated reductions in grey matter volume (GMV) in the prefrontal cortex (PFC), orbitofrontal cortex (OFC), and subcortical limbic regions suggestive of morphological integrity abnormalities in individuals with cocaine use disorders (iCUD). Functional studies have implicated these regions in learning (reward/punishment), goal-directed behavior, and cognitive control. However, a gap in this literature pertains to a primary focus on men at the exclusion of women. The timely narrowing of this gap is especially important given that the prevalence rate of substance use disorders is increasing more rapidly in women compared with men as shown in recent national studies. A more gender-balanced (or gender-focused) study could also help uncover the mechanisms underlying a seemingly divergent drug use phenomenology as that seen in cocaine use disorders, where women have greater addiction severity than men. Indeed, preclinical and human literature indicates that chronic drug use is associated with sexually dimorphic alterations in specific brain regions, especially those that exhibit higher levels of sex-steroid receptors during development, which are also implicated in the addiction phenomenology. Thus, the goal of this study was to evaluate GMV in iCUD and healthy controls as a function of diagnosis and sex.

Methods: Participants were 22 iCUD (13M/9F) and 25 healthy controls (12M/13F). Groups did not differ on age, education, race, or estimates of verbal and non-verbal intelligence. All iCUD met current or lifetime DSM-IV criteria for cocaine abuse or dependence. Participants' drug use histories were assessed by a semi-structured clinical interview and baseline craving and cue-induced drug-seeking were assessed with the Cocaine Craving Questionnaire. T1-weighted anatomical images were acquired with a 3D MPRAGE sequence using the 3 T Siemen's Skyra. Normalized, nonmodulated grey matter maps were smoothed with an 8-mm full-width at half maximum Gaussian kernel in an SPM8 toolbox.

The independent and interactive effects of diagnosis and sex on GMV were examined in SPM8 using a whole-brain 2 (diagnosis: iCUD, control) x 2 (sex: Male, Female) ANOVA. Comparisons focused on examining sex-differences were further separately carried out using t-tests within iCUD and controls. Significant clusters were defined as those with >20 contiguous voxels, with a Puncorr<0.005 search threshold.

Results: Relative to healthy controls, iCUD had reduced GMV in the bilateral OFC (and right mid temporal gyrus), consistent with prior research that reliably showed OFC structural abnormalities in addiction. Comparisons within groups yielded important preliminary results as follows: (A) Within iCUD, compared with men, women had greater GMV in the bilateral amygdala and dorsal anterior cingulate cortex (BA 24) (while the opposite effect was only observed in visual areas including the calcarine sulcus). The left amygdala GMV was positively associated with baseline craving (r = 0.662 p = 0.037) and the likelihood to use cocaine if in a triggering environment (r = 0.687 p = 0.028) in female iCUD only. (B) Within females, compared to controls, iCUD had lower GMV in the right superior and right mid frontal gyrus (BA 8 and 9) and left OFC (BA 11). (C) Within men, iCUD had lower GMV in the right rectus gyrus/ventromedial PFC compared to controls. Note, analyses are ongoing to replicate these effects in an independent dataset containing comparable sample sizes for all subgroups.

Conclusions: Our results confirm prior findings of decreased GMV in the PFC and OFC in iCUD as compared to healthy controls. Importantly, however, the current study suggests that these PFC differences may be driven by female (more than male) iCUD. Our results also highlight the importance of the amygdala, potentially suggestive of addiction-mediated changes in the brain's stress/alarm system that may contribute to or potentiate aversive or stress-like affective states, directly associated with drug craving in women iCUD. Indeed, stress is a known risk factor for drug abuse and relapse, particularly for women. Future longitudinal studies can test whether these sex differences in GMV in addicted individuals could reflect a predisposition to drug use (i.e., whether they predate addiction) and/or morphologic changes secondary to the chronic drug use (that may be accentuated in women). Taken together, despite a small sample size, our findings

support a growing body of evidence of sex-specific differences associated with chronic cocaine use and speak to the importance of studying women substance users in future research.

Keywords: sex differences, cocaine addiction, women, brain structure, neuroimaging

Disclosures: Nothing to disclose.

W220. Alterations of Glial Glutamate Transporters and Certain Neurotransmitters in Alcohol Withdrawal Syndrome Using Alcohol Preferring Rat Model

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Background: Alcohol withdrawal syndrome (AWS) comprises of series of signs and symptoms (autonomic/ neuropsychiatric), which appear up to 48 hours of abrupt cessation of binge ethanol intake. The ultimate target of the most neurochemical agents used to treat AWS is to restore the balance between excitatory and inhibitory neurotransmission. But less is known about the contribution of glial glutamate transporters to hyper-neuroexcitation in AWS. In this study, we used a model of AWS through abrupt cessation of binge ethanol intake in male alcohol preferring (P) rats to investigate the effects of AWS on glial glutamate transporters, and tissue content of glutamate, glutamine, dopamine and serotonin in prefrontal cortex (PFC) and nucleus accumbens (NAc).

Methods: To simulate AWS model, P rats were exposed to free choice of ethanol (15% and 30%) and/or water drinking for two weeks. P rats received then water or ethanol (4 g/kg/ gavage) through oral gavage three times a day for three days followed by 48 hours of withdrawal period. P rats were further re-exposed to voluntary drinking of ethanol and/or water for one-week. P rats were then euthanized and brain regions such PFC and NAc were dissected. Western blot analysis was used to determine the expression of GLT-1, xCT and GLAST in PFC and NAc. Tissue content of glutamate, glutamine, dopamine and serotonin in both PFC and NAc were quantified using HPLC-EC system. Glutamine synthetase (GS) activity was measured through γ-glutamyl transfer assay.

Results: We found downregulation of glutamate transporter 1 (GLT-1) in both PFC and NAc after ethanol withdrawal. The expression of cystine/glutamate exchange transporter (xCT) and GLAST was unchanged in both PFC and NAc. The tissue content of glutamate was significantly lower in these two regions in withdrawal rats whereas tissue of glutamine was significantly higher in PFC but unchanged in NAc. Furthermore, the tissue content of dopamine was significantly lower in both PFC and NAc of ethanol withdrawal rats. However, the tissue content of serotonin and the GS activity were unchanged in these brain regions.

Conclusions: Alcohol withdrawal altered the glutamate-glutamine cycle, which was associated with downregulation of GLT-1 expression in both PFC and NAc. This suggests the important role of GLT-1 in AWS.

Keywords: Alcohol withdrawal syndrome, GLT-1, xCT, glutamate, Alcohol-preferring rats

Disclosures: Nothing to disclose.

W221. Tolerance to Alcohol-Stimulated GluR1 Phosphorylation in the Central Amygdala in the Context of Nicotine Dependence

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Background: Nicotine and alcohol are two of the most commonly abused substances. Nicotine use increases alcohol drinking, suggesting that the combination of these drugs may produce synergistic effects in activating reward circuitry. Alternatively, excessive use of either of these drugs may facilitate the development of cross-tolerance to the other to promote intake escalation. Mechanisms underlying these interactions are largely unknown, and there is little information on neuronal signaling events that occur in animals exposed to both drugs.

Methods: In the present experiment, adult male Wistar rats were exposed to room air (non-dependent group) or intermittent nicotine vapor (nicotine-dependent group) for 12 hours per day (resulting in 12 hours of withdrawal per day) for three weeks. This treatment regimen has been shown to produce symptoms of nicotine dependence as evidenced by hyperalgesia and elevated nicotine self-administration during withdrawal. On the final day of nicotine exposure, at 10 hours of withdrawal, animals were challenged with either saline or alcohol (1 g/kg, IP) and euthanized 15 minutes later. Brains were snap frozen and dissected for processing via Western analysis. We examined changes in the phosphorylation status of glutamate channel subunits in stress- and reward-related brain regions since excitatory neuroadaptations are heavily implicated in both alcohol and nicotine dependence. All experimental protocols were approved by the Institutional Animal Care and Use Committee and were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Results: We discovered differential alterations in glutamate channel subunit phosphorylation following nicotine withdrawal and alcohol challenge. For example, alcohol significantly increased PKA phosphorylation of GluR1 (a subunit of the AMPA receptor) across multiple brain regions, including the hippocampus, cingulate cortex, and central amygdala. However, this neuroadaptation was largely absent following alcohol challenge in the central amygdala of animals exposed to chronic intermittent nicotine vapor exposure (significant interaction between alcohol challenge and nicotine exposure). Interestingly, PKA-mediated GluR1 phosphorylation was also increased in the dorsal striatum following alcohol challenge, although pGluR1 levels were reduced as a main effect of nicotine dependence, indicating an antagonistic relationship between withdrawal from chronic nicotine and acute alcohol exposure in this region.

Conclusions: These data suggest a functional tolerance to alcohol-stimulated phosphorylation of GluR1 (a subunit of the AMPA receptor) in the central amygdala and dorsal striatum in the context of nicotine dependence. As a consequence, more alcohol may be required to influence glutamatergic signaling within these regions in

the nicotine-dependent state, and this may in part facilitate increased drinking in heavy smokers.

Keywords: Alcohol, Nicotine dependence, AMPA glutamate receptors, Central nucleus of the amygdala, Tolerance

Disclosures: Nothing to disclose.

W222. Resting State Functional Connectivity in Rat Brain during Extended Daily Access to Cocaine and Abstinence

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Background: Large-scale neuronal synchrony across distributed brain regions plays a significant role in memory and high-order associative processing, and it may serve in part to enhance the efficiency of neuronal communication. Disruption of this synchrony may underlie various psychiatric diseases, including addiction. Changes in resting state network activity could represent one neurobiological mechanism impeding neural processing, affecting thought, motivation, and motor circuitry in addiction. Interpreting human functional connectivity findings in the context of addiction remains challenging, however, because of difficulties in controlling patient characteristics such as duration of drug use, polysubstance use, and comorbid psychiatric conditions. To address these challenges, we used functional magnetic resonance imaging (fMRI) in a rat model to evaluate brain functional connectivity across stages of cocaine self-administration and abstinence.

Methods: Adult male Long-Evans rats underwent surgery to implant intravenous catheters. One week later, a subset of these rats (n = 8) proceeded to self-administer cocaine (1.0 mg/kg/infusion) in 6 h sessions for 14 consecutive days. The remaining rats (n = 5, control group) performed the same behavioral response to obtain oral access to a sucrose solution. Rats were imaged at three time points: prior to surgery, after 2 days of abstinence, and after 14 days of abstinence. This experimental design allowed for both within- and between-subjects comparisons. Resting state networks have been observed under a variety of conditions in both rodents and primates under anesthesia. To minimize the time inside the MRI scanner and to maintain a uniform imaging procedure across subjects, rats were scanned for 30-40 min under anesthesia (0.02 mg/kg dexmedetomidine (i.p.) 30 min prior to the start of scanning and maintained under 0.5% isoflurane). Rats were scanned on a 4.7 Tesla MRI. A fast spin echo scan was acquired first, followed by two 10-minute fMRI scans (TR = 1 sec; TE = 50ms; 32 mm2 x 12 mm field of view; twelve 1.5 mm slice; 64 x 64 data matrix). Functional images were drift- and motion-corrected and band pass filtered (0.01-0.1Hz) to remove higher frequency signal components. White matter and CSF signals were also removed. Seed ROI's were chosen from the brain reward system based on a rat brain atlas template. Time series from seed ROIs were used for correlating with the rest of the brain on a voxel-by-voxel basis. Correlation maps (z transformed) were group-analyzed using analysis of variance (p < 0.05, cluster size corrected).

Results: Rats escalated their cocaine intake over the course of the 14 self-administration sessions (from approximately 55 to 75 mg/kg/day). Prior to surgery, baseline resting state connectivity maps for 150 ROIs were qualitatively similar between the two groups. At 2 days after self-administration, cocaine rats showed increased functional connectivity across a number of brain regions in the 'Salience Networks', including the Anterior Cingulate (ACg), Prelimbic Cortex, Insular Cortex (Ins), Dorsal Striatum (DS), and Amygdala. Of note was an increase in Insula-to-Dorsal Striatum connectivity that was observed only in cocaine but not in control (sucrose) rats. This was also observed for the Anterior Cingulate and Dorsomedial Striatum seeds. ACg-to-Lateral Hypothalamus connectivity and DS-to-Ins connectivity were also observed to be significant in cocaine but not control rats (p < 0.05, cluster size corrected). Increased connectivity for regions involving the Dorsomedial Striatum and Ins showed a trend towards persisting 14 days after self-administration in cocaine compared to control rats. Additional analyses of the dorsal attention network and default mode network are ongoing.

Conclusions: Several recent neuroimaging studies have reported alterations in synchronous neural activity across brain regions in cocaine users, and similar findings have been reported in rats. Here we show that, using a longitudinal design, 14 days of long access cocaine self-administration produces an increase in functional connectivity in neural networks involving insula, anterior cingulate, and dorsal striatum. This increase in functional connectivity was most evident soon after self-administration, although some components persisted through at least two weeks of abstinence. Importantly, control procedures suggest that the altered functional connectivity was not readily attributable to the surgical or instrumental learning components of cocaine self-administration. Previous work has shown that this cocaine self-administration regimen produces robust and persistent impairments in cognitive flexibility and decision-making. Hence, the cocaine-induced changes in functional connectivity reported here may contribute to the well-described deficits in these cognitive domains in chronic cocaine users.

Keywords: cocaine seeking, Resting State Functional Connectivity, fMRI Functional Connectivity, salience network, cocaine addiction

Disclosures: Nothing to disclose.

W223. Characterization and Associated Risk-Factors of a Human Model of Chronic-Heavy-Intermittent-Drinking

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Background: Alcohol misuse is the leading risk factor for premature death and disability among people between the ages of 15 and 49. In 2013, 16.6 million adults in the United States reported having an Alcohol Use Disorder (AUD). Currently, the DSM-V recognizes AUD as falling on a continuum to account for the many different drinking phenotypes associated with functional impairment. However, many drinking phenotypes have not been identified and characterized.

Methods: Participants were selected from all screening protocols at the National Institute on Alcohol Abuse and Alcoholism (NIAAA). A total of 1,547 screened participants had 90-day Alcohol Timeline Follow-Back (TLFB) data. Drinking groups were formed based off of patterns of alcohol consumption found in the TLFB. For the present study we characterized a human model of chronic-heavy-intermittent-drinking (CHID) using the TLFB, where the drinking phenotype is mainly described by cycles of heavy drinking (defined as consuming ≥ 4 or ≥ 5 standard drinks per day in females or males, respectively) and abstinence. Specifically, CHIDs were categorized as those who reported heavy drinking for between 23 and 45 days, who did not report drinking alcohol for 8 or more consecutive days, and who did not report having 15 or more consecutive non-drinking days. For comparison, we also defined a constant-drinking (CD) group and a light-social-drinking (LSD) group. CDs were defined as those who reported drinking for at least 75 days, and critically, the mean of the total drinks consumed over the 90 day period matched that of the CHID group. Therefore, the main difference between the CHID and CD groups is the pattern of drinking, not the total amount of alcohol consumed over the 90-day period. Finally, LSDs were defined as those who reported 0 heavy drinking days and who drank a total of between 14 and 90 drinks over the 90 day period.

Results: A total of 106 participants met criteria for CHID, where the average total drinks consumed was 336.80, average number of heavy-drinking-days was 33.49, and average number of no-drinking-days was 49.12. A total of 105 participants met criteria for CD, where the average total drinks consumed was 336.76, average number of heavy-drinking-days was 32.67, and average number of no-drinking-days was 6.13. A total of 110 met criteria for LSD, where the average total drinks consumed was 37.93, all persons reported 0 heavy drinking days, and average number of no-drinking-days was 68.22.

Conclusions: Consistent with the goal of developing more personalized treatments, the characterization of CHIDs is a first step to understanding how the pattern of drinking may lead to the identification of specific phenotypic characteristics. Currently, we are analyzing group differences in psychological and behavioral measures, blood biomarkers and structural differences in key brain regions associated with reward and appetitive motivation. Future studies should test how manipulating measures specifically associated with CHIDs may lead to changes in drinking outcome measures.

Keywords: alcohol use disorder, drinking, at-risk, epidemiology

Disclosures: Nothing to disclose.

W224. Intravenous and Smoked Methamphetamine in Women – "It's Like Two Different Drugs"

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Background: Methamphetamine (meth) is one of the most addicting and dangerous drugs used around the world for non-medical purposes. Long-term meth use can lead to paranoia, hyper-sexuality and symptoms of Parkinson's disease. Some studies also suggest that long-term meth use can produce neurotoxicity, especially in dopamine-rich brain regions. A major statistic that separates meth from many other drugs of abuse, even similar stimulant drugs such as cocaine, is that women are as likely to use meth as men are. This differs from other drugs where men are often two or three times more likely to use them than women are. Despite this fact, there is a relative paucity in the data on the effects of meth in females in both the preclinical and clinical literature. In addition, there are few studies in the literature that have compared the route of meth administration and the perceived subjective effects of the drug. Most reports simply lump all routes of administration into a single category. This study was designed to determine potential differences in the subjective effects of intravenously administered (IV) meth when compared to other routes of administration in women using unstructured interviews.

Methods: After obtaining IRB approval from LSU Health in Shreveport, potential subjects were recruited through the Council on Alcoholism and Drug Abuse for Northwest Louisiana (CADA). After reviewing instructions on client confidentiality and other related HIPAA considerations with CADA staff, they were asked to determine if any current or former clients were interested in participating in a research study involving an interview where they would discuss their experiences with meth. A consent letter was provided for potential subjects to read, but they were not required to sign it so that confidentiality could be maintained. The inclusion criteria were that the potential subjects were female, over the age of 18, and with meth as their primary drug of choice. CADA staff was also asked to focus on recruiting IV meth users. Once a client agreed to participate and reviewed the consent letter, an interview was scheduled. Subjects were instructed that the interview was going to be conducted by a scientist, not a psychiatrist or a counselor. Subjects were assured that they did not have to answer any question and that they were free to end the interview at any time. They were told that the interview was being conducted to learn more about their experiences with meth. During the unstructured interview, the subjects were allowed to speak freely; there was not a specific set of questions that was asked. Subjects were simply asked, "How does meth make you feel?" Obvious examples (e.g., did your heart beat faster) were sometimes provided to help them focus on how meth made them feel internally, but they were not lead or coached in any way and were allowed to describe how meth made them feel in their own words. Notes were written down after the interview but were not taken during the interview in order maintain a conversation-like atmosphere. When these unstructured interviews were completed, the interview notes were searched for the emergence of common themes regarding differences between IV and smoked or snorted meth using grounded theory, whereby key points were extracted from information contained in the interview notes.

Results: Fifty-two women participated in the 2.5-year study. The mean age of the subjects was 34.5 ($\pm$ 10.2; range: 18 to 56). Forty-seven (90%) of the participants had used meth IV, and most reported that they had either smoked, snorted or ingested meth before they used it IV. Twenty-three women said that they would experience "vapors" following

an IV injection of meth, resulting in an immediate "cough" or a "taste" of the drug in the back of the throat. Of the 47 women who used meth IV, 41 (87%) reported the perception of an immediate sexual feeling indistinguishable from an orgasm following an injection of sufficient purity. None of the participants reported a similar response when they smoked or snorted the drug. The subjects also reported several additional subjective and physiological responses experienced only when they used meth by the IV route that will be discussed in this presentation.

Conclusions: The major finding of this study is that the IV administration of meth produces subjective (and physiological) responses that are readily perceived as different from the responses experienced when meth is smoked or snorted. One possible explanation for this surprising effect may be pharmacokinetic in nature. Although users report experiencing some of the effects of meth within seconds regardless of whether the drug was snorted, smoked or injected, the time to peak plasma concentrations (TMAX) varies depending on the route. In a 2009 review by Cruickshank and Dyer, it was reported that the Tmax for IV meth is 6 minutes, which is similar to the Tmax for IV cocaine (2.4 min). However, while the Tmax for smoked cocaine is virtually identical to the Tmax for IV cocaine (2.4 min; Cone, 1995), the Tmax when meth is smoked increases to 150 min (2.5 hr). Thus, the shorter Tmax following IV meth may contribute to the different subjective effects experienced when smoked (or snorted) meth is compared to IV. Regardless of the mechanisms involved, the dramatic differences in the subjective responses to meth depending on the route of administration in women suggest that this factor should be taken into consideration when designing therapies for the treatment for meth addiction in women.

Keywords: Methamphetamine, women, intravenous administration, subjective effects

Disclosures: Nothing to disclose.

W225. Development of a Novel Rodent Model of THC Self-Administration

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Background: Cannabis is the most frequently used illicit drug worldwide with highest consumption occurring in economically developed countries like the United States. Among cannabis users there is a long-standing perception that the drugs is both harmless and non-addictive. In contrast, the US federal government has since the 1970's classified cannabis as a Schedule I controlled substance indicating a high potential for abuse. In recent years, however national attitudes have shifted with legalization or decriminalization of the drug on the rise. Thus it is becoming increasingly important to establish the effects of long-term cannabis exposure on the brain. Unfortunately preclinical research on the drug's effects has been hampered by both the disparity between public and private attitudes of the drug as well as the lack of a tractable animal model. Investigators have been unable to show that rats will maintain self-administration of isolated Δ9-tetrahydrocannabinol (THC), the drug's main psychoactive component. THC on its own can produce unpleasant side effects including increased anxiety, but cannabis contains over 60 cannabinoids and more than 400 additional chemicals. Cannabidiol (CBD), a non-psychoactive cannabinoid, can counter some of the aversive properties of THC by producing anxiolytic and anti-psychotic effects. Thus in the present investigation, we employed several strategies in order facilitate acquisition of THC self-administration. Namely, we 1) used a lower unit dose of THC than has previously been employed in rodent studies, 2) pre-exposed rats to THC vapor before initiating operant intravenous self-administration, and 3) combined THC with CBD in a proportion previously demonstrated to neutralize a THC conditioned place aversion.

Methods: Adult Sprague-Dawley rats were pre-exposed to THC and CBD vapor (10:1 ratio of THC:CBD) using a Volcano vaporizer to deliver THC:CBD (10 mg THC vapor per rat) to animals in enclosed plastic boxes (42 x 29 x 14 cm3) with an incubation time of 10 minutes per day for 4-5 consecutive days. Following vapor pre-exposure rats were trained to acquire operant responding for food pellets in a single 2-hour session without cues prior to beginning THC self-administration. Finally, subjects were trained to self-administer THC:CBD on an FR1 schedule (1.5 hrs per day) where responses on an active lever resulted in a drug infusion (2 μg/kg/0.05 ml infusion) paired with discrete light and tone cues for 10 days. Self-administration was followed by 10-14 days of extinction where lever pressing no longer had any programmed consequences. Finally animals were tested for reinstatement of drug seeking to drug-paired cues, or a THC prime (1 mg/kg). All experimental protocols in animal studies were approved by the Institutional Animal Care and Use Committee at MUSC and were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Results: Pre-exposure to THC vapor provided a physiologically relevant dose of THC as we were able to measure a decrease in core body temperature 1 hr after exposure $T(30) = 5.813$, $p < 0.0001$. After vapor pre-exposure, rats self-administered intravenous THC on an FR1 schedule. Two-way ANOVA of lever pressing behavior during the acquisition phase revealed a main effect of self-administration day $(F(9,324) = 3.375$, $p = 0.0006)$, lever (active vs inactive; $(F(1,36) = 11.58$, $p < 0.0017)$, and subjects (matching) $(F(36,324) = 5.282$, $p < 0.0001)$. During self-administration, lever discrimination was observed with greater than 2-fold preference for the drug-associated lever. The rats sustained low levels of responding with a cumulative average of 7.5 infusions per day. Finally, THC-conditioned cues successfully elicited reinstatement responding above an extinction baseline $(F(1.184,29.59) = 21.72$, $p < 0.0001$; RM one-way ANOVA with Geisser-Greenhouse correction). Likewise, a THC-priming injection promoted reinstatement responding above extinction $(F(1.675,35.17) = 13.41$, $p = 0.0001$; RM one-way ANOVA with Geisser-Greenhouse correction).

Conclusions: We have established a rodent model of THC self-administration and reinstatement allowing us to evaluate THC-dependent brain changes relevant to addiction. Innovations in our approach included using a THC

vapor pre¬-exposure prior to i.v. THC self-administration as previous studies have shown that THC pre-exposure facilitates development of a conditioned place preference for the drug rather than an aversion and thus was predicted to facilitate acquisition of operant responding. Pulmonary administration (inhalation) of THC provides the most rapid onset of subjective effects and higher bioavailability compared to parenteral (i.p. or i.v.) or enteral (oral) dosing. Additionally, the combination of CBD with THC in both the vapor and intravenous solution was used to mitigate some of the unpleasant subjective effects of THC. The development of this model affords the opportunity to initiate reverse-translational investigations of therapeutic targets for cannabis addiction including compounds such as n-acetylcysteine (NAC) that have shown efficacy in clinical studies of marijuana dependence. Additional studies will examine biomarkers of altered glutamatergic synaptic plasticity in the nucleus accumbens after THC self-administration to evaluate the overlap and distinctions in THC-dependent brain changes compared to other more well characterized drugs of abuse.

Keywords: delta9-tetrahydrocannabinol, Self-Administration, cue reinstatement, Cannabidiol
Disclosures: Nothing to disclose.

W226. Lower Brain Responses during Cognitive Inhibition of Food Craving Elicited by Food Stimulation in Obese Subjects

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Background: Obesity is associated with a higher risk for impaired executive cognitive function. Here we assessed if obese men had decreased brain responses during attempted inhibition during food stimulation.
Methods: Sixteen obese male subjects (32 $\pm$ 8.7 y/o) with BMI of 38.6 $\pm$ 7.2 were compared with 10 age-matched non-obese male subjects (BMI 24.2 $\pm$ 2.5) using PET and FDG. Brain metabolism was evaluated in food deprived (19 hrs) subjects during no stimulation and food stimulation with attempted inhibition and with no inhibition on 3 separate days. Absolute metabolic images were analyzed using a full factorial SPM8 design (p = 0.005, cluster size = 100 voxels). Self-reports for hunger were recorded.
Results: During attempted inhibition non-obese and obese men had lower hunger scores compared to no inhibition (-37 $\pm$ 29%, p < 0.0001). Food stimulation compared to no stimulation activated metabolism in inferior frontal and superior frontal gyrus, default mode network and cerebellum in non-obese men. There was a stimulation x group interaction effect such that compared to no stimulation, food stimulation produced greater activation in anterior cingulate, and left orbitofrontal cortex, superior frontal gyrus, left inferior temporal lobe, left hippocampus/parahippocampus, and cerebellum, for non-obese than for obese subjects. A second interaction effect was observed. Compared to food stimulation, attempted inhibition suppressed

brain glucose metabolism in anterior cingulate, left insula, left inferior frontal, left orbitofrontal cortex, superior frontal gyrus, left amygdala, left hippocampus/parahippocampus, pons and cerebellum, more strongly in non-obese than in obese men.
Conclusions: Compared to non-obese men, obese men had lower activation in brain regions involved with motivation and reward during food stimulation. They were also unable to suppress activation during food stimulation in brain regions involved with emotional regulation, conditioning and motivation. These results suggest that this is the mechanism by which attempted inhibition decreases the desire for food, which is consistent with greater vulnerability of obese men to uncontrolled eating when food is readily available.
Keywords: neuroimaging, obesity, cognitive control
Disclosures: Nothing to disclose.

W227. Rapid Changes in CB1 Receptor Availability in Cannabis Dependent Males after Abstinence from Cannabis

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Background: The widespread use of cannabis, the increasing legalization of "medical" cannabis, the increasing potency of cannabis and the growing recreational use of synthetic cannabinoid 1 receptor (CB1R) full agonists underscores the importance of elucidating the effects of cannabinoids on the CB1R system. Exposure to cannabinoids is known to result in CB1R downregulation. However, the precise time course of changes in CB1R availability in cannabis dependent subjects (CDs) following short and intermediate term abstinence has not been determined.
Methods: Using High Resolution Research Tomography (HRRT) and [11C]OMAR, CB1R availability as indexed by the volume of distribution (VT) [11C]OMAR was measured in male CDs (n = 11) and matched healthy controls (HCs) (n = 19). CDs were scanned at baseline (while they were neither intoxicated nor in withdrawal), and after 2 days and 28 days of monitored abstinence. HCs were scanned at baseline and a subset (n = 4) was rescanned 28 days later.
Results: Compared to HCs, [11C]OMAR VT was 154.85% lower in CDs (effect size Cohen's d = -1.11) at baseline in almost all brain regions. However, these group differences in CB1R availability were no longer evident after just 2 days of monitored abstinence from cannabis. There was a robust negative correlation between CB1R availability and withdrawal symptoms after 2 days of abstinence. Finally, there were no significant group differences in CB1R availability in CDs after 28 days of abstinence.
Conclusions: Cannabis dependence is associated with CB1R downregulation, which begins to reverse surprisingly rapidly upon termination of cannabis use and may continue to increase over time.

Disclosures: Nothing to disclose.

W228. Cocaine Mediated Molecular Regulation of Mitochondrial Dynamics in Nucleus Accumbens Projection Neuron Subtypes

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Background: Altered brain energy homeostasis is a hallmark adaptation occurring in the cocaine-addicted brain. This includes alterations in glucose metabolism, glutamate homeostasis, and oxidative stress. Recent studies demonstrate that mitochondria dysfunction is associated with psychiatric disorders. However, mitochondrial dynamics have not been thoroughly addressed in cocaine abuse. We focus on the two main nucleus accumbens (NAc) projection medium spiny neuron (MSN) subtypes, those enriched in dopamine D1 vs. D2 receptors, since they display critical but antagonizing roles in cocaine-mediated behaviors. Previous studies demonstrate differential plasticity and signaling processes in D1-MSNs vs. D2-MSNs with both contingent and non-contingent chronic cocaine. Given these findings it is plausible that these MSNs have different energy demands, which could lead to altered mitochondrial dynamics in each MSN subtype.

Methods: We first examined mitochondria biogenesis and function genes in the NAc of rodents that self-administer cocaine (FR1 schedule, 1mg/kg/infusion) and in postmortem NAc of cocaine dependent individuals. We next examined mitochondrial biogenesis and function genes in the two MSN subtypes, after repeated cocaine (7 days, 20 mg/kg), using the RiboTag methodology to isolate ribosome-associated mRNA from D1-MSNs and D2-MSNs. Additionally, we have examined the active form of dynamin-related protein 1 (Drp1), a GTPase that directly binds to the outer mitochondrial membrane to promote mitochondria division. To determine if Drp1 activity is important for mediating behavioral responses to cocaine we then used a Drp1 inhibitor, Mdivi-1, during cocaine (7.5mg/kg) conditioned place preference (CCP).

Results: Our data demonstrate that genes important for mitochondria biogenesis and function are upregulated in nucleus accumbens (NAc) of rodents that self-administer cocaine and in postmortem NAc of cocaine dependent individuals. When we examine ribosome-associated mRNA in MSN subtypes we observe an upregulation of many mitochondrial biogenesis and function genes in D1-MSNs but a decrease in D2-MSNs after repeated cocaine. We also observe the active form of Drp1 upregulated in the NAc after repeated cocaine. This is consistent with an increase in Drp1 mRNA in D1-MSNs, in NAc of rodents that self-administer cocaine, and in postmortem NAc of cocaine dependent individuals. Finally, we observed a blunted cocaine CPP response after treatment with the Drp1 inhibitor, Mdivi-1.

Conclusions: Our study establishes a direct role for altered mitochondrial dynamics in cocaine abuse. Both human cocaine dependents and rodents that self-administer cocaine display increased mitochondrial biogenesis and function genes in the NAc. These contingent cocaine effects likely occur through D1-MSNs since we observe an increase in these genes in D1-MSNs after non-contingent cocaine. Our studies also implicate differential energetic states in MSN subtypes since D2-MSNs display a reduction in mitochondrial biogenesis and function genes after repeated cocaine. Finally, our studies establish a direct role for mitochondrial fission in mediating rewarding responses.

Disclosures: Nothing to disclose.

W229. Ecological Momentary Assessments and Attentional Bias Modification for Postpartum Smoking

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Background: Maternal smoking is the leading cause of cancer mortality in the mother, and is associated with adverse pregnancy outcomes and increased infant morbidity and mortality. Nearly half of women who smoke prior to pregnancy achieve abstinence during pregnancy. Unfortunately, nearly 80% of abstinent women relapse within a year after delivery. Despite considerable study, there are few strategies that effectively combat smoking relapse in this population. Smokers exhibit an "attention bias" for smoking-related cues. Attentional bias plays a role in the maintenance or escalation of smoking by increasing subjective cravings and directly affecting cigarette seeking behavior. Attentional bias modification (ABM) using the visual probe task (VP) has been used experimentally to reduce attentional bias (AB) and blunt cue-provoked craving. The goal of this project was to assess AB in postpartum abstinent smokers, and test a novel technique, the use of smartphones to administer ABM, as an intervention for relapse prevention in postpartum women.

Methods: We first conducted an observational pilot study using ecological momentary assessments (EMA) administered on a personal digital assistant (PDA) to assess AB and the role of affective and situational stimuli on the relapse process in 10 postpartum smokers who achieved abstinence during pregnancy. Women were asked to complete 4 daily random assessments (RAs) that collected data on craving, AB (utilizing the smoking Stroop), affect and environmental factors for 2 weeks after delivery. We then conducted a follow-up study with 11 abstinent pregnant smokers who were asked to carry around a smartphone as they went about their daily lives for 1-2 weeks in their last month of pregnancy and for 2 weeks immediately postpartum. Participants were randomized to either receive ABM (N = 6) or attentional control (N = 5). The smartphone randomly alerted the participants (4 times/day) to respond to questions assessing subjective states, followed by the

ABM (or control) procedures utilizing the visual probe task. Outcome measures included attentional bias for smoking and self-reported craving.

Results: In the EMA only study, 2 subjects relapsed 1 week after delivery, 4 reported one lapse, and the remaining 4 remained abstinent. As expected, the subjects who lapsed/relapsed reported higher levels of craving both prior to delivery and at 2-weeks postpartum, along with heightened anxiety and parenting stress. The median compliance was 75.0% (number of completed assessments/total number of assessments presented by the PDA). The 10 participants completed 394 assessments and exhibited a smoking Stroop effect (AB) that was associated (p < .05) with craving; when craving was higher, so was AB. In the ABM study, women completed 444 trainings/assessments (60% pregnancy; 40% postpartum), and 2.92 trainings/assessments per day. Craving significantly increased from pregnancy (M = 1.40, SD = 1.23) to postpartum (M = 2.28, SD = 2.27); postpartum participants reported having smoked "since the last assessment" on 12.64% of assessments, and 8 of the 11 women reported smoking at least 1 cigarette during the study. AB assessed on the smartphone was more negative in the ABM group (n = 35 assessments, M = -52.6 ms, SD = 122) vs. controls (n = 44 assessments, M = 18.5 ms, SD = 146) (p < 0.05, using LMM), suggesting that ABM reduced AB to smoking cues and that women in the ABM group were attending away from smoking cues.

Conclusions: These pilot studies demonstrate that ABM can be administered on a mobile device with good compliance in perinatal smokers. We confirmed that that women experience heightened craving postpartum, which is associated with AB. Our findings also show that ABM can reduce AB to smoking cues in perinatal women. The control of attentional processes can play a key role in preventing relapse among abstinent smokers. Our intervention addresses two major barriers to treatment in perinatal women, the use of medication and convenience of treatment delivery. Further research is needed to test the efficacy of ABM as an innovative approach to smoking relapse-prevention in postpartum women.

Keywords: smoking, perinatal, attentional bias modification

Disclosures: Nothing to disclose.

W230. The Relationship between Pain and Prescription Drug Use Disorders: A National Prospective Study

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Background: The high prevalence of chronic pain and serious adverse events attributed to prescribed opioids has focused attention on potentially complex relationships between pain and prescription opioid abuse and dependence. The goal of this study was to examine prospective associations between moderate and more severe pain and prescription opioid use disorders in the general population using structural equation models to ascertain the direction of causality.

Methods: We used structural equation modeling to assess the interdependency of pain and prescription opioid use

disorder at Waves 1 and 2 of the National Epidemiologic Survey on Alcohol and Related Conditions (Wave 1, 2001–2002; Wave 2, 2004–2005), a nationally representative sample of US adults, age ≥ 18 years, interviewed 3 years apart. Pain was measured with a 5 point scale of pain related interference in daily activities dichotomized at moderate and more severe. Prescription opioid use disorders were measured with a structured interview (AUDADIS-IV). Other covariates included age, sex, anxiety or mood disorders, and family history of drug, alcohol, and behavioral problems.

Results: In the adjusted structural equation model, pain interference at Wave 1 predicted prescription opioid use disorder at Wave 2 (p = 0.02) and pain interference and Wave 2 (p < 0.001). Prescription opioid use disorder at Wave 1 predicted prescription opioid use disorder at Wave 2 (p = 0.002), but not pain interference at Wave 2 (p = 0.14). Younger age (p < 0.001) and a family history of behavioral problems (p = 0.02) also predicted prescription opioid use disorder at Wave 2 while older age (p < 0.001), female sex (p = 0.03), family history of alcohol (p = 0.007), family history of depression (p = 0.004) and personal history of mood or anxiety disorders (p < 0.001) each predicted pain interference at Wave 2.

Conclusions: Painful conditions contribute to the risk of prescription opioid use disorders. To help reduce the incidence of prescription opioid abuse and dependence among adults with moderate to severe pain, careful monitoring and consideration of non-opioid alternative treatments is warranted.

Keywords: chronic pain, Prescription drug abuse, national representative sample, national study

Disclosures: Nothing to disclose.

W231. Role of TAAR1 in the Mesolimbic Regions in Cocaine-Seeking Behavior

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Background: A novel G-protein coupled receptor, trace amine-associated receptor 1 (TAAR1), has been shown to be a promising target to prevent stimulant relapse. Our recent studies showed that systematic TAAR1 agonist decreased abuse-related effects of cocaine and methamphetamine. However, the neural mechanisms underlying the effect of TAAR1 in drug addiction are largely unknown.

Methods: Here, we assessed the effects of microinjection of a selective TAAR1 full agonist RO5166017 into the mesolimbic regions, i.e. the ventral tegmental area (VTA), the medial prefrontal cortex (mPFC), and the nucleus accumbens (NAc), on cue- and drug priming-induced cocaine seeking in rats with a history of cocaine self-administration. Rats underwent extinction after cocaine self-administration training. RO5166017 (5 μg/ 0.5 μl/ side) or Vehicle (0.5 μl/ side) was microinjected into each brain regions immediately before cue- and drug priming-induced cocaine seeking test.

Results: Microinjection of RO5166017 into the VTA and the prelimbic cortex of mPFC decreased both cue- and drug priming-induced cocaine seeking. However, cocaine-seeking behaviors were not affected by RO5166017 when

microinjected into the infralimbic cortex of mPFC. Furthermore, microinjection of RO5166017 into the NAc core only inhibited cue-induced drug seeking, but had no effect on drug priming-induced cocaine seeking. RO5166017 microinjected into all above brain regions did not affect locomotor activity.

Conclusions: Together, these results indicated that TAAR1 in different mesolimbic regions distinctly contribute to cue- and drug priming-induced cocaine-seeking behavior.

Keywords: cocaine seeking, reinstatement, self-administration, trace amine-associated receptor 1

Disclosures: Nothing to disclose.

W232. Prescription Opiate Dependent Patients Display Anhedonia and Differential Processing of Reward Stimuli Following Withdrawal: An fNIR Study

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Background: The incidence of prescription opiate dependence has grown considerably since the turn of the century. Anhedonia has been identified as a potential risk factor for relapse in opiate dependence; however, our understanding of the biological markers of anhedonia remain limited. The purpose of this study was to evaluate the extent to which recently withdrawn prescription opiate dependent patients (PODP) might experience anhedonia. We postulated that anhedonia would be evident in a PODP population undergoing residential treatment relative to controls. We employed an integrated approach to test this hypothesis, using traditional self-report, affect-modulated acoustic startle response (AMSR), and functional near infrared spectroscopy (fNIRs).

Methods: Recently withdrawn prescription opiate patients (n = 36) were recruited at the Caron Foundation; a residential treatment facility. Patients had been abstinent from opiates for 10-14 days. Healthy controls (n = 10) were recruited from the Milton S. Hershey Medical Center. All participants underwent a laboratory session where the completed the Snaith-Hamilton Pleasure Scale (SHAPS), AMSR (viewing stimuli with affective valence that was positive, negative, or neutral), and a cue-response task using fNIRs. During the fNIRs cue-response task, participants viewed pictures of three types of natural rewards: highly palatable food, positive social interactions (e.g. family at a dinner table), and emotionally intimate stimuli (e.g. couples embracing – non-erotic). Hemodynamic response from the prefrontal cortex (PFC) was recorded during stimulus presentations. Groups were compared using t-tests and regression analyses; data were processed using SPSS.

Results: PODP scored higher on the SHAPS compared to controls (t(44) = 3.55, p < .01). In addition, AMSR evoked an amplified startle response to positive stimuli (indicative of a reduced valuation of positive stimuli) in PODP compared to controls (t(40) = 2.87, p < .01). When viewing positive stimuli in the fNIRs cue-response task, POPD displayed decreases in neural activity in left ventrolateral PFC (VLPFC; t(40) = 2.04, p < .05). In response to food stimuli, POPD displayed decreased neural activity in the left lateral rostral PFC/left VLPFC (t(42) = 2.6, p = .01), right VLPFC (t(40) = 2.39, p = .02), and left medial RPFC (t(39) = 2.24, p = .03). No differences were found in response to the emotionally intimate images.

Conclusions: This study used three separate measures which, taken together, offer evidence that varying levels of anhedonia are present among PODP in the early stages of recovery. Relative to control participants, PODP endorsed higher levels of anhedonia on a validated inventory of hedonic tone on the day of testing. PODP also showed reduced startle suppression while viewing positive stimuli relative to controls. PFC response during neuroimaging suggests that recently withdrawn PODP are devaluing natural rewards, namely food and social interactions. The specificity of response to distinct categories of natural reward may be of clinical importance since the fNIRs device could easily be used in a clinical setting. There is a pressing need to understand and identify biological markers of risk for relapse; continuation of this work will examine anhedonia as a predictor of treatment outcome.

Keywords: fNIR, anhedonia, opioid dependence

Disclosures: Dr. Scott Bunce owns shares in fNIR Devices, LLC, a company that manufactures fNIR instruments for research

W233. Telomere Length in Crack/Cocaine Use Disorder with Early Life Stress

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Background: Early life stress and crack-cocaine addiction are related to cell aging and with age-related problems. Shorter telomere length (TL) has been described as a marker of biological aging with women showing longer lifespans and lower telomere shortening. Despite that the role of TL in crack-cocaine addiction has never been studied. The aim of this study is to investigate TS in adult female crack cocaine users reporting exposure to ELS and compare them with a community-based elderly female sample as a reference group of senescence.

Methods: This study included treatment seeking crack-cocaine women dependents (n = 122) and elderly women with no psychiatric disorder (ELD, n = 50). The crack-cocaine sample was split in two groups according with their Childhood Trauma Questionnaire (CTQ) scores: crack users with a history of childhood abuse and neglect (CRACK-ELS) and without childhood maltreatment history (CRACK). TS was obtained on DNA from peripheral blood samples and measured using qPCR assay through T/S ratio. The TL comparisons were controlled for age, years of education, body mass index and depression symptoms.

Results: Differences on TL were found between all groups. Crack users (CRACK and CRACK-ELS) exhibited shortened TL in comparison to ELD group, despite their younger age. Among the crack users, CRACK-ELS group had significantly

shorter telomeres than CRACK group. Correlation analysis within crack-cocaine groups indicated that TL was negatively correlated with CTQ total scores, including emotional abuse and neglect subtypes, as well craving symptoms severity.

Conclusions: Our findings are supported by previous studies that found shorter telomeres in ELS and drug addiction samples and offer a partial explanation to the complex relation regarding ELS and psychiatry disorders. Considering that crack/cocaine addiction are associated with age-related trajectories, the ELS may induce an increase in the biological aging of drug dependents.

Keywords: Telomere, Child abuse and neglect, Substance-related disorders, aging

Disclosures: Nothing to disclose.

W234. Calcium Permeable AMPA Receptors Mediate Synaptic Plasticity at Synapses onto Nucleus Accumbens Parvalbumin Expressing Interneurons

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Background: Maladaptive circuit function within the reward system is likely a key component to susceptibility to drug addiction. The nucleus accumbens (NAc) core is a key brain region involved in mediating aspects of addiction-related behaviors and is comprised of parallel output loops (D1 and D2 dopamine receptor expressing medium spiny neurons (MSNs)) whose activity is governed by excitatory drive and by often overlooked inhibitory interneuronal microcircuitry. Parvalbumin (PV) expressing fast spiking interneurons (FSIs) are GABAergic cells that regulate the activity of networks through inhibition of local projection neurons. Through feed-forward inhibition, activity of PV-FSIs is thought to gate excitatory (glutamatergic) drive within the NAc to correctly process goal-directed behaviors. Relative to excitatory synapses onto NAc MSNs, little is known regarding excitatory transmission onto NAc INs, including PV-INs. Our results indicate PV-INs functionally express calcium permeable AMPA receptors that are capable of inducing long term depression of excitatory postsynaptic currents (EPSCs).

Methods: Male mice ages 6-10 weeks were utilized and experiments were performed in accordance with Vanderbilt University IACUC approved protocols. Targeted whole-cell patch-clamp recordings of EPSCs were obtained from PV-INs and D1 or D2 dopamine expressing MSNs in NAc core slices. PV-INs were labelled with D1tdTomato by crossing PV-cre mice with Ai9 mice, a cre dependent Rosa26 tdTomato reporter line. In acute slices from D1-tdTomato bacterial artificial chromosome mice, MSNs were identified as D1-tdtomato positive (D1-MSN) or negative (D2-MSN). Excitatory afferents were stimulated at the border between the NAc core and cortex dorsal to the anterior commissure. All recordings were done in presence of picrotoxin (50μM) to block GABAA receptor mediated inhibitory synaptic currents. For experiments examining long term depression (LTD), recordings from control cells were interleaved with recordings from cells undergoing experimental manipula-

tions including bath application of agonists/antagonists. Comparisons between different experimental manipulations were made using a two tailed Student's t test with $p < 0.05$ considered significant.

Results: Here we show that NAc PV-INs have similar presynaptic properties to D1 and D2 MSNs, however, excitatory synapses at PV-INs have a greater AMPAR/NMDAR ratio. Most notably, in contrast to D1 or D2 MSNs, PV-INs express robust AMPAR rectification at positive holding potentials. Investigation of synaptic plasticity mechanisms in the PV-INs revealed that, similar to indirect pathway MSNs in the NAc, low frequency stimulation (LFS: 10 Hz, 5 min) produces robust LTD in the PV-INs. However, the mechanisms underlying this LFS-LTD are distinct from that reported in the D2 MSNs. LFS-LTD is absent at D1 MSNs and dependent upon mGluR5/CB1R/TRPV1 receptor function at excitatory synapses onto D2 MSNs. Unlike D2 MSNs, LFS-LTD at excitatory synapses onto PV-INs is independent of mGluRs. It is also independent of NMDARs. Interestingly, LFS-LTD at PV-IN excitatory synapses is sensitive to NASPM, a blocker of calcium permeable AMPA receptors.

Conclusions: The results of this study and previous work demonstrate that excitatory input onto NAc neurons can have differential effects depending on neuronal target. LFS at 10 Hz has no effect on D1 MSNs, elicits mGluR5/CB1R/TRPV1 dependent LTD at D2 MSN synapses and CP-AMPAR dependent plasticity at PV-INs. Thus, integration of excitatory input in the NAc core can be cell-type specific. LTD of excitatory synapses onto PV-INs could attenuate feed-forward inhibition, thus alleviating a break on MSN output. Considering these neuronal subtypes receive similar excitatory inputs, we suggest that differential integration of excitatory drive onto PV-INS gates MSN output maintaining normal physiological circuit dynamics. Disruption of this feedforward mechanism may be a key component underlying NAc-dependent disease states.

Keywords: Nucleus Accumbens, parvalbumin interneurons, Synaptic Plasticity

Disclosures: Nothing to disclose.

W235. Acute Effects of Alcohol and Nicotine on Perfusion and Functional Connectivity in Reward and Cognitive-Control Brain Circuitry

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Background: 20 Million Americans are alcohol dependent or regularly drink alcohol in harmful quantities and nearly 50 million Americans smoke cigarettes. As many as 88-96% of alcoholics are smokers and approximately 60% of smokers consume alcohol in significant quantities. Individuals who are both alcohol (ALC) and nicotine (NIC) dependent generally have heavier use of both drugs and more severe dependence. Despite the ramifications of co-abuse of these substances, very little is known about how this drug combination acts within the brain to become so strongly paired. Notably, there are no neuroimaging studies

of the combined effects of ALC and NIC on brain function. We report the first neuroimaging study of the acute effects of alcohol, nicotine, and their combination to investigate their combined effects on physiology and function of reward- and cognition-related brain circuitry that may underlie co-use.

ALC and NIC have complex interactions on the brain's mesocorticolimbic dopamine system (MDS), which is strongly implicated in reward and addiction. ALC also impairs cognitive performance, whereas NIC may enhance cognitive performance. Thus, during ALC intoxication, we might expect to see additive effects of NIC and ALC in MDS structures and opposing effects of NIC on ALC-related alterations in cognitive control regions. To test this, we conducted a pharmacologic magnetic resonance imaging (phMRI) study of the acute effects ALC and NIC on cerebral blood flow (CBF) and resting state functional connectivity (RSFC). Drugs can cause both global and localized changes in CBF and can alter the function of large-scale brain networks, which can be measured using functional MRI (FMRI). Notably, when using FMRI to study drug effects, it is critical to measure CBF to control for drug-related global CBF changes that can give rise to non-neural changes in the FMRI signals. We assessed global CBF and focal CBF in key MDS structures (orbitofrontal cortex, nucleus accumbens (NAcc), ventral tegmental area/substantia nigra (VTA/SN) and in cognitive-control brain regions in the salience network (SN), e.g., dorsal anterior cingulate cortex (dACC), ventromedial prefrontal cortex (vmPFC) and insula, and in medial PFC (mPFC), which is a key node of the default mode network (DMN). DMN activity is high during mentation, in the absence of goal-directed behavior, and the SN must switch the DMN off and alert cognitive control networks to come online to perform tasks. We also assessed RSFC as connectivity between these circuits is dysregulated in psychopathology and, recently, was also shown to be impaired in abstinent chronic heavy smokers.

Methods: 7 healthy male light/moderate smokers – moderate/heavy drinkers participated in this within-subjects placebo-controlled phMRI study of ALC, NIC, and NA. Simultaneous BOLD/CBF data were collected on a 3T Seimens Tim Trio using a dual echo PCASL sequence with: TE1/TE2/TR = 10ms/25ms/3.5 sec, whole brain, 3.44x3.44x7 mm3, ~8.5 min. Study sessions were conducted on separate visits, with scans pre- and post-ALC drinking (in the scanner). For each study session, a placebo NIC or 14 mg NIC patch was placed on the arm. After a three-hour uptake period, participants underwent imaging. After baseline BOLD/CBF scans participants drank an ALC beverage (vodka and orange juice, 0.7 g/kg of alcohol in 400 ml total volume), which was followed by post-Gdrinking scans after a 20 min uptake period. Data were analyzed using FSL to calculate CBF in units of ml/100g/min and to assess RSFC of brain circuits using group independent component analysis with dual regression during placebo, NIC, ALC, and NA conditions and to compare drug effects relative to placebo.

Results: Participants reached peak BAL of ~0.08, with NIC decreasing the time to peak of ALC effects. ALC increased positive ratings of drug effects, including drunkenness, ARCI: MGB, and POMS:Vigor ($p < 0.05$) and NA showed further enhancement of these effects. Assessment of global CBF changes for drug relative to placebo showed that NIC cause a large decrease in global CBF ($p < 0.02$, corrected). After rescaling to account for variation in global CBF across subjects/conditions, NIC did not alter CBF in MDS structures, likely due to the route of administration (patch). ALC showed robust CBF increases in all MDS structures ($p < 0.05$) and NA showed larger increases in right NAcc and VTA/SN relative to ALC alone. In a whole-brain analysis of CBF, large CBF increases were observed with ALC relative to placebo in frontal cortex ($p < 0.05$, uncorrected) and the effects of ALC in these areas were significantly reduced with NIC on board. RSFC of mPFC, left NAcc, and left insula with the SN was decreased by ALC ($p < 0.05$ corrected), with these effects being diminished with NIC on board.

Conclusions: NIC patch caused a global decrease in CBF, but did not alter CBF appreciably in key MDS regions. This is likely due to the route of administration, which removes all behavioral aspects of NIC use that might contribute to enhance rewarding effects of NIC. ALC drinking increased CBF in all key MDS structures and in frontal cortical regions that overlap with key areas in the SN and DMN. The effects of NIC on ALC drinking were to enhance ALCs effects in NAcc and to diminish ALCs effects in frontal cortex. These alterations are consistent with an enhanced reward with co-use of ALC and NIC relative to each drug alone, and with NIC diminishing the impairing effects of ALC. Together, our findings provide support that these effects play a key role in co-use of ALC and NIC and co-morbid alcohol and nicotine dependence.

Keywords: nicotine, Alcohol, Resting State Functional Connectivity, Cerebral Blood Flow, FMRI

Disclosures: Nothing to disclose.

W236. The 5-HT7 is Potential Target for the Suppression of Alcohol Craving: Modulation of Context and Cue-Induced Alcohol Seeking Behaviors

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Background: The high rate of relapse in alcoholics has been linked to the ability of conditioned cues to stimulate alcohol craving. The transition from moderate alcohol consumption to alcoholism is associated with an increase in the saliency of conditioned cues to evoke craving and a 'loss of control' over the effects of conditioned cue. The 5-hydroxytryptamine 7 receptor (5-HT7) is a novel target that has been shown to regulate 'behavioral self-control'. 5-HT7 agents modulate 'impulsive' and/or 'compulsive' behaviors; two possible predisposing factors in drug-taking and seeking. In addition, the efficacy of anti-ADHD medicine is correlated with alterations in the 5-HT7 receptors system within the nucleus accumbens shell (AcbSh). The object of the current experiments was to determine if manipulating the 5-HT7 receptors within AcbSh would alter context- and/or cue-induced alcohol-seeking behaviors.

Methods: Previous work in our lab has demonstrated that P rats will readily express a context-induced alcohol-seeking behavior and we have also shown that olfactory discrimi-

native cues can enhance (conditioned excitation; CS+) or suppress (conditioned inhibition; CS-) these behaviors. We have shown that presentation of the CS+ increases dopamine and serotonin levels in the AcbSh, while presentation of a CS- reduces these neurotransmitter levels. The initial experiment examined the peripheral effects of the 5-HT7 antagonist SB269970 on context- induced alcohol-seeking. We then examined the local effects of SB269970 within the AcbSh and AcbC. In the third study, we examined the local effects of 5-HT7 agonist LP-12 within the AcbSh on context-induce seeking.

Results: The results indicated that context-induced alcohol-seeking is enhanced by peripheral injections of a 5HT7 antagonist. Similarly, a 5HT7 antagonist microinjected into the AcbSh increased alcohol-seeking. In contrast, micro-injection of a 5HT7 agonist into the AcbSh reduced alcohol-seeking. Thus, the data indicated that modulating the activity at the 5HT7 receptor within the AcbSh can bidirectionally alter alcohol-seeking. Previously we have shown that conditioned cues can enhance (excitatory) or reduce (inhibitory) alcohol-seeking. Therefore, we determined if microinjections of a 5HT7 antagonist into the AcbSh would block the reduction of alcohol-seeking produced by presentation of the inhibitory conditioned cue (CS-). The data indicated that the ability of a 5HT7 antagonist to stimulate alcohol-seeking was influenced by the presence of the CS-; rightward shift to the dose response curve.

Conclusions: Collectively, the data indicate that 5-HT7 receptors within AcbSh mediate alcohol-seeking behaviors. Activating 5HT7 receptors reduces drug-seeking, while inhibiting the receptors promotes drug-seeking. In addition, stimulating the 5HT7 receptor in the AcbSh can overcome the behavioral inhibition produced by presentation of a CS-. Overall, the data consistently indicated that the 5HT7 receptor is a potential pharmacological target for treatment regulating drug craving.

Keywords: Serotonin 5-HT7, Nucleus accumbens shell, Alcohol-seeking, Alcohol-preferring (P) rats

Disclosures: Nothing to disclose.

W237. Adolescent Alcohol Exposure and Persistent Effects on LSD1-Mediated Chromatin and Synaptic Remodeling in the Amygdala

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Background: Binge drinking during adolescence appears to be an important risk factor for the development of alcoholism and other psychiatric disorders in adulthood. Epigenetic processes due to changes in histone acetylation in the amygdala have been shown to play a role in adolescent alcohol exposure-induced anxiety and alcohol drinking behaviors in adulthood. We investigated the role of histone methylation mechanisms in the persistent effects of adolescent intermittent ethanol (AIE) exposure in adulthood.

Methods: Adolescent male Sprague Dawley rats were exposed to 2g/kg of ethanol (2 days on/off) or intermittent

n-saline (AIS) intraperitoneally during post-natal days (PND) 28-41. Brains were collected 1 and 24 hr after last AIE for measurement of histone methylation-modifying enzymes in the amygdala and bed nucleus of the stria terminalis (BNST). Another cohort of rats was allowed to grow to PND 92 to investigate the lasting effects of AIE on histone methylation mechanisms in the amygdala. We also measured expression of several synaptic plasticity-associated genes (NeuroD1, NeuroD2, Homer1, Neurogranin, and Synaptophysin) in the amygdala of AIE and AIS exposed adult rats. We measured anxiety-like behaviors using the elevated plus-maze test. The quantitative real-time PCR and in situ PCR were used to measure mRNA levels of various genes in the amygdala and BNST. Protein levels of lysine demethylase 1(LSD1), dimethylated histone H3K4 and H3K9 in the amygdaloid structures were measured by gold immunolabeling procedure.

Results: We found that AIE exposure increased Lsd1 mRNA in the amygdala 1 hr after last AIE but started decreasing during early withdrawal in adolescence. AIE rats displayed increased anxiety-like behavior and decreased mRNA and protein levels of Lsd1 and mRNA levels of Lsd1+8a (a neuron-specific splice variant), in specific amygdaloid structures compared to AIS exposed rats in adulthood 53 days after last AIE. Interestingly, AIE increased H3K9 dimethylation (H3K9me2) in the central (CeA) and medial nucleus of amygdala (MeA) in adulthood without producing any change in H3K4me2 protein levels. We also found a decrease in the mRNA levels of H3K9 demethylase Kdm4c and synaptic plasticity-associated genes (NeuroD1, Neu-roD2, Homer1, Neurogranin, and Synaptophysin) in the amygdala of AIE exposed adult rats.

Conclusions: These novel results indicate that adolescent alcohol exposure produces persistent effects on epizymes (Lsd1+8a) involved in H3K9 dimethylation in the amygdala, and possibly is responsible for AIE-induced chromatin and synaptic remodeling and adult psychopathology.

Keywords: Histone methylation, Amygdala, Anxiety, Adolescent Alcohol, LSD1

Disclosures: Nothing to disclose.

W238. Gene X Smoking Interactions in the Ventromedial PFC: Alpha 5 Nicotinic Cholinergic Receptor Gene Variation and Smoking Effects on Adolescent Grey Matter

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Background: The rs16969968 single nucleotide polymorphism (SNP) of the alpha 5 nicotinic receptor has A/G as minor/major alleles. The risk allele A has been previously associated with nicotine dependence. However, the neural mechanism, should one exist, associating this SNP to smoking behavior is still unknown. Here, we investigate structural and functional brain correlates of smoking, the rs16969968 genotype effect and the smoking*genotype interaction in a large sample of 14 years old adolescents.

Methods: Voxel-based morphometry (VBM) and the Monetary Incentive Delay (MID) fMRI reward task were used to determine the brain structural and functional correlates of nicotine in 1,737 adolescents on whom whole-genome genotyping and behavioral data were acquired. A nicotine score was calculated from the European School Survey Project on Alcohol and Drugs questionnaire, scoring as follows (Score (Lifetime occurrences)): 0(0), 1(1-2), 2(3-5), 3(6-9), 4(10-19), 5(20-39), 6($\geq$40 cigarettes).

An association analysis was performed to assess the relationship between genotype and nicotine exposure. Clusters identified from the whole-brain VBM analysis were used to calculate the mean activity from the MID task (both reward anticipation and reward outcome) and for an ROI-level ANOVA to test the smoking*genotype interaction on grey matter volume (GMV). In these analyses subjects were grouped by genotype (AA; GA; GG) and by smoking status as smokers (scores:1-6) or non-smokers (score=0). Age, sex, handedness, scanner site, puberty status, total GMV, socioeconomic status and IQ were included as nuisance covariates.

Results: Nicotine exposure was significantly associated with a higher frequency of the risk genotype in the 1,737 adolescents (p=0.03). The between-group VBM comparison between 389 non-smokers and 93 average and advanced smokers (scores 3-6) yielded significantly less GMV in smokers in the ventromedial prefrontal cortex (vmPFC) following a p<0.05 cluster-wise correction. The VBM regression that included 816 subjects (non-smokers and smokers with low, average and high nicotine exposure levels (scores:1-6)), showed a significant negative linear correlation between GMV and nicotine exposure in the same vmPFC region. There was also a nicotine*genotype interaction on the vmPFC volume (p <0.03), with no main effect for genotype (p>0.05). The vmPFC volume decrease in smokers was largest in the carriers of the nicotine-related high-risk genotype (AA). Further, there was a similar nicotine*genotype interaction on reward anticipation and outcome in the MID task where, similar to the GMV effects, activation was reduced in smokers with the largest effect in the AA carriers.

Conclusions: This gene*environment interaction in the vmPFC, a brain region known to be involved in value calculations and decision making processes, suggests a possible neurobiological mechanism that underlies both a genetic predisposition towards smoking and the detrimental effects of smoking. The linear relationship between cigarette use and vmPFC volume suggests effects at very low nicotine exposure levels. The reward-related hypoactivity in the smokers with the high-risk genotype suggests a genetic predisposition combining with nicotine exposure to produce a reward-blunted phenotype which, in turn, may increase the reinforcing effects of nicotine. These observations further support the need for strong regulatory protections of adolescents from cigarette smoking.

Keywords: nicotine addiction, vmPFC, nicotinic acetylcholine receptors, alpha 5

W239. Effects of Chronic Alcohol Drinking on Circadian Gene Expression

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Background: Clinical and pre-clinical studies provide evidence that circadian genes and rhythmic processes play an important role in alcohol use disorders. Circadian rhythms are generated and maintained in the suprachiasmatic nucleus (SCN) by a molecular clock that is composed of a series of transcriptional and translational feedback loops. Elements of the molecular clock are found throughout the brain and periphery. We have previously shown disruption (via mutation or ventral tegmental area knock down) of the circadian gene, Clock, can promote alcohol intake in mice. Other work has shown that mutations in the circadian genes, Period 1 and Period 2, also increase alcohol intake in mice. While these findings suggest that there exists an important relationship between alcohol abuse and circadian gene disruptions, there are no studies of the effects of alcohol intake on diurnal circadian gene expression (multiple time points). Furthermore, no studies have examined whether changes in circadian gene expression are correlated across multiple brain regions. It would be of significance to determine this for regions known to be important for circadian rhythms and alcohol intake.

Methods: We performed two studies to assess the effects of chronic alcohol on circadian gene expression. The first study was designed to complement behavioral findings from Seggio et al. (2009) showing that chronic no-choice alcohol drinking (but not voluntary choice alcohol drinking) altered circadian rhythms. This study was carried out in C57BL/6J mice subjected to either 28 days of drinking water only or alcohol (10%) only. Two days after the end of the study, mice were euthanized at 4 time points (6 hours apart, over a 24 hour period). Fresh frozen tissue from nucleus accumbens (NAc) was processed for quantitative real time PCR (qRT-PCR; n=5-6 mice/treatment/time point). Data was analyzed using the ddCT relative gene expression method. The second study was designed to determine the effects of chronic binge-like alcohol drinking on diurnal rhythms (8 time points). This study was carried out in mice selectively bred to achieve high blood alcohol levels after a short binge-drinking session (High Drinking in the Dark, HDID-1 mice). Mice were subjected to 8 weeks of limited access 20% alcohol drinking (or water) for 4 days/week using the Drinking in the Dark paradigm. One day after the end of the study, mice were euthanized at 8 time points (3 hours apart, over a 24 hour period). Fresh frozen tissue from NAc and SCN has been collected and is being processed for qRT-PCR (n=6 mice/treatment/time point).

Results: We found that one month of no choice alcohol (10%) intake results in a significant up-regulation of Period 1 and Period 2 genes as compared with water drinking mice (two-way ANOVA, main effect of treatment for Per1 ($F(1,23) = 13.03$, p<0.01) and for Per2 ($F(1,23) = 9.42$, p<0.01). Data from the SCN are not available for this study. This study was designed to complement behavioral findings from Seggio et al. (2009) showing that the same ethanol paradigm resulted in shortened free-running period in

constant darkness and support the hypothesis that chronic binge-like drinking will alter diurnal expression of circadian genes. Results from the second study show that HDID-1 mice reliably consumed 1.5-4.5g/kg ethanol per binge-drinking session. qRT-PCR data collection and analysis is currently ongoing for SCN and NAc brain tissue in this experiment. We hypothesize that alcohol-experienced mice will exhibit increased levels or amplitude of Period gene expression, perhaps with a reduced circadian period.

Conclusions: Addicted individuals display disrupted rhythms, and chronic rhythm disruption may increase the risk for substance abuse and relapse. These foundational studies identify significant brain regional effects of chronic alcohol on rhythmic expression of circadian genes. Ultimately, these results guide ongoing studies aimed at determining whether pharmacologically inhibiting a key regulator of the clock period reduces binge-like drinking. Further, we plan to test whether this treatment "re-sets" the molecular clock, ameliorating alcohol-induced changes in rhythmic gene expression.

Keywords: Alcohol, circadian, gene expression, genetic mouse model

W240. Chronic Stress Exposure During Early Withdrawal Enhances Incubation of Cocaine Craving

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Background: A major challenge for treating cocaine addiction is the propensity for abstinent users to relapse. Once abstinence is achieved, both stressful life events and cues associated with prior drug use can be powerful triggers for relapse and human studies indicate that exposure to chronic adverse life events is associated with increased relapse vulnerability. However, little is known about interactions between the effects of chronic stress and drug withdrawal on cue-induced cocaine craving and the underlying neural mechanisms driving this behavior. To study the effect of chronic stress on withdrawal-dependent changes in relapse vulnerability, we used the incubation model of craving and relapse in which cue-induced drug seeking progressively intensifies ("incubates") during withdrawal from extended-access cocaine self-administration.

Methods: Repeated restraint stress was used as a chronic stressor. Rats self-administered cocaine under extended-access conditions (6 h/d for 10 d) that have been shown to produce incubation of craving. On the day after the last self-administration session [withdrawal day (WD) 1], rats received a test for cue-induced cocaine seeking, during which nose-pokes resulted in presentation of the light cue but not cocaine. Rats were then divided into 2 groups destined for either control or stress conditions. Rats exposed to repeated restraint stress underwent 7 daily restraint sessions (20 min) over a 9 day period from WD6 to WD14 and received a seeking test on WD15, a day after the last repeated restraint session. Controls were placed in a cage with bedding on the same schedule.

Results: As expected, we found that controls showed greater cue-induced cocaine seeking on WD15 compared to WD1 (i.e. incubation of craving). Interestingly, rats exposed to repeated restraint stress showed a more robust increase in seeking on WD15, indicating acceleration or facilitation of incubation. Separate studies showed that the enhanced cocaine seeking observed was due to repeated restraint stress sessions and not a single restraint stress exposure.

Conclusions: These data indicate that chronic stress during early withdrawal facilitates incubation of cocaine craving, which is thought to contribute to enhanced relapse vulnerability. Future studies will assess the synergistic effects of cocaine and chronic stress exposure during withdrawal on cellular and behavioral measures and, using a stress resilience model, identify neuroadaptations that can reverse such effects. Together, these studies will ultimately bring us closer to developing effective pharmacotherapies to prevent relapse.

Keywords: cocaine seeking, chronic stress, incubation

Disclosures: Nothing to disclose.

W241. Sex Differences of Insula Volume in Cannabis Dependence and Association with Cognition and Emotion

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Background: Growing evidence has suggested sex differences in many aspects of cannabis use disorders. Results from a national representative sample of U.S. adults that were diagnosed with cannabis dependence (CD) found that women with CD had more mood and anxiety disorders and were at greater risk for externalizing disorders, while men with CD had a higher incidence of comorbidity of other psychiatric disorders and other substance use disorders. These findings triggered our interest in looking at potential sex differences in brain morphology, and their associations with behavioral features in individuals with CD. To date, only two studies have looked at sex differences in brain structure in cannabis users. One study reported group by sex interaction of amygdala volumes and the other reported marginal group by sex interaction of prefrontal cortex (PFC) volumes, while neither detected further group or sex effect. The first study found larger amygdala volumes in female chronic cannabis users compared to healthy females, with no difference in male users compared to healthy males. The second study reported larger PFC volumes in female chronic cannabis users compared to healthy females, and smaller PFC volumes in male users compared to healthy males. The current study investigated potential sex differences in insula morphometry. The insula is a core constituent of the salience network (SN), a key brain network responsible for detecting, integrating and filtering

relevant interoceptive, autonomic and emotional information and coordinating activity between other brain networks to perform tasks. Disruption of SN function is implicated in psychopathology and addiction and lesions to the insula specifically have been linked to disruption of smoking addiction. We also investigated sex differences in the association between insula volume and measures of emotion and cognition in men and women with CD.

Methods: Structural imaging data and clinical/behavioral data for 12 females and 12 males with CD, as well as healthy control subjects matched for demographic features were drawn from the data from the Human Connectome Project (HCP). The HCP data are a large comprehensive dataset collected in nearly 900 individuals to map the human brain connectome. These data are available to the public, along with extensive clinical, demographic, and behavioral data (http://humanconnectome.org). Insula volumes for each subject were extracted from FreeSurfer processed imaging data. Depression and anxiety symptom scores measured using DSM criteria, emotion regulation measures from the Penn Emotion Regulation Test (PERT), and cognitive performance measures from the Dimensional Change Card Sort (DCCS) were also extracted from the database. Relative volumes of bilateral insula corrected for intracranial volume were used for all the analyses. ANOVAs were conducted for the comparison of insula volume and clinical/behavioral measures, with group as fixed variable, and sex as a covariate. Spearman correlation coefficients were used to test associations of insula volume with clinical/behavioral measures in each sex separately.

Results: For insula volumes, there was a significant group by sex interaction of right insula volume ($p = 0.040$) but not left insula volume ($p = 0.500$). Further analysis found that the group by sex interaction was driven by sex effect ($p = 0.036$), not by group effect ($p = 0.316$). Females with CD had largest right insula volume among the four subgroups. For PERT assessments, there was a significant sex effect for "number of correct neutral identifications" ($p = 0.044$, females scored lower than males) and a trend for a sex effect for "number of correct anger identifications" ($p = 0.054$, females scored higher than males). There was also a trend for a group by sex interaction for the scale score in DCCS ($p = 0.081$). Further correlation analysis of right insula volume with the clinical/behavioral variables showed that there were significant positive correlations of insula volumes in females with DSM depression (rho = 0.587, $p = 0.003$) and anxiety (rho = 0.474, $p = 0.019$) symptom scores, but not in males, a trend for a negative correlation with "number of correct anger identification" in the performance of PERT in females with CD (rho = -0.514, $p = 0.087$) but not males with CD, and significant negative correlation with the DCCS score in males with CD (rho = -0.617, $p = 0.017$) but not females with CD.

Conclusions: Our results show that right insula appears to be vulnerable to CD, especially in women. Factors relating to poorer emotion regulation, including symptoms of depression and anxiety, are associated with insula volume in women. In men, right insula volume is negatively correlated with cognitive performance. Given the key role of insula as a constituent of the salience network, our findings that sex-specific factors are associated with insula volume in CD – with affective regulation being important in females and cognitive function important in males – shed insight into sex specific differences that might guide clinical practice in treating CD as well as other comorbid mental illnesses. Further imaging studies including different modalities are needed to identify mechanisms of insula and insula related network dysregulation in men and women with CD.

Keywords: Cannabis Dependence, sex difference, Insula

Disclosures: Nothing to disclose.

W242. 5-HT1B Receptor Agonism Has Different Effects on Cocaine-Induced Locomotion and Dopamine Neuron Activity in the Vta Depending on Time of Testing after a Repeated Injection Regimen in Mice

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Background: We have previously shown that in rats trained to self-administer cocaine, 5-HT1B receptor (R) agonism modulates cocaine reinforcement in opposite directions when rats are tested 24 h versus 3 weeks after their final self-administration session. In this study, we found that C57BL/6 mice treated daily with either saline or cocaine (15 mg/kg, IP) for 21 days also exhibited different responses to the 5-HT1BR agonist CP94253 (5.6 mg/kg, SC) depending on whether testing occurred 24 h or 21 days after the last daily treatment. Specifically, CP94253 given 24 h after the last injection increased locomotion, whereas the same treatment given 21 days after the last injection decreased locomotion and prevented expression of cocaine sensitized locomotion. We hypothesized that the change in 5-HT1BR agonist effects involves changes within the ventral tegmental area (VTA) circuitry that modulates activity of mesolimbic dopamine neurons.

Methods: To test this hypothesis, we treated mice with an identical regimen as that used for testing locomotor activity. Then either 24 h or 21 days after the final injection of the regimen, the mice were anesthetized with chloral hydrate anesthesia and single unit recordings of VTA dopamine neurons were made following administration of CP94253, and subsequently administration of cocaine.

Results: CP94253 produced a biphasic change in firing in saline-injected control mice where there was an initial decrease in firing rate and burst spikes followed by an increase in these measures. In contrast, CP94253 showed only a slight decrease in firing rate and burst spikes in cocaine-injected mice. Neuronal responses to cocaine also varied depending on repeated drug treatment and time of testing. In saline-injected controls, cocaine decreased firing rate and burst activity at both time points and produced a less marked decrease in these measures in cocaine-injected mice tested 24 h after the last repeated injection. However, in cocaine-injected mice tested 21 days after the last repeated injection, 0.5 mg/kg, IV cocaine produced a severe decrease in firing rate and burst spiking whereas as this same dose in saline-injected mice produced only a mild attenuation in these measures.

Conclusions: These findings suggest that changes in dopamine neuron activity in the VTA may be involved in

the switch in functional effects of 5-HT1BR agonism during the course of abstinence from chronic cocaine exposure.
Keywords: cocaine, Serotonin, addiction
Disclosures: Nothing to disclose.

W243. Nuclear HDAC5 Suppresses Cocaine Reward-Like Behaviors Through Repression of Its Target Gene, Npas4, in the Nucleus Accumbens

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Background: Drug addiction is a devastating disease that is mediated, at least in part, by molecular mechanisms that promote maladaptive changes in brain reward function. Chronic drug abuse is associated with physiological and psychological problems including comorbidity with other psychiatric illnesses, criminal behaviors and even mortality. Drug addiction is a chronic relapsing disorder characterized by drug seeking and taking behaviors even after long period of abstinence, and the high incidence of relapse is a major clinical challenge for the treatment of addiction. Understanding the genes and molecules that control the encoding and persistence of drug-associated memories may provide critical new therapeutic targets for the treatment of drug addiction. Epigenetic mechanisms, including changes in histone acetylation, are engaged by chronic drug taking and are thought to modulate addiction-related behaviors. Cocaine exposure induces the transient nuclear accumulation of the class IIa histone deacetylase, HDAC5, through a signaling pathway involving the D1 dopamine receptor, cAMP signaling and PP2A phosphatase (Taniguchi et al., 2012). In addition, we find that expression of nuclear HDAC5 reduces reinstatement of drug seeking after a cocaine priming procedure. In the present study, we identified novel genomic targets of HDAC5 and demonstrate that one of its genomic targets, the Neuronal PAS Domain Protein 4 (Npas4), is important for cocaine reward-like behavior.
Methods: We identified HDAC5 binding peaks in striatal neurons using chromatin immunoprecipitation followed by high-throughput sequencing (ChIP-seq). Npas4 gene and protein expression and HDAC5 association on the Npas4 enhancer region were examined in nucleus accumbens (NAc) and striatal tissues after exposure to novelty, stress and/or cocaine or in cultured primary striatal neurons. Enhancer characterization was performed using a luciferase reporter gene. HDAC5 and Npas4 manipulations in vivo were accomplished with adult mice (or rats) receiving intra-NAc infusions of adeno-associated viruses (AAVs) expressing HDAC5 (3SA, WT or control), Cre recombinase, or Npas4 shRNA. Mice were either WT (C57BL/6) or homozygous floxed-Npas4 (C57BL/6 backcrossed), as appropriate. We examined cocaine behaviors using several behavioral paradigms, including rat intravenous self-administration (IVSA), mouse cocaine conditioned place preference (CPP) and mouse locomotor sensitization.

Control behavioral testing also included the sucrose preference test, contextual fear conditioning, and the open field test.
Results: AAV-mediated expression of nuclear HDAC5 (S259A/S279A/S498A or 3SA) in the rat medial NAc significantly reduced cocaine-prime induced drug seeking in cocaine IVSA compared to animals that received viruses expressing either HDAC5 (WT) or GFP. In contrast, no significant effects were observed on stable cocaine intake or extinction training. To identify HDAC5 target genes that mediate its role in cocaine reward-related behavior, we performed ChIP-seq and identified numerous candidate genes, including Npas4. Npas4 is an immediate early gene that is strongly induced by glutamatergic synaptic activity and that regulates inhibitory and excitatory synapse balance in a cell-type specific manner. We found that exposure to either cocaine or a novel context induced transient Npas4 mRNA and protein expression in a small neuronal subpopulation throughout the NAc. We identified a ∼400 bp enhancer region in the 5' end of the Npas4 gene that is necessary and sufficient to mediate depolarization-induced activation of Npas4, and that HDAC5 3SA expression blocks the activity-dependent induction of Npas4 via this enhancer region. Interestingly, HDAC5 KO mice show elevated levels of Npas4 mRNA in the NAc, and endogenous HDAC5 is recruited to the Npas4 enhancer region in a delayed fashion following cocaine administration. Consistent with an important role of nuclear HDAC5 in cocaine reward behaviors, reduction or genetic deletion of Npas4 in the adult NAc significantly reduces cocaine CPP, but does not alter contextual fear conditioning, sucrose preference, cocaine-induced locomotor sensitization or anxiety-like behavior.
Conclusions: In this study, we found that expression of dephosphorylated, nuclear HDAC5 reduces prime-induced drug seeking after abstinence. We identified Npas4 as an HDAC5 target gene, and found that nuclear HDAC5 antagonizes its activity-dependent induction via association with a defined enhancer element. Our findings also demonstrate that Npas4 is induced in response to novelty and drug exposure, and that its expression in the adult NAc is required for cocaine reward-like behavior. Together, our findings suggest that nuclear HDAC5 reduces cocaine behaviors, likely in part, through repression of activity-induced Npas4 expression in the adult NAc.
Keywords: cocaine, Epigenetic, transcription factor
Disclosures: Nothing to disclose.

W244. Psychosocial Stress and Consumption of a High Calorie Diet in Female Monkeys Alters Brain Neurochemistry and Functional Connectivity: A Model of Food Addiction?

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Background: Exposure to chronic stress is implicated in the etiology of adverse health outcomes. For instance, stress exposure in women is associated with increased risk

emotional eating, food addiction and obesity. However, the biological signals underlying the link between stress and the development of emotional eating and food addiction are not understood. One potential mechanism involves alterations in dopamine signaling in cortico-limbic-striatal circuits, as dopamine 2 receptor (D2R) binding potential is reduced as a consequence of chronic stress, a neurochemical phenotype also observed in psychostimulant abuse and obesity. It is uncertain whether alterations in prefrontal-limbic-striatal D2R influence brain connectivity, specifically in prefrontal-striatal circuits critical for executive function. Using an animal model of chronic psychosocial stress, we hypothesized that chronic stress and high calorie diet (HCD) induce decreases in D2Rs and altered functional connectivity (FC) between prefrontal, amygdala and ventral striatal regions.

Methods: Socially housed adult female rhesus monkeys that had access to two dietary conditions were studied longitudinally. Social subordination in these groups is a well-established model of chronic psychosocial stress in females. The present study assessed how exposure to social subordination in adult female rhesus monkeys and access to a HCD affects D2R binding potential (D2R-BPND) and FC in and within prefrontal-limbic-striatal regions. One group cohort of females (n = 18) was fed a typical low calorie primate laboratory diet (LCD), and a second cohort (n = 16) had access to both the LCD and the more palatable HCD (high in fat and sugar). Half of the animals were of high social status (dominant) and the other half of low status (subordinates). All animals were fed ad lib using an automated feeding system that allows for the constitutive assessment of caloric intake previously validated. After 48 wks of diet exposure, each subject received a PET scan using [18F]-fallypride to assess D2R-BPND. Structural images were obtained within 3 weeks of the PET scan using a 3T magnet (Siemens Trio) for co-registration of PET, and calculation of D2R binding potential in cortico-limbic-striatal regions of interest (ROI). rs-fMRI scans were also collected in a subset of females within each dietary condition (n = 16), using an echo planar imaging (EPI) sequence sensitive to BOLD contrast (TR/TE = 3s/30ms, voxel size = 1.5mm isotropic, 2x15 min scans). ROI analysis used FSL functions adapted for the rhesus brain, and images were corrected for artifacts, smoothed, and signal intensity normalized per experimental block. Individual EPI data was registered to the structural image in standard space (F99) and resting state BOLD time series extracted and correlated ROI by ROI for each animal to create correlation (FC) matrices.

Results: In the dietary choice condition, all animals preferred the HCD and consumed more calories compared with females in the LCD only condition (p = .001). Subordinates in the choice condition consumed more total calories over time (p = .04). These differences in caloric intake were associated with a greater increase in body weight (p = .027) and body fat (p = .004) over time. Additionally, D2R BPND in the right oribitofrontal cortex (OFC) was significantly affected by a status by diet interaction (p < .001), wherein dominant females in the LCD only condition had significantly greater D2R BPND in the OFC compared to all other groups of females. rs-MRI analyses also revealed an interaction of status and dietary environment on FC between the NACC and the OFC (right,

11m). Specifically, in the LCD condition, more submissive behavior predicted increased FC between these regions (r = 0.824; p = .012), while in the HCD condition, submission predicted reduced FC between them (r = -0.762; p = .028). Higher NACC D2R BPND, but not OFC D2R BPND, predicted increased NACC-OFC FC in both the choice (r = .739, p = .036) and no choice conditions (r = .704, p = .051). In addition, reduced D2R BPND in the left nucleus accumbens (NACC; r = -0.535, p = .07), left OFC (r = -0.525, p = .08), right anterior cingulate (ACC; r = -0.577, r = .049), and right dorsal lateral PFC (r = -0.610, p = .035) predicted more intake of the HCD for females in the choice condition. Finally, increased amygdala-NACC (r = .714, p = .046) and amygdala-OFC FC (r = .711, p = .048) predicted greater intake of the HCD.

Conclusions: Taken together, these preliminary data indicate that social subordination and access to a HCD impact brain dopamine function through changes in D2R BPND as well as FC between PFC, NACC and amygdala. The findings suggest that social subordination stress and consumption of a HCD decrease D2R BPND in the OFC, and that reduced D2R BPND in the NACC and in PFC sub-regions is associated with more intake of a HCD. Furthermore, stress effects on FC between the OFC and NACC varies as a function of dietary environment, as FC is reduced in subordinate females in the dietary choice condition. Overall, the current data suggest that the effects of social stress on brain connectivity and dopamine function are significantly influenced by the diet the animals are consuming. Ongoing analysis of PFC connectivity to the NACC and amygdala on HCD consumption will provide insights into the pathophysiology of food addition in females.

W245. Dysregulated NMDA NR1 Signaling in the Infralimbic Cortex Contributes to Increased Impulsivity during Protracted Alcohol Abstinence

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Background: Abstinent alcoholics exhibit persistent impairments in the capacity for response inhibition, and this form of impulsivity is significantly associated with heightened relapse risk. Brain imaging studies implicate aberrant function of the ventromedial prefrontal cortex (vmPFC) in this behavioral pathology, though the underlying mechanisms are not understood. Here, we present evidence that that in rats, deficient activation of glycine (GLY) and serine (SER) release in the infralimbic cortex (IL) contributes to increased motor impulsivity during protracted abstinence from chronic alcohol exposure.

Methods: Male Wistar rats were trained in the 5-Choice Serial Reaction Time Task (5-CSRTT) using standard

procedures (100 trials/session; 2 sec visual stimulus duration; 2 sec limited hold; 5 sec inter-trial interval). Two groups of equal task performance were subsequently formed; one was given exposure to chronic intermittent EtOH vapor inhalation (EtOH group: 12h EtOH/day; 3 weeks; average BAL of 246 ± 32 mg%) and the other group received similar handling without EtOH exposure (CON group). All rats were subsequently tested in the standard 5-CSRTT for 3 weeks, with microdialysis tests occurring in the 4th week of EtOH abstinence. IL microdialysates (5 min intervals) were collected during either a cognitively challenging varITI task (ITI varied between 5, 7, 9 and 11 sec; 1 sec SD; EtOH = 14; CON = 13) or a standard 5-CSRTT session (EtOH = 6; CON = 6). Sessions were extended to 250 trials to enable sufficient samples for temporal profiling. Dialysate levels of GLY, SER, aspartate (ASP), glutamate (GLU), γ-amino butyric acid (GABA), dopamine (DA), NE, 5-HT, taurine (TAU), histamine (HIS), and glutamine (GLN) were quantified by LC-MS/MS. Based on evidence that increased premature responding by EtOH-exposed rats is associated with diminished IL GLY and SER recruitment and the loss of an inverse correlation between these neurotransmitters and premature responding, we hypothesized that deficient activation of the NMDA NR1 co-agonist site contributes to impaired behavioral inhibition during protracted EtOH abstinence. To test this, we evaluated the effects of intra-IL infusion of GLY transport inhibitor ALX5407 (2 ng/side; EtOH = 8; CON = 8), the NMDA GLY site antagonist L-701,324 (either alone or in combination with ALX5407; each drug 2 ng/side; ALX5407 + L-701,324 = 5; ALX5407 alone = 7; L-701,324 alone = 5; VEH = 6), or vehicle (EtOH = 6; CON = 10). Intra-IL infusions (0.6 μl over 2 min, injectors removed 2 min post-infusion) were administered 90 min prior to varITI testing. All procedures strictly adhered to the NIH Guide for Care and Use of Laboratory Animals and were approved by The Scripps Research Institute IACUC.

Results: Consistent with our prior observations, there were no group differences in 5-CSRTT performance with standard task parameters either during a 3 week post-EtOH period or during microdialysis tests employing these parameters. In contrast, during microdialysis tests conducted with the varITI parameters EtOH-exposed rats elicited significantly more premature responses than did CON rats, though no group differences were evident in any other aspect of task performance. No significant group differences in baseline neurotransmitter levels were evident in samples collected prior to either standard or varITI 5-CSRTT sessions. 5-CSRTT performance recruited distinct patterns of neurotransmitter recruitment in the EtOH and CON groups, and group-related differences in the neurotransmitter response to the varITI vs. standard 5-CSRTT were also evident. Alcohol-exposed rats exhibited blunted task-related recruitment of IL glycine and serine release, and loss of an inverse relationship between levels of these neurotransmitters and premature responding normally evident in alcohol-naïve subjects. Intra-IL administration of the glycine transport inhibitor ALX5407 attenuated excessive premature responding by alcohol-exposed rats, and this was reliant on NMDA glycine site availability. Alcohol-exposed rats also exhibited dysregulation of task-related increases in IL aspartate, acetylcholine, dopamine

and norepinephrine and the possible contribution of these disruptions to motor impulsivity under cognitively challenging conditions is considered.

Conclusions: In humans, restraint of improper responses relies on the function of the vmPFC and rodent studies also demonstrate an important influence of the vmPFC in the constraint of premature responding in the 5-CSRT. The present findings reveal that in drug-naïve rats premature responding in the varITI 5-CSRTT is inversely correlated with task-related increases in GLY and SER in the IL cortex. EtOH-exposed rats elicit significantly more premature responses than controls in the varITI task, and this is associated with diminished recruitment of IL GLY and SER and loss of the negative relationship between GLY and SER levels and premature responding. Intra-IL administration of the glycine transport inhibitor ALX5407 ameliorates excessive premature responding by EtOH-exposed rats, through a mechanism reliant on NMDA glycine site availability. These results suggest that deficient signaling at the NMDA receptor NR1 co-agonist site contributes to increased motor impulsivity during protracted EtOH abstinence.

Keywords: impulsivity, Alcohol dependence, infralimbic cortex, NMDA glycine-site receptor

W246. Novel Treatments for Cocaine Bingeing and Relapse: Progesterone and Exercise

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Background: Cocaine and other drug abuse have been a costly source of morbidity and mortality in the US. Many treatments efforts have failed to reduce the most damaging aspects of cocaine and other drug addiction. The research reported here involves the use of two treatments that have been tested on rats trained to self-administer iv cocaine. They were selected because initial data from our laboratory indicated that they were effective in reducing drug-seeking, bingeing, and relapse to cocaine seeking. They were also selected for the possibility of being self-sustaining when translated to the human population. The first is progesterone (PRO), and numerous studies from our laboratory have shown that PRO suppresses cocaine seeking in females, and to some extent males, over several phases of the addiction process. The translation to humans would be that many adolescent and young adult females already receive PRO in the form of oral contraceptives especially the PRO-only formulations. The second treatment is physical exercise in the form of wheel running for rats, that translates to cardiovascular exercises that are often self-maintained in humans such as running, swimming, sports, etc. The purpose of these studies was to examine PRO and exercise (and their combination) in female and male rats as a means to reduce cocaine-seeking behavior.

Methods: Female and male rats were trained to self-administer iv cocaine infusions (0.4 mg/kg) during daily 6-h sessions for 10 days. Cocaine and cocaine cues were discontinued for a 3 - 30 day withdrawal periods, and subsequently rats were reintroduced cues associated with cocaine self-administration and/or an ip injection of cocaine, and responses on the lever previously associated with cocaine were compared under different treatment conditions, physical exercise (wheel running) during the withdrawal period, or were given PRO injections prior to presenting stimuli previously associated cocaine in a relapse model. Some groups received PRO and exercise treatments, and the results were compared to each treatment alone and control treatments (locked wheel, or vehicle injection instead of PRO). In the impulsivity model rats responded for cocaine infusions under a delay-discounting task (Evenden and Ryan 1996), whereby they chose a small-immediate amount or a large-delayed amount of cocaine.

Results: Females responded more for cocaine than males during cocaine access. Access to the exercise wheel reduced extinction responses and cocaine-primed reinstatement in females but not in males. In male rats the combined wheel + PRO treatment was more effective than either wheel or P alone. When incubation of cocaine seeking (craving) was treated with exercise, rats with wheel access for 3 days showed more relapse behavior than rats treated with 30 days of wheel access. The locked-wheel controls showed moderate relapse after 3 days and much higher relapse responding after 30-days of incubation. Thus, exercise blocked the incubation of 'craving' resulting in the same lower level of relapse as the 3-day group. In the impulsivity study, PRO reduced impulsive responding for iv cocaine infusions compared to vehicle controls. However, PRO had no effect on the same delay-discounting task when sucrose pellets were used as rewards. Thus PRO was selectively effective for reducing impulsive responding for cocaine, females showed more drug-seeking than males, PRO and wheel running were more effective treatments in females than males, and the combination of PRO and exercise produced a greater reduction in cocaine seeking than either treatment alone in males.

Conclusions: The novel treatments, PRO and wheel running exercise in male and female rats each decreased cocaine seeking in both females and males, but more in females. Exercise reduced the incubation of cocaine seeking over 30 days in rats, and this may be an important strategy to reduce long-term craving after drug abstinence is achieved in humans. The combination of the novel treatments, PRO an exercise, reduced cocaine seeking more than either treatment alone, supporting a multi-pronged approach to reducing relapse. Finally, PRO selectively reduced impulsivity for iv cocaine (vs sugar pellets). Targeting a fundamental characteristic of addiction, impulsivity, appears to be a useful strategy for reducing cocaine seeking. The significance of these findings was that PRO is a commonly used substance in female humans and exercise commonly occurs in male and female, adolescent and adult humans, suggesting that these treatments might be self-sustaining after an initial intervention.

Keywords: exercise, progesterone, treatment, cocaine addiction, sex differences

Disclosures: Nothing to disclose.

W247. Social Economic Status Predicts Dopamine D2/3 Receptor Availability in Healthy Volunteers but Not Cocaine Abusers

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Background: Positron emission tomography (PET) studies in monkeys and humans have shown that social status is associated with striatal Dopamine D2/3 receptor (D2/3R) availability. That is, higher social hierarchy and higher scores on questionnaires assessing social status (i.e., the Multidimensional Scale of Perceived Social Support [MSPSS] and Barratt Simplified Measure of Social Status [BSMSS]) correlated positively with striatal D2/3R availability in monkeys (Grant et al., 1998; Morgan et al., 2002) and humans (Martinez et al., 2010) respectively. Further, subordinate monkeys were vulnerable to the reinforcing effects of cocaine (Morgan et al., 2002), suggesting that alternations in social hierarchy can change D2/3R availability and vulnerability to cocaine reinforcement. To date it has not been investigated whether SES also predicts striatal D2/3R availability in cocaine abusers.

Methods: Here, we investigated whether socioeconomic status (SES) measured with the Hollingshead scale predicts D2/3R availability in the human striatum using [11C]Raclopride PET in N = 38 cocaine abusers (mean age = 43.86 ± 4.50SD years; 4 female) and N = 42 healthy controls (mean age = 42.17 ± 4.54SD years; 4 female), matched for age and education. We calculated regional Bmax/KD values for hand-drawn caudate, putamen and ventral striatum (VS) ROIs. The ratio of the distribution volume in striatal regions to that in the cerebellum was computed to obtain the non-displaceable binding potential (BPND), which was used as a quantification of D2/D3R availability. Regressions analyses were performed to determine whether SES was a predictor of striatal D2/3R availability in both groups. Since D2/D3R availability reduces with age, age was included as a covariate to the analyses.

Results: Compared to controls, cocaine abusers showed lower D2/3R availability in the caudate, putamen, and ventral striatum (all p ≤ .001). Despite matching for education, SES scores were lower in cocaine abusers than controls (p < .001). In the control group only, SES was a significant predictor of D2/3R availability in the caudate (p < .05) and putamen (p < .05) but not in the VS (p > .1), which replicates previous findings in a larger sample of volunteers, but with a different questionnaire for social status. There were no associations between SES and striatal D2/3R availability in the group of cocaine abusers (p > .1).

Conclusions: The study confirms that SES predicts striatal D2/3R availability in healthy human volunteers. However, individual differences in D2/3R availability in cocaine abusers may be driven by factors other than SES, for example history of cocaine use (Volkow et al., 2012).

Keywords: Dopamine (D2, D3) receptors, social, cocaine addiction

Disclosures: Nothing to disclose.

W248. "Too Much of a Good thing?" A Heightened Brain Response to 6 Second Cocaine Video Cues Predicts Poor Drug Use Outcomes

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Background: From an evolutionary perspective, individuals with a greater sensitivity to signals for reward (food, sexual opportunity, attachment) had a survival advantage – turning both animals and humans into exquisite "reward detectors". Ironically, our highly-conserved, survival-driven sensitivity to reward signals may have a potential dark side. For addicted individuals, a heightened sensitivity to drug reward cues could be "too much of a good thing", putting them at greater risk for relapse. We have hypothesized that individual differences in relapse vulnerability may be traced to two, interactive, brain systems: the brain's incentive motivational ("GO!") circuitry, triggered by rewards and their signals, and the brain's modulatory ("STOP!") circuitry, responsible for inhibiting and managing the pull of incentive stimuli. When encountering cues signaling a drug reward, either an over-responsive "GO!" circuit, or an under-responsive "STOP" circuit, or both, could increase the likelihood of relapse. We directly tested this hypothesis in a new cohort of cocaine-addicted individuals, using a task that probed brain responses both while cocaine patients simply watched drug reward cues, and while they actively attempted to inhibit their responses to the cues. We predicted that individuals with poor (future) drug use outcomes would have a stronger brain response (especially in motivational circuitry) to the cocaine cues, and that they would not be able to inhibit this response.

Methods: Our participants were selected from a new cohort (n = 39) of cocaine-dependent patients in large study focused on brain predictors of relapse. Each individual received inpatient stabilization, followed by a functional magnetic resonance imaging (fMRI) session with several probes. The inpatients were then discharged into 12 weeks of outpatient treatment, with twice weekly urine samples. For the current outcome-based comparisons, we selected two phenotypic extremes, "POOR" outcome individuals (more than 90% urines cocaine positive/missing) and "GOOD" outcome individuals (30% or fewer urines cocaine postiive/missing). Each patient underwent BOLD (Blood Oxygen-Level-Dependent) fMRI imaging to examine the brain response to a pseudo-random series of 6-second video clips. The videos represented three conditions (6 clips per condition): WATCH ("Just watch" the cocaine video), DOWN (Please try to reduce your feelings to the cocaine video), or NEUTRAL ("Just watch" the Neutral video). Data were smoothed, normalized, realigned and batch-analyzed within SPM 8, using canonical HRF as the basis function. Pre-planned contrasts compared the brain response to the WATCH vs. the NEUTRAL condition, and to the DOWN vs. NEUTRAL condition, for the whole task, and for the first and second halves of the task (to allow us to examine for change in response as the task progressed). Statistical parametric (t) maps for the second-level (group) analyses (whole group, and for the two phenotypic extremes, "GOOD" and "POOR") were thresholded at t > 2 < 5 for display.

Results: For the WATCH (cocaine video) vs. NEUTRAL comparison, cocaine-addicted individuals who would proceed to a POOR outcome showed a dramatic, widespread brain activation that included not only the classical nodes for motivational processing (e.g., ventral tegmental area, amygdala, ventral striatum/pallidum, medial orbitofrontal cortex, mOFC), but also strong activation in the dorsal cortex, the posterior cingulate cortex and visual cortices. In stark contrast, cocaine patients who would later go onto GOOD outcomes had remarkably "quiet" brains during the cocaine video. Results from the "DOWN" (cocaine video) vs. Neutral condition generally echoed those in the "WATCH" analyses: the POOR outcome group showed massive activation in the mesolimbic circuitry and the visual cortices during "DOWN" (and this activation pattern was even more striking in the second half of the task), while the GOOD outcome group showed much "quieter" brains with only minor activations (in ventral striatum, and in dorsolateral prefrontal cortex, DLPFC, a modulatory region) by the final half of the DOWN task.

Conclusions: These findings offer a clear demonstration that drug cue-provoked brain responses may be able to predict future relapse in addicted individuals. The difference between the two phenotypic extremes was dramatic, with the POOR outcome group showing an impressive, widespread brain response to the drug cues...even when attempting to inhibit. The results have several implications. From an evolutionary perspective, individuals with heightened reward sensitivity may be very "fit" – but they could be at a disadvantage in contemporary environments offering easy access to energy-dense foods, to sexual partners with transmissible infections, and to compelling drugs of abuse. From a mechanistic perspective, these data suggest that the brain response to drug cues in incentive motivational (GO!) circuits is likely to be an important relapse substrate. From a practical perspective, the brain response to drug cues may be a very useful research tool, allowing us to screen candidate medications for their ability to engage relapse-relevant brain targets – offering new hope for individuals with "too much of a good thing".

Keywords: fMRI, relapse, cue-reactivity

Disclosures: Nothing to disclose.

W249. Homer2 Regulates Sensitivity to Methamphetamine Reward

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Background: Homer2 is a post-synaptic scaffolding protein involved in regulating glutamate receptor function that has been highly implicated in drug-induced neural plasticity. Withdrawal from repeated methamphetamine injections increases Homer2 protein expression within the nucleus accumbens (NAC). Moreover, both idiopathic & genetic

vulnerability to high MA reward is associated with elevated Homer2 expression within NAC subregions.

Methods: Herein, we employed methamphetamine-induced place-conditioning procedures [4 pairings of 1 or 2 mg/kg] to assay the effects of constitutive Homer2 gene knock-out (KO) mice and shRNA-mediated knock-down of Homer2b within the NAC core of C57BL/6J mice.

Results KO mice expressed conditioned place-aversion to an environment paired repeated with low-dose methamphetamine (1 mg/kg). While not fully recapitulating the effects of the null mutation, Homer2 knock-down within the NAC core attenuated the expression of a methamphetamine-conditioned place-preference and this effect was observed when animals were tested in under both methamphetamine-free and -primed states.

Conclusions: Together, these results provide novel evidence that Homer2 contributes to the rewarding properties of methamphetamine & poses Homer2-dependent regulation of glutamate transmission within the NAC as a neurobiological substrate in the etiology of MA addiction.

Keywords: Methamphetamine, conditioned place preference, homer, glutamate, Stimulant Abuse

Disclosures: Nothing to disclose.

W250. Essential Role for Arc in Cocaine Addiction-Related Behaviors and Synapse Plasticity

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Background: Exposure to drugs of abuse induces lasting alterations in the brain function of vulnerable individuals, and these changes are thought to maintain maladaptive behaviors characteristic of addiction. Understanding the molecules that mediate such responses to drug experience is critical to developing successful treatment and prevention strategies. Acute cocaine exposure transiently upregulates mRNA and protein expression of the immediate early gene, activity-regulated cytoskeleton-associated protein (Arc), in reward-related brain regions of rodents, an effect that becomes more persistent after multiple exposures. As a key regulator of structural and functional synaptic features that are altered by cocaine treatment and withdrawal, including AMPA receptor surface expression, and as an early responder to cocaine exposure, Arc is well positioned to mediate aspects of drug addiction.

Methods: Separate cohorts of mice lacking Arc (Arc knockout; KO) and their wild-type (WT) littermates underwent testing in multiple behavioral, biochemical and electrophysiological assays, including locomotor sensitization and conditioned place preference. In drug experience-dependent experiments, mice were administered cocaine or saline daily for 7 days and given a moderate withdrawal period prior to undergoing cocaine conditioning for place preference testing. Given the reported learning and memory deficits in Arc KO mice, learning and memory of a fear-associated context were measured using fear conditioning, and preference/operant responding for natural rewards were assessed by sucrose preference and food operant conditioning assays. Biochemical cross-linking methods and electrophysiological recordings were used to assess surface AMPA receptor expression and synaptic strength. Finally, mouse intravenous drug self-administration is currently in progress to assess Arc's potential role in drug seeking and taking.

Results: Consistent with Arc's reported role in activity-dependent endocytosis of synaptic AMPA receptors, we find that Arc KO mice display increased AMPA receptor surface expression and synaptic strength on medium spiny neurons of the nucleus accumbens (NAc). We also observed that Arc KO mice show significantly enhanced locomotor activity in response to moderate doses of cocaine compared to WT littermates, both upon acute and repeated drug administration. Interestingly, naïve Arc KO mice demonstrate normal place preference to various doses of cocaine compared to WT littermates. However, Arc KO mice receiving repeated cocaine administration followed by 10 days of drug abstinence display a significantly sensitized cocaine place preference score, whereas WT mice show no difference between drug naïve and cocaine-experienced mice. Given Arc's importance for learning and memory, the observation of normal, and especially enhanced, place conditioning was unexpected; however, consistent with previous studies, we find that these mice have deficits in contextual fear conditioning, suggesting a unique role for Arc in drug-related learning and memory. When we examine the response of Arc KO mice to natural rewards, they do not differ from WT littermates in the sucrose two-bottle choice assay, nor in the acquisition of sweetened liquid food self-administration. Ongoing studies indicate possible differences between WT and Arc KO mice in the cocaine self-administration assay.

Conclusions: Together, our results demonstrate that mice lacking Arc have enhancements in basal AMPA receptor function in the NAc, as well as in reward-related behavioral plasticity following prior cocaine experience. Future studies addressing the role of this protein in behaviors that are thought to drive continued abuse and addiction, such as cue-induced craving and relapse, are warranted.

Keywords: Arc, cocaine, Reward, Synaptic Plasticity, sensitization

Disclosures: Nothing to disclose.

W251. Sign-Tracking is More Resistant to Extinction than Goal-Tracking, but More Sensitive to Spontaneous Recovery

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Background: Individuals differ in the extent to which reward cues instigate and support goal-directed behavior. Cue-reactive behavior is thought to be a central component of behavioral disorders such as addiction, in which exposure to drug-related cues is a common trigger for craving, relapse, and other pathological behaviors. Conditioned responses to cues can be reduced or eliminated by

extinction procedures, in which the cue is presented repeatedly in the absence of the reward. However, clinical trials using extinction therapies of this sort to treat addiction have been largely disappointing. Pavlovian conditioning in which a neutral stimulus is repeatedly paired with reward delivery can produce two different conditioned responses: sign-tracking responses, which involve approach toward the conditioned stimulus, and goal-tracking responses, which involve approach toward the site of impending reward delivery. Individuals that are prone to develop more sign-tracking than goal-tracking responses are also more susceptible to cue- and drug-induced reinstatement of drug-seeking behaviors. Sign-tracking, like addictive behavior, is also associated with measures of impulsivity and appears difficult to restrain, even in the face of adverse consequences. In this study, we compared sign-tracking to goal-tracking behavior in terms of sensitivity to extinction, reward-induced reinstatement, and spontaneous recovery. In addition, we tested whether sign-tracking rats would be more or less sensitive than goal-tracking rats to extinction, reinstatement, and spontaneous recovery of an operant task.

Methods: 95 male, Sprague-Dawley rats were given Pavlovian conditioned approach training for seven daily sessions, each session consisting of 25 presentations of a retractable lever for 8 s followed immediately by response-independent delivery of a food pellet into a magazine. Following Pavlovian training, rats were divided into two groups. The first group underwent daily extinction sessions in which the lever was presented 25 times without food pellet delivery. Following extinction, the reinstatement of sign- and goal-tracking responses was tested by presenting a non-contingent food reward followed by repeated lever presentations. Finally, the rats underwent extinction again and 2 weeks later underwent a spontaneous recovery test in which the lever was presented 25 times without food delivery. The second group of rats underwent operant training in which they learned to nose-poke for a different food reward. This was followed by extinction, and tests for reinstatement and spontaneous recovery using procedures similar to those described above for the Pavlovian tasks.

Results: For the Pavlovian task, extinction of the sign-tracking response was significantly slower than extinction of the goal-tracking response, even in animals that performed both responses. Sign- and goal-tracking rats did not differ in the reinstatement of the conditioned response; however rats that predominantly goal-tracked showed statistically significant reinstatement relative to their own baseline responding, while sign-tracking rats did not. The spontaneous recovery test showed that conditioned responses to the lever increased after 2 weeks for sign-trackers but not goal-trackers, and for animals that performed both responses the sign-tracking response showed spontaneous recovery while the goal-tracking response did not. For the operant task, sign-tracking rats had comparable extinction rates and less reinstatement of operant behavior than goal trackers. There was no significant spontaneous recovery of operant behavior after 2 weeks for any of the rats.

Conclusions: These results show that the sign-tracking response is more resistant to extinction than the goal

tracking response, and it is more sensitive to spontaneous recovery. This was not true for operant tasks, suggesting that the differences between sign- and goal-tracking observed in this study are specific to the behaviors themselves, rather than due to individual rats with a general deficit in extinction learning. The reinstatement effects were less clear, but suggestive that sign-tracking is actually less sensitive to reward-induced reinstatement, perhaps because of increased salience of the cue relative to the reward in these individuals. To the extent that Pavlovian sign-tracking responses are involved in addictive behaviors, these results could help to explain why drug-seeking is so persistent in certain individuals despite extinction therapies and even after extended periods of abstinence.

Keywords: Reward, incentive salience, individual differences, Pavlovian conditioning, associative learning
Disclosures: Nothing to disclose.

W252. Transcriptional Profiling and Behavioral Phenotyping of Cell Populations in the Mouse Interpeduncular Nucleus during Nicotine Exposure

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Background: Recently the medial habenula (MHb)-interpeduncular nucleus (IPN) has been implicated in nicotine addiction and withdrawal, primarily because of the high expression of the CHRNA5-CHRNA3-CHRNB4 gene cluster of nicotinic receptors within this pathway. Mutations in CHRNA5 that lead to decreased function of a5* nicotinic acetylcholine receptors (nAChR) have been linked to human nicotine addiction, while studies in rats and mice demonstrate that the receptor is responsible for some of the aversive effects of nicotine consumption. While a5 is highly expressed in the IPN, relatively little is known about the distribution of a5 among the poorly characterized cell populations within the IPN, nor is it known to what extent each of these populations contribute to nicotine aversion and/or withdrawal.

Methods: We utilized several BAC-transgenic Cre lines generated by GENSAT to selectively target distinct cell populations within the IPN, including Chrna5-Cre mice. By crossing BAC-transgenic Cre mice with Cre-dependent GFP-tagged ribosome mice, we were able to perform translational affinity purification (TRAP) and determine which genes are actively used by each cell population, both at baseline and after nicotine exposure. Further, by using Cre-dependent viral vectors, we were able to silence different populations within the IPN and determine their role in nicotine consumption and withdrawal.

Results: We determined that the Chrna5 + population within the IPN is heterogeneous and composed of two mutually exclusive subpopulations. Transcriptional profiling also revealed that the Chrna5-Cre mouse overexpresses all of the nAChRs in the CHRNA5-CHRNA3-CHRNB4 gene cluster, limiting its utility in behavioral studies of nicotine consumption and withdrawal. Silencing of one Chrna5 + subpopulation affected both nicotine intake and withdrawal

behaviors, while silencing the other subpopulation had little effect.

Conclusions: Nicotine consumption and withdrawal are modulated by Chrna5 + cells within the IPN. We found that a subset of Chrna5 + IPN cells are necessary for limiting intake of nicotine and for modulating severity of nicotine withdrawal. Understanding the role of the IPN in nicotine-dependent behaviors could lead to more effective therapies for smoking cessation, and perhaps more generally for cessation of other drugs of abuse.

Keywords: nicotine, mRNA, interpeduncular

Disclosures: Nothing to disclose.

W253. Ibudilast, a Novel Neuroimmune Modulator, Decreases Alcohol Craving and Increases Positive Mood in an AUD Population

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Background: The few available medications for the treatment of alcohol use disorder (AUD) have limited efficacy. Therefore, the development of efficacious medications for AUD remains a high research priority, and there is a need to identify new molecular targets for alcoholism treatment. Preclinical data have indicated that neuroinflammation, as indicated by microglial activation and increases in related proinflammatory cytokine signaling, is critically involved with alcohol's acute behavioral effects, alcohol consumption, and alcohol-induced neurotoxicity. This evidence suggests the neuroimmune system may be a potential novel treatment target for AUD. Ibudilast (IBUD) is a medication that inhibits phosphodiesterases -4 and -10 and macrophage migration inhibitory factor and reduces glial cell activation, thereby attenuating neuroinflammatory processes. Recently, IBUD has been shown to reduce alcohol consumption in rats, but its efficacy as a potential AUD pharmacotherapy has not been tested in humans. Therefore, the objective of this randomized, double-blind, placebo-controlled within-subject crossover Phase I/Phase II study was to determine the safety, tolerability, and initial human laboratory efficacy of IBUD (50 mg BID) in a sample of 24 non-treatment individuals who meet criteria for AUD.

Methods: Participants completed two separate 6-day medication regimens (50 mg BID or placebo, in a randomized, counterbalanced order) separated by a 5-10 day washout period between each condition. Participants were titrated on IBUD as follows: 20 mg twice a day on days 1-2 and 50 mg twice a day on days 3-6. On day 5 of each regimen, participants completed a guided imagery stress exposure in the afternoon. On day 6, participants completed an alcohol cue reactivity task followed by an alcohol infusion paradigm. After completion of the infusion, participants remained in the UCLA CTRC for an overnight stay and were discharged the following morning (i.e., day 7). Mood and alcohol craving measures were assessed on a daily basis, as well as during the cue reactivity and stress procedures. Alcohol craving and subjective response were measured during the alcohol infusion paradigm.

Results: The stress, cue reactivity, and alcohol infusion procedures produced the expected changes in mood, craving, and subjective response to alcohol. While IBUD did not affect subjective measures during alcohol infusion, IBUD did significantly block the reduction in positive mood that was observed during the placebo condition in both the cue reactivity and stress procedures (med x trial, $p < 0.05$). After the 6 days of treatment, IBUD, but not placebo, significantly reduced tonic levels of craving (med x day, $p < 0.05$, Day 1 vs. Day 7). Additionally, IBUD, vs. placebo, was associated with a trend toward higher basal cortisol levels (med, $p = 0.07$). Finally, IBUD did not significantly differ from placebo in the number or severity of reported adverse effects over the 7 days of the study.

Conclusions: The results of the present study indicate that IBUD may be a safe and promising treatment for AUD. Ibudilast reduced basal levels of daily alcohol craving, produced a modest increase in basal cortisol levels, and promoted a sustained elevation in positive mood during exposure to alcohol-related cues and stressful imagery. These findings suggest that IBUD may be useful for AUD treatment through mechanisms related to mood enhancement, which are consistent with a hypothesized role for neuroinflammation in mood regulation. Given the excellent safety profile demonstrated in the current study, additional studies of IBUD for AUD treatment appear warranted in larger studies, using chronic dosing and additional markers of efficacy (e.g., alcohol self-administration in the laboratory or real world drinking behavior).

Keywords: alcohol use disorder, ibudilast, Early Phase Drug Development, neuroimmune

Disclosures: Lara Ray is a paid consultant for GSK and has received medication from Pfizer and Medicinova.

W254. Epigenetic Regulation of Memory System Competition Following Withdrawal from Cocaine

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Background: Learning and memory is not a unitary process but includes different kinds mediated by different brain systems. Evidence suggests that the different memory systems of the brain can compete for the control of behavior and that dysfunction in these systems may contribute to the persistence of addiction. In humans, deficits in tasks requiring the use of hippocampal-dependent memory processing predict poor treatment outcomes in cocaine users and preference towards the use of striatal-dependent learning strategies has been associated with addictive drug use (Aharonovich et al., 2006; Bohbot et al., 2013). More recent data suggests that oral cocaine self-administration selectively impairs hippocampal-dependent extinction learning and that dorsal striatal-dependent memory is enhanced by repeated cannabinoid exposure (Gabriele et al., 2015; Goodman and Packard, 2015). The neural mechanisms supporting the dissociable effects of drugs of abuse on memory systems in the brain remain poorly understood. Evidence suggests that epigenetic mechanisms contribute to the establishment and main-

tenance of aberrant neuronal gene programs and behaviors associated with drug exposure. Here we investigated molecular and behavioral effects of prior cocaine exposure in a dual-solution plus maze task that can be acquired using either hippocampus-dependent or dorsal striatal-dependent learning strategies.

Methods: To determine the extent to which withdrawal from repeated cocaine exposure biases competition between memory systems, adult male Long-Evans rats were given daily injections of cocaine (20 mg/kg · 14 days) or saline vehicle. Three weeks later rats were trained and tested in the dual-solution plus maze task (Packard & McGaugh, 1996). Following prolonged withdrawal rats were trained in daily sessions to approach a consistently baited goal arm from the same start arm. Probe trials in which rats were started from the opposite start arm were administered to test whether rats adopted a hippocampal-dependent spatial strategy or a dorsal striatum-dependent response strategy to solve the task. The dorsal striatum and hippocampus were collected 45 minutes after behavioral testing to assess differential regulation of gene transcription and chromatin structure associated with learning strategy bias.

Results: Consistent with previous findings, saline treated rats adopted a "cognitive" hippocampal-dependent learning strategy early in training. In contrast, repeated cocaine followed by extended withdrawal biased rats towards the immediate use of a "stimulus response/habit" learning strategy dependent on the dorsal striatum. Investigations into the molecular mechanisms underlying the effects of prior cocaine experience on memory system bias are underway and will be presented.

Conclusions: These findings suggest that prior cocaine exposure causes a general bias towards the use of striatal-dependent "habit" learning strategies at the expense of more flexible, "cognitive" hippocampal-dependent strategies. We hypothesize that this shift is in part mediated through epigenetic regulation of gene inducibility in both the dorsal striatum and hippocampus.

Keywords: Epigenetics, cocaine addiction, learning and memory

Disclosures: Nothing to disclose.

W255. Discerning the Contribution of Negative Affective-Like and Somatic Symptoms of Withdrawal to the Etiology of Escalated Alcohol Self-Administration in Alcohol Dependence: Role of Nucleus Accumbens Shell Kappa-Opioid Receptors

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Background: Following chronic intermittent alcohol vapor exposure, the neuroadaptive responses in dynorphin (DYN) A-like peptide expression and heightened kappa-opioid receptor (KOR) signaling in the central nucleus of the amygdala (CeA) differentiates alcohol-dependent from non-dependent phenotypes (Kissler et al. 2014). 'Self-medication' hypotheses predominantly explain escalation of alcohol self-administration (ASA) through reductions of aversive states present during withdrawal (Markou et al. 1998). Understanding the nature of the aversive stimulus that drives dependence-induced escalation of ASA during acute withdrawal is important for therapeutic development efforts to treat alcohol dependence. Indeed, it has been shown that in the CeA, which together with the nucleus accumbens shell (AcbSh) and bed nucleus of the stria terminalis form the extended amygdala (Alheid and Heimer 1988), KOR antagonism dissociates enhanced motivation for alcohol from somatic withdrawal symptoms in alcohol-dependent Wistar rats (Kissler and Walker 2015). These data establish that aversive somatic withdrawal symptoms are insufficient to drive escalated ASA. Conversely, KOR antagonism in the CeA is sufficient to rescue escalated ASA, instead suggesting that negative affective-like states may be the stimuli that promote escalated ASA. Given AcbSh involvement in bidirectional signaling of hedonic information (Wheeler and Carelli 2009), AcbSh KOR involvement in depressive-like phenotypes (Mague et al. 2003) and intra-AcbSh KOR-mediated escalated ASA (Nealey et al. 2011), the present experiment tested the hypothesis that intra-AcbSh KOR antagonism in alcohol-dependent animals would produce coincident reductions in escalated ASA and negative affective-like behavior (i.e., 22-kHz ultrasonic vocalizations; USVs) that would be dissociable from reductions in somatic withdrawal symptoms.

Methods: All animal experimentation was conducted according to the Guide for the Care and Use of Animals (National Research Council et al. 2011) and approved by the WSU Institutional Animal Care and Use Committee. Following operant ASA training and stability, adult male Wistar rats were implanted with intra-AcbSh guide cannula and following recovery, were exposed to chronic intermittent alcohol vapor exposure that resulted in escalated ASA, increased 22-kHz USVs and elevated physiological withdrawal signs during acute withdrawal. Once acute withdrawal-mediated escalated ASA was stable, an intra-AcbSh infusion of the KOR antagonist nor-binaltorphimine (nor-BNI; 0, 2, or 6 μg) occurred prior to operant ASA, 22-kHz USV measurement and assessment of somatic withdrawal symptoms during acute withdrawal.

Results: The results verified that during acute withdrawal from chronic intermittent alcohol vapor exposure, animals showed escalated ASA, increased 22-kHz USVs and increased signs of somatic withdrawal. Site-specific KOR antagonism in the AcbSh dose-dependently attenuated escalated ASA and rescued increases in 22-kHz USVs during acute withdrawal in alcohol-dependent Wistar rats, without impacting somatic withdrawal signs.

Conclusions: These results dissociate escalated ASA and increased negative affective-like behavior from somatic withdrawal through KOR antagonism in the AcbSh and identify that pro-negative affective neuroadaptations in KOR signaling promote escalated ASA without impacting somatic withdrawal. These data clarify the nature of the AcbSh stimulus that drives escalated ASA during acute withdrawal and identify an important concept for alcohol dependence-targeted therapeutics; namely that in the face of escalated alcohol intake, targeting negative affective behavior appears to have increased efficacy for reducing escalated alcohol intake in comparison to targeting somatic withdrawal signs since escalated alcohol self-administration

was rescued by KOR antagonism in the presence of increased somatic withdrawal signs.

Keywords: kappa opioid receptor, Alcohol dependence, Phenotypes, alcohol self-administration, negative emotionality

Disclosures: Brendan Walker is a consultant for H. Lundbeck A/S and within the last three years has received honoraria for providing lectures on the kappa-opioid receptor-related neurobiology of nalmefene efficacy in alcohol dependence at Lundbeck-hosted scientific events.

W257. Dependence of Nucleus Accumbens Shell Dialysate Dopamine from the Activity of Apamin-Sensitive Slow-Conducting Ca2 + -Activated K + Channels

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Background: Dopamine (DA) neurons fire in two modes, single spike and burst firing and these two firing modes have been proposed to be the substrate of two different modalities of DA transmission, tonic and, respectively, phasic (Grace, Neuroscience, 41, 1, 1991). Within this frame, extracellular DA assayed by brain microdialysis has been assumed to provide an estimate of the tonic modality of DA transmission (Grace, ibidem, 1991). This, however, is at odds with the fact that drugs activating DA burst firing also increase dialysate DA and that single spike firing is poorly effective in promoting exocytotic release of DA. Burst firing in DA neurons is controlled by apamin-sensitive Ca + + -activated K + (SK) channels. Blockade of SK channels by apamin increases DA burst firing while activation reduces it (Ji and Shepard, Neuroscience, 140, 623, 2006; Herrik et al, Frontiers Pharmacol. 3,11, 2012). However, Steketee and Kalivas (JPET, 254, 711, 1990), failed to observe an increase in dialysate DA in the accumbens (NAc) after intra-ventral tegmental area (VTA) infusion of apamin but they aimed their probes to the NAc core, thus probably sparing the shell. Given the well known differences in the responsiveness of these two subdivisions of the NAc to drugs and motivational stimuli, we have now re-investigated the effect of intra-VTA apamin on dialysate DA by distinguishing shell from core. In addition we studied the effect of systemic administration of the allosteric activator of SK channels, cyclohexyl-[2-(3,5-dimethyl-pyrazol-1-yl)-6-methyl-pyrimidin-4-yl]-amine (CyPPA) (Herrik et al, ibidem, 2012) on the increase of dialysate DA in the NAc shell and core induced by systemic raclopride, a pure D2 antagonist that activates DA burst firing (Andersson et al, Naunyn Schmiedebergs Arch Pharmacol.352, 374, 1995) and increases dialysate DA in the NAc shell and core (Tanda et al, Psychopharmacol, 232, 1427, 2015).

Methods: Male Sprague-Dawley rats (Harlan) weighing 275–300 g were utilized. All procedures and experiments were carried out according to Italian laws (D.L. 116/92 and 152/06) and European directives (609/86 and 63/2010) and were approved by the Ethical Committee for Animal Experiments of the University of Cagliari. Rats were anaesthetized with Equitesin and stereotaxically implanted with guide cannulae unilaterally in the VTA (A − 5.3 mm, L ± 0.9 mm from bregma, V − 8.0 mm from dura). For microdialysis, rats were unilaterally implanted on the same side of the VTA implants with probes in the NAc shell (A + 1.8 mm, L ± 1.1, from bregma, V − 5.8 mm from dura) or in the NAc core (A + 1.6 mm, L ± 1.8 mm, from bregma, V − 5.5 mm from dura). Rats to be injected i.v.were implanted with a catheter in the right jugular vein. Microdialysis experiments were performed 24 hours after probe implant on freely moving rats according to Tanda et al, (2015). Apamin (Sigma-Aldrich) and CyPPA (Tocris) were dissolved in saline and 2% Tween 80 in saline respectively. Raclopride (Sigma-Aldrich) was dissolved in acetic acid and saline and taken to pH 7.4 with NaOH. Apamin was infused in the VTA in 1μl at a speed of 0.5μl/min, at three dose levels: 1.7, 3.3 and 6.6 pmol. CyPPA was administered i.v at doses of 3.3 mg/kg i.v. by itself or as pretreatment, being followed after 5 min by 75 mg/kg i.v. of raclopride. Two-way repeated measure ANOVA with pretreatment (apamin and CyPPA or vehicle) and time as factors was applied to the data obtained from serial assays of DA normalized as percentage of basal DA values of each experimental group. Basal DA values were the means of three consecutive samples differing no more than 10 %. Results from treatments showing overall changes were subjected to post-hoc Tukey's test with significance at p < 0.05.

Results: Intra-VTA apamin, at doses of 1.7 and 3.3 pmol, dose-dependently increased dialysate DA in the shell. Doses of 3.3 pmol of apamin increased NAc shell DA by 75% over basal after 40 min, reaching a plateau of 100-125% over basal after 80 min and up to 140 min. post-drug. Apamin 3.3 pmol increased dialysate DA also in the NAc core but after a longher latency (90 min) than in the NAc shell. After a dose of 6.6 pmol apamin in the VTA, shell DA increased after a shorter latency but returned to basal values by 140 min post-drug. Thus, a 3.3 pmol dose of apamin was the most effective one in raising NAc shell DA. CyPPA, given i.v.at doses of 3.3 mg/kg did not affect basal dialysate DA but reduced to half the maximal increase of NAc shell DA induced by raclopride (75 μg/kg i.v.). CyPPA did not affect the increase of dialysate DA induced by raclopride in the NAc core.

Conclusions: Our observation that intra-VTA apamin increases dialysate DA in the NAc shell and to a lesser extent in the core provides an explanation for the failure of Steketee and Kalivas (ibidem 1990) to observe an increase of DA in the accumbens after intra-VTA apamin. In fact the lateral coordinates of their probe placements (L 1.7 from the midline) as well the histology (Fig.7) shows that microdialysis was centerd to the NAc core and probably spared the shell. Our results are consistent with an important contribution of SK channels and DA burst-firing to dialysate DA. Selective reduction of the raclopride-induced increase of dialysate DA in the NAc shell by systemic CyPPA suggests that dependence of dialysate DA from burst firing is particularly relevant for NAc shell DA transmision and less so for NAc core. This might provide a basis for the higher responsiveness of NAc shell dialysate DA to conventional and drug rewards.

Keywords: Dopamine, KCa2 channels, Nucleus Accumbens Shell, NAcore, Microdialysis

Disclosures: Nothing to disclose.

W258. The Interaction of Food Intake with Voluntary Alcohol Intake: Effects of Macronutrient Deprivation, Incentive Motivation, and Galanin Microinjection

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Background: Research from several approaches suggest the interaction of mechanisms that mediate alcohol abuse and dependence with those that control food intake and nutrition. Alcohol is a calorically rich food as well as a drug of abuse. Food deprivation increases alcohol intake and preference. The mechanisms underlying these increases are not well understood. We explore the role of vital macronutrients in the increase in alcohol intake. In addition to these nutritional aspects of the motivation to drink, we explored the motivational state in the initial exposure to alcohol on later motivation for alcohol intake.
Methods: Experiment 1. Rats were given initial access to alcohol under either food deprivation or non-deprivation conditions. Amount of alcohol during this exposure was controlled such that all animals received the same amount of alcohol during this initial exposure. They were then tested for alcohol intake and preference using the two bottle alcohol-water choice method. Experiments 2, 3, and 4.: Animals were exposed to selective deprivation of fats, carbohydrates and protein respectively and then tested for alcohol intake and preference. Experiment 5: Animals were given microinjection of the orexigenic neuropeptide galanin in the paraventricular nucleeus (PVN) under food deprivation or ad lib food and then tested for alcohol intake and preference.
Results: 1. Rats given initial access to alcohol under deprivation consumed more and preferred alcohol over water more than non-deprived controls. 2. Selective deprivation of fat as well as carbohydrates and protein did not increase alcohol intake or preference. 3. Micro-injection of galanin in the PVN increased alcohol intake in animals under deprivation and ad lib food availability. There was not difference in the maginitude of the effect with either of these food intake conditions.
Conclusions: These data indicated that 1. Initial exposure to alcohol under deprivation conditions increases the incentive motivation for alcohol; 2. Selective deprivation of the fat and the other two macronutrients does not increase motivation to consume alcohol. That the concurrent deprivation of all three nutrients appears to be necessary for the increase in motivation to consume alcohol; 3. The increase in alcohol consumption by the orexigenic peptide galanin appears to be equally potent in increasing alcohol intake under both deprivation and ad lib food conditions. These confirm the interaction of mechanism controlling food intake in the motivation to consume alcohol and these mechanisms may be play a significant role in alcohol use disorder.
Keywords: Alcohol consumption, Incentive motivation, Maronutrient, Galanin
Disclosures: Nothing to disclose.

W259. Acute Responses to Marijuana: Effects of a Strain High in Cannabidiol

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Background: Over the last several years, the United States has witnessed enormous changes concerning the acceptance of marijuana. Colorado, Washington, Oregon, Alaska, and D.C. have legalized recreational use of marijuana with several other states likely to follow in 2016.There are a number of critical limitations in the extant literature that must be addressed if scientists are to influence the regulation of marijuana. First and foremost, scientists need to understand the effects of commonly used marijuana strains (e.g., high THC potency), as they are used in everyday life, as opposed to relying solely on testing the effects of government grown marijuana in controlled laboratory experiments. In addition, scientists need to conceptualize the effects of marijuana as the compound action of different cannabinoids, some that are more inherently harmful than others. For example, cannabidiol (CBD) appears to mitigate some of the effects of tetra-hydrocannabinol (THC), and the ratio of CBD to THC may have a large impact on the effects of the marijuana, which in turn may have important implications for harm reduction. To that end, the overarching objective of this program of research is to advance a more nuanced understanding of the potential harm associated with different strains of marijuana, using a naturalistic design with high external validity, thereby avoiding many of the pitfalls and limitations of previous research.
Methods: A preliminary study was conducted in a sample of 24 regular marijuana users. After a 7 day washout period, the regular users were asked to switch from their normal THC ($\sim$18%) strain to a common strain with THC similar to what they normally use (THC $\sim$ 18%; CBD 0%) or to a strain with lower THC but high CBD (THC $\sim$ 9%; CBD $\sim$12%). The users were instructed to use the new strain at home as they normally would for three days. On the third day, the participants used the marijuana one last time before being transported to the lab for assessments of mood, reward, and verbal memory. Blood samples were collected before and after the trial with the new strain to verify that participants had used the correct strain and to test the association been THC blood levels and the dependent measures.
Results: The blood levels of THC and CBD indicated that two of the subjects did not follow the procedures; these subjects were eliminated from the analyses. In the group that used the normal high potency THC strain, there were significant positive associations (range: $r = .38$ to $r = .74$) between THC blood levels and measures of drug reward, positive mood, and errors on verbal memory task. Conversely, there were no significant associations between THC blood levels and these variables in the subjects who used the high CBD strain. If anything, effect sizes suggested a modest inverse relationship (range: $r = -.35$ to $r = .15$) between THC level and the dependent variables in the high CBD strain group.

Conclusions: The data suggest that the harmful effects of marijuana may differ across strains with different compositions of cannabinoids. In particular, the ratio of THC to CBD may be important in terms of some of these effects. However, it is important to note that this study is preliminary and has a number of limitations. The sample size is small and the results need to be replicated in a larger study. In addition, the variable length of time for transporting subjects back to the lab is a limitation that needs to be addressed in future studies. Despite these limitations, this program of research is important for informing harm reductions strategies in an increasingly post-legalization landscape.

Keywords: Cannabidiol, THC, Harm reduction

Disclosures: Nothing to disclose.

W260. Imaging the Effect of Deep rTMS on Brain Activity in Chronic Cannabis Use

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Background: Chronic cannabis dependence presents a public health challenge, as about 10% of regular users develop dependence, and there are currently no biological treatments available. There is preliminary evidence that repetitive transcranial magnetic stimulation (rTMS) with standard figure-8 coils applied to the dorsolateral prefrontal cortex (DLPFC) in cocaine and tobacco users reduces drug craving and drug self-administration. In this study we used functional magnetic resonance imaging (fMRI) and a laboratory model of cannabis self-administration to evaluate an innovative type of rTMS in human volunteers as a potential treatment approach to cannabis dependence. We used the H7 coil, allowing for the stimulation of deeper brain structures, directed at the dorsomedial prefrontal cortex (dmPFC) and anterior cingulate cortex (ACC), areas shown to be affected in addiction, including in cannabis dependence. Our hypothesis was that active rTMS administration would normalize activity in the PFC and ACC and reduce the choice the self-administer cannabis.

Methods: To this date, 10 medically healthy non-treatment-seeking participants with chronic daily cannabis use (CD, age 35.9 ± 8. 5,2 females , 8 males) were admitted to our research unit for a 21 day study. Following 4 days of inpatient monitored abstinence, the participants underwent baseline cannabis self-administration sessions. Laboratory based self-administration sessions were conducted at baseline and after the complete period of rTMS to provide a model of withdrawal and relapse. Participants were given 6 opportunities to choose 0, 1, 2, or 3 puffs of standardized marijuana cigarettes containing 5.5% Δ9-tetrahydrocannabinol (THC) at 1.5 hour intervals, at $0.5 per puff, deducted from their study earnings. Subjective effects of cannabis and craving were rated. Participants were then randomly assigned to two groups: high frequency (n = 5) and sham (n = 5). Participants underwent a total of 11 treatments (over 2 weeks) with rTMS using the H7 coil at 110% of motor threshold at a frequency of 10 Hz; sham controls underwent the same scenario, but with a coil set to produce noise artefacts only. The cannabis self-administration sessions were repeated after 5 and after 11 rTMS sessions, and the outcome measure was the number of puffs chosen. Using fMRI and the Rapid Simon interference task, we also compared baseline BOLD activation in the PFC and ACC post-treatment activation. The Rapid Simon task assesses response to conflicting stimuli and response inhibition, functions subserved by the ACC and DLPFC. An SPM8 analysis was performed of the congruent and incongruent stimuli and hypotheses were tested with Random Field Theory correction for multiple comparisons set to a significance level of p < .05. Participants also completed neurocognitive assessments of processing speed, reaction time and verbal learning at baseline and after the complete treatment.

Results: There were no differences in age or ethnicity between groups. The results showed that the active frequency group decreased their cannabis intake from 9.6 ± 2.7 puffs to 6.8 ± 2.8 (p = 0.14 one tailed ttest). Meanwhile, the sham group showed no change in their choices for cannabis: baseline 4.4 ± 3.8 puffs vs post rTMS: 4.2 ± 4.5 (p = 0.82 one tailed ttest). This corresponded to an effect size of 0.8.

FMRI analysis showed that the conflict-related neural activity detected by the contrast cI vs cC (incongruent trials preceded by congruent trials versus congruent trials preceded by congruent trials) in 5 cannabis users who performed the Simon task before and after active rTMS that participants had deactivated activity within the inferior frontal gyrus (IFG) at baseline. After active rTMS, the activity in IFG and ACC were normalized. In addition, rTMS also activated the activity in caudate and putamen, regions related to cognitive control, suggesting that rTMS can normalize the top-down and bottom-up networks that govern performing a task related to self-regulation.

There were no differences in reaction time or memory pre- and post-treatment or between groups in this preliminary sample.

Conclusions: This report presents the first study using rTMS as novel tool to treat chronic cannabis use. The preliminiary results in participants receiving active rTMS suggest that rTMS can normalize the task-related activity in brain regions related to substance use, including inferior frontal gyrus and anterior cingulate cortex, the regions known to mediate conflict processing. Further, these results suggest that high frequency rTMS using the H coil to the mPFC/ACC may influence cannabis self-administration using a laboratory paradigm to model relapse, and may thus aid in re-inforcing abstinence from clinically relevant drug use with greater efficacy than any pharmacological treatments, of which there currently are no reliable ones available. The results from this study will provide important pilot- data for a future larger study using this novel method as an imaging probe and as a potential and can be used to evaluate potential treatments for drug dependence.

Keywords: transcranial magnetic stimulation, cannabis dependence, translational imaging

Disclosures: Nothing to disclose.

W261. The Effect of Oxytocin on Methylphenidate-Induced Stimulation of Dopamine Levels: Importance of Route of Administration for Oxytocin

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Background: Oxytocin (OXT), a nonapeptide hormone synthesized in the hypothalamus, has been associated with numerous psychological and physiological processes. OXT plays a role in appetite and food regulation as well as social recognition, pair bonding and social anxiety. It has been shown that the behavioral and neuroplastic changes that occur during social bonding parallel those that occur in addiction and recent research suggests that OXT may be involved in these processes. The hormone has been shown to alter behaviors related to the administration of drugs of abuse, including methamphetamine, cocaine, psychostimulants and alcohol. OXT has also been shown to interact with the dopaminergic pathways believed to influence the development of addiction. In this study, we investigated the effect of OXT on methylphenidate (MPH)-induced dopamine release in the nucleus accumbens in rats.

Methods: We analyzed the effect of various doses of OXT on basal striatal dopamine levels (via microdialysis) following MPH administration as a function of different routes of administration: intraperitoneal (IP) injection, intranasal (IN) administration (with application to rhinarium) and an alternative IN administration using a Pressurized Olfactory Device, which permits the drug to be delivered directly to olfactory region of the nasal cavity. OXT was administered 10 minutes prior to injections of MPH which occurred at timepoints 0, 30 and 60 minutes at doses 0.1, 0.32 and 1.0 mg/kg, respectively. Microdialysis data was collected continuously over 10 minute increments. The effect of OXT alone on basal dopamine levels was analyzed by pretreatment with 2.0 mg/kg OXT, followed by injections of saline at timepoints 0, 30 and 60 minutes.

Results: Successive injections of MPH result in a dose-dependent increase in stimulated dopamine levels. Pretreatment with 2 mg/kg OXT IP resulted in a significantly greater dopamine stimulation as compared to both 1 mg/kg OXT and saline (p < 0.05). Additionally, IN administration, both rhinarium application and via the Pressurized Olfactory Device, yielded no significant changes in dopamine stimulation. OXT alone did not affect basal dopamine levels.

Conclusions: Although OXT alone did not stimulate dopamine levels, these results indicate that OXT, administered IP, potentiates MPH-stimulated striatal dopamine levels in a dose-dependent manner. The results from this study indicate that OXT IP (but not IN) administration results in the modulation of basal dopamine levels following MPH injection. This study provides a platform for a planned translational clinical PET study with 11[C] raclopride to look at the effects of OXT on striatal dopamine signaling in humans.

Keywords: oxytocin, Psychostimulant, Dopamine, addiction, Microdialysis

Disclosures: Nothing to disclose.

W262. Arithmetic and Local Circuitry Underlying Dopamine Prediction Errors

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Background: Dopamine neurons signal prediction error, or the difference between actual and predicted reward. Prediction errors are thought to be crucial for both adaptive learning and the development of addiction. However, despite two decades of investigation, little is known about how prediction errors are calculated in the brain.

Methods: To determine how dopamine neurons calculate prediction error, we combined optogenetic manipulations with extracellular recordings in the ventral tegmental area (VTA) while mice engaged in classical conditioning. Our techniques allowed us to tag recorded neurons as either dopaminergic or GABAergic, and selectively stimulate or inhibit these neurons while recording from other neurons in the circuit. We performed 4 experiments, with a total of 33 mice and 632 VTA neurons.

Results: We demonstrate that dopamine neurons perform subtraction, a computation that is ideal for reinforcement learning but rarely observed in the brain. Furthermore, selectively exciting and inhibiting neighboring GABA neurons in the VTA reveals that these neurons are a source of subtraction: they inhibit dopamine neurons when reward is expected, casually contributing to prediction error calculations. In particular, we found that stimulating VTA GABA neurons subtracts from dopamine reward responses (P < 0.001, t-test), as if reward is more expected, and that inhibiting VTA GABA neurons increases dopamine reward responses (P < 0.001, t-test), as if reward is less expected. Finally, bilaterally stimulating VTA GABA neurons dramatically reduces anticipatory licking to conditioned odours (P < 0.001, mixed effects linear model), consistent with an important role for these neurons in reinforcement learning. VTA GABA neurons, therefore, help put the "prediction" in "prediction error".

Conclusions: Together, our results uncover the arithmetic and local circuitry underlying dopamine prediction errors. This provides a framework for understanding how alterations in the circuitry–in particular, inhibition of VTA GABA neurons–can stimulate a vicious cycle leading to substance addiction.

Keywords: Dopamine, Reinforcement learning, Ventral tegmental area (VTA), GABAergic interneurons, reward prediction error

Disclosures: Nothing to disclose.